RECIPE

Therapeutic Drug Monitoring (TDM) of Antibiotics by LC-MS/MS

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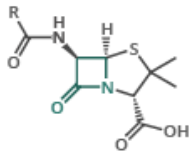
DIFFERENT CLASSES OF ANTIBIOTICS - AN OVERVIEW

Key: COMMONLY ACT AS BACTERIOSTATIC AGENTS, RESTRICTING GROWTH & REPRODUCTION — COMMONLY ACT AS BACTERICIDAL AGENTS, CAUSING BACTERIAL CELL DEATH

ß-LACTAMS

MOST WIDELY USED ANTIBIOTICS IN THE NHS



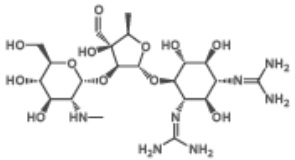
All contain a beta-lactam ring

EXAMPLES
Penicillins (shown) such as amoxicillin and flucloxacillin; *Cephalosporins* such as cefalexin.

MODE OF ACTION
Inhibit bacteria cell wall biosynthesis.

AMINOGLYCOSIDES

FAMILY OF OVER 20 ANTIBIOTICS



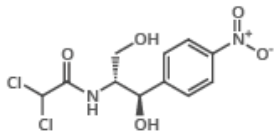
All contain aminosugar substructures

EXAMPLES
Streptomycin (shown), neomycin, kanamycin, paromomycin.

MODE OF ACTION
Inhibit the synthesis of proteins by bacteria, leading to cell death.

CHLORAMPHENICOL

COMMONLY USED IN LOW INCOME COUNTRIES



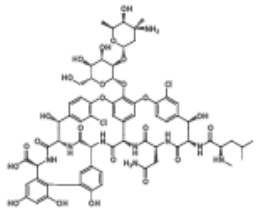
Distinct individual compound

MODE OF ACTION
Inhibits synthesis of proteins, preventing growth.

No longer a first line drug in any developed nation (except for conjunctivitis) due to increased resistance and worries about safety.

GLYCOPEPTIDES

COMMON 'DRUGS OF LAST RESORT'



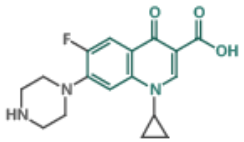
Consist of carbohydrate linked to a peptide formed of amino acids

EXAMPLES
Vancomycin (shown), teicoplanin.

MODE OF ACTION
Inhibit bacteria cell wall biosynthesis.

QUINOLONES

RESISTANCE EVOLVES RAPIDLY



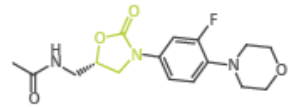
All contain fused aromatic rings with a carboxylic acid group attached

EXAMPLES
Ciprofloxacin (shown), levofloxacin, trovafloxacin.

MODE OF ACTION
Interfere with bacteria DNA replication and transcription.

OXAZOLIDINONES

POTENT ANTIBIOTICS COMMONLY USED AS 'DRUGS OF LAST RESORT'



All contain 2-oxazolidone somewhere in their structure

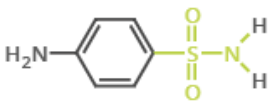
EXAMPLES
Linezolid (shown), posizolid, tedizolid, cycloserine.

MODE OF ACTION
Inhibit synthesis of proteins by bacteria, preventing growth.

DISCOVERY 1930 1940 1950 1960 1970 1980

SULFONAMIDES

FIRST COMMERCIAL ANTIBIOTICS WERE SULFONAMIDES



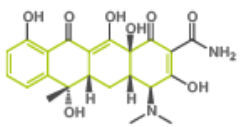
All contain the sulfonamide group

EXAMPLES
Prontosil, sulfanilamide (shown), sulfadiazine, sulfisoxazole.

MODE OF ACTION
Do not kill bacteria but prevent their growth and multiplication. Cause allergic reactions in some patients.

TETRACYCLINES

BECOMING LESS POPULAR DUE TO DEVELOPMENT OF RESISTANCE



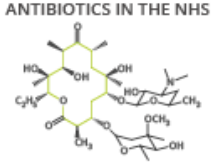
All contain 4 adjacent cyclic hydrocarbon rings

EXAMPLES
Tetracycline (shown), doxycycline, limecycline, oxytetracycline.

MODE OF ACTION
Inhibit synthesis of proteins by bacteria, preventing growth.

MACROLIDES

SECOND MOST PRESCRIBED ANTIBIOTICS IN THE NHS



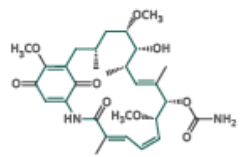
All contain a 14-, 15-, or 16-membered macrolide ring

EXAMPLES
Erythromycin (shown), clarithromycin, azithromycin.

MODE OF ACTION
Inhibit protein synthesis by bacteria, occasionally leading to cell death.

ANSAMYCINS

CAN ALSO DEMONSTRATE ANTIVIRAL ACTIVITY



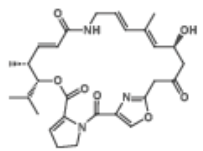
All contain an aromatic ring bridged by an aliphatic chain.

EXAMPLES
Geldanamycin (shown), rifamycin, naphthomycin.

MODE OF ACTION
Inhibit the synthesis of RNA by bacteria, leading to cell death.

STREPTOGRAMINS

TWO GROUPS OF ANTIBIOTICS THAT ACT SYNERGISTICALLY



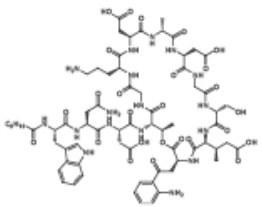
Combination of two structurally differing compounds, from groups denoted A & B

EXAMPLES
Pristinamycin IIA (shown), Pristinamycin IA.

MODE OF ACTION
Inhibit the synthesis of proteins by bacteria, leading to cell death.

LIPOPEPTIDES

INSTANCES OF RESISTANCE RARE



All contain a lipid bonded to a peptide

EXAMPLES
Daptomycin (shown), surfactin.

MODE OF ACTION
Disrupt multiple cell membrane functions, leading to cell death.

RECIPE

Antibiotics: Division

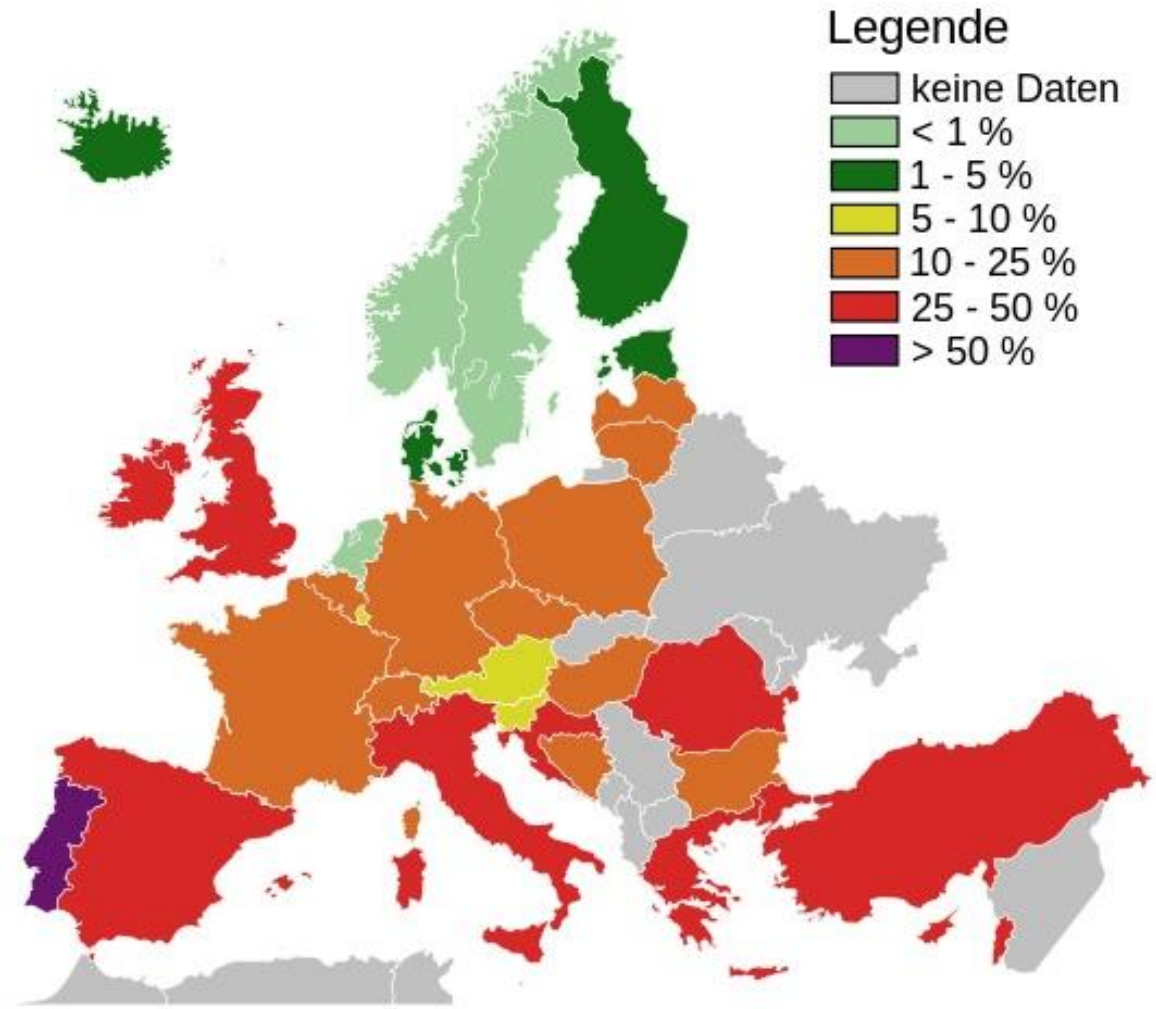
Most prescribed Antibiotics (ICUs Gernmay):

1. Piperacillin/Tazobactam
2. Imipenem
3. Meropenem
4. Ampicillin/Sulbactam
5. Ciprofloxacin
6. Levofloxacin
7. Ceftriaxon
8. Metronidazol (Nitroimidazole)
9. Clarithromycin
10. Vancomycin

Antibiotic class	Examples	Mechanism of action
Beta Lactam	Penicillins, Cephalosporins	Inhibit peptidoglycan synthesis in bacterial cell walls
Glycopeptides	Vancomycin	Inhibit peptidoglycan synthesis in bacterial cell walls
Aminoglycosides	Kanamycin, Gentamicin	Target bacterial ribosomal subunit (30S)
Tetracyclines	Tetracycline, Doxycycline	Target bacterial ribosomal subunit (30S)
Macrolides Lincosamides	Azithromycin Clarithromycin Erythromycin Clindomycin	Target bacterial ribosomal subunit (50S)
Quinolones	Ciprofloxacin Moxifloxacin	Inhibit bacterial DNA replication
Rifampin	Rifampicin	Inhibit bacterial RNA transcription
Sulfonamides	Sulfa Drugs	Inhibit folate synthesis

Resistence to Antibiotics

Share of MRSA in all staphylococcal infections in 2008. Institute for Public Health and the Environment (RIVM), Bilthoven, NL. CC BY-SA



European study (2018): 33,000 deaths a year from antibiotic resistant infections

How to avoid Antibiotic Resistance?

- Roberts et al. *Clinic Iinfect Dis* **2014**; 58 (8): 1072 – 833 (Studie about β-Lactam in ICUs)
  - 16 % of the patients have low drug concentrations
  - 30 % No improvement
  - about 50 % of patients are overdosed
- *Piperacillin concentration in relation to therapeutic range in critically ill patients - a prospective observational study.* Zander et al. *Critical Care* (**2016**) 20: 79
  - **Piperacillin**: 50 – 60 % < therap. range

Patients in ICU differs in Pharmokinetik due to
- organ dysfunctions, infusions, additional drugs…

=> Change in strategy from „ one-dose-fits-all“ to personalized montitoring of the drug concentration in the blood

How to monitor at best?

Different drug different target

C_{max}/MIC

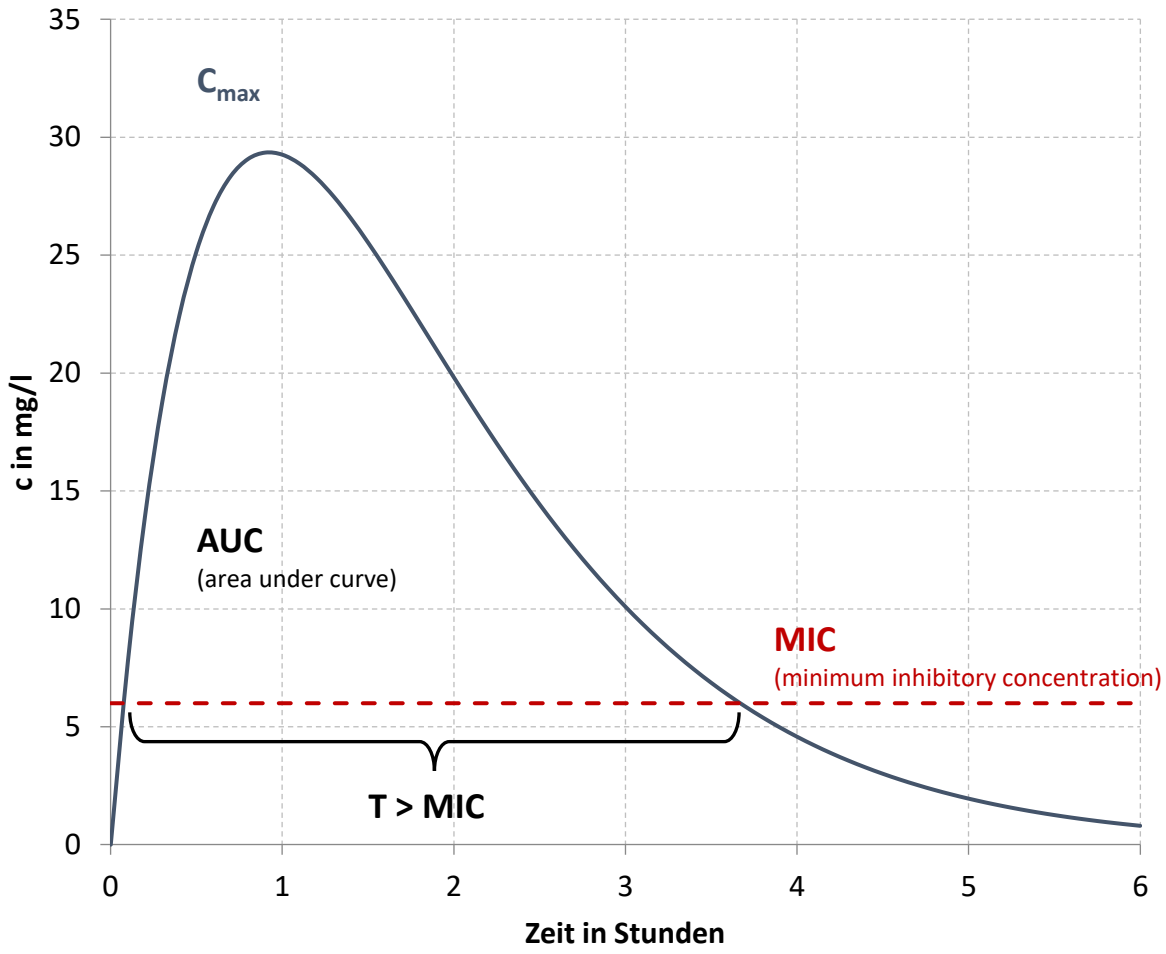
- z. B. Aminoglykoside, Fluorchinolone, Daptomycin

AUC/MIC

- Area under 24 hour-Concentration above MIC
- z. B. Linezolid, Tigecyclin, Azithromycin, Glykopeptide, Colistin

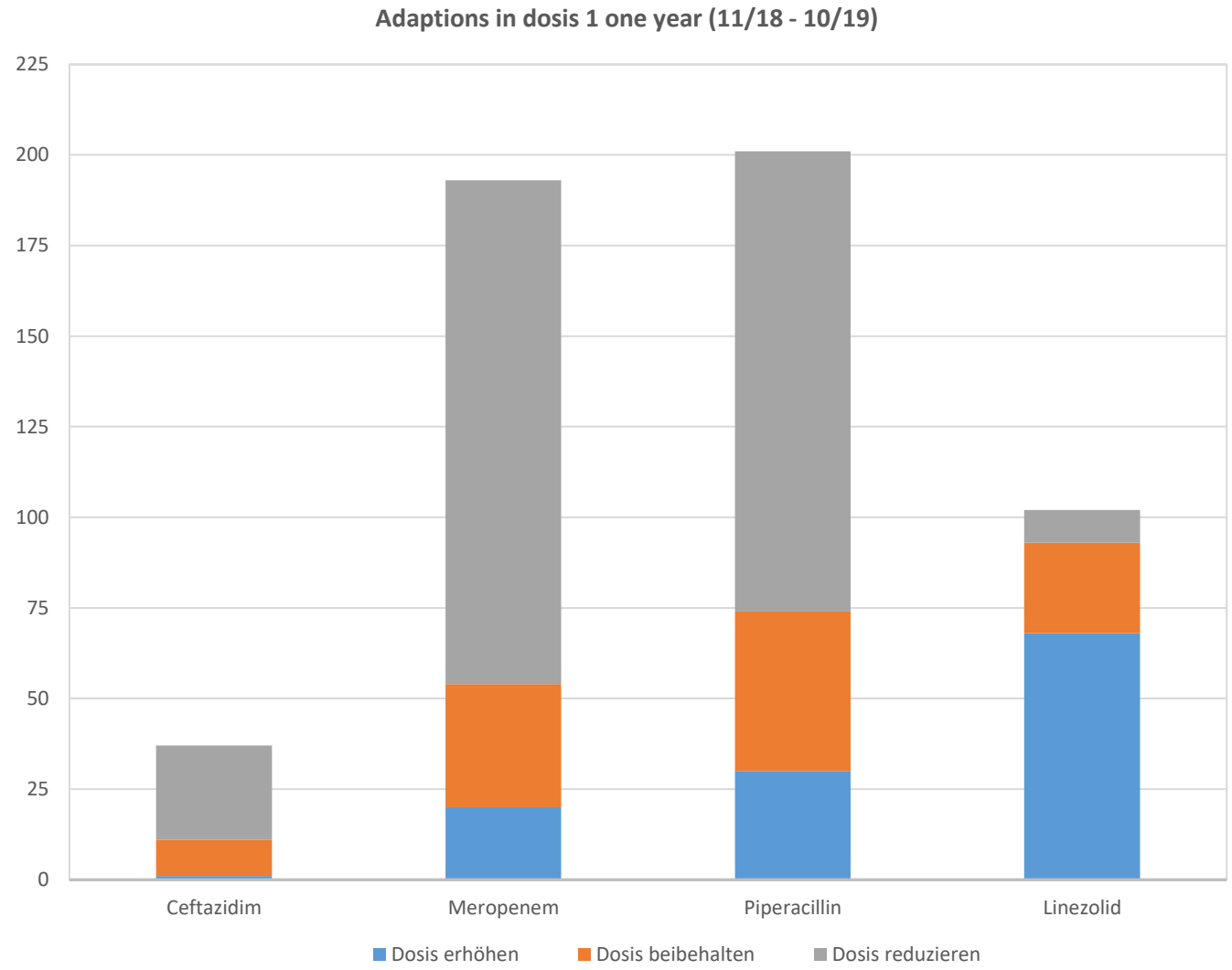
T > MHK

- % of time after dosing while c > MHK
- E.g.. β-Lactam-Antibiotics, Clindamycin, Fidaxomicin



Results pilot study in KH Chemnitz 2018/2019

Very positive feedback from ICU => They want to keep the monitoring and enlarge the panel



ClinMass TDM Kit System: Add-on Antibiotics MS9700

The ClinMass® TDM Kit System is based on the universal ClinMass® TDM Platform (MS9000) that can be used for a huge number of TDM analytes.

- **<u>Currently available ClinMass® Add-on Sets:</u>**

- **MS9100 for 15 Tricyclic Antidepressants (TCA)**
- **MS9200 for 26 Antiepileptic Drugs (AED)**
- **MS9300 for 28 Neuroleptics (NLP)**
- **MS9400 for 37 Antidepressants (ADP)**
- **MS9500 for 36 Benzodiazepines (BZP)**
- **MS9600 for 11 Antimycotics (AMC)**
- **MS99100 for 2 Mycophenolic Acids (MPA)**
- ***MS99200 for 4 Immunosupressants (ISD)***
- **and NEW for 15 Antibiotics (ABx)**
-

ClinMass TDM Kit System: Add-on Antibiotics MS9700

List of analytes and the related internal standards

Analyte	IS	Analyte	IS
Ampicillin	Ampicillin-d_5	Flucloxacillin*	Clindamycin-d_3
Cefazolin*	Piperacillin-d_5	Linezolid	Linezolid-d_3
Cefepim	Cefepim-d_3	Meropenem	Meropenem-d_6
Cefotaxim*	Clindamycin-d_3	Piperacillin	Piperacillin-d_5
Cefuroxim*	Tazobactam-$^{15}N_3$	Sulbactam	Sulbactam-d_5
Chloramphenicol	Chloramphenicol-d_5	Tazobactam	Tazobactam-$^{15}N_3$
Clindamycin	Clindamycin-d_3	Vancomycin*	Clindamycin-d_3
Daptomycin	Daptomycin-d_5		

Sample Pretreatment

Precipitation	**50 µl Plasma (Calibrator, control, patient)**	**100 µl Internal Standard IS (reconstituted in Precipitant P)**

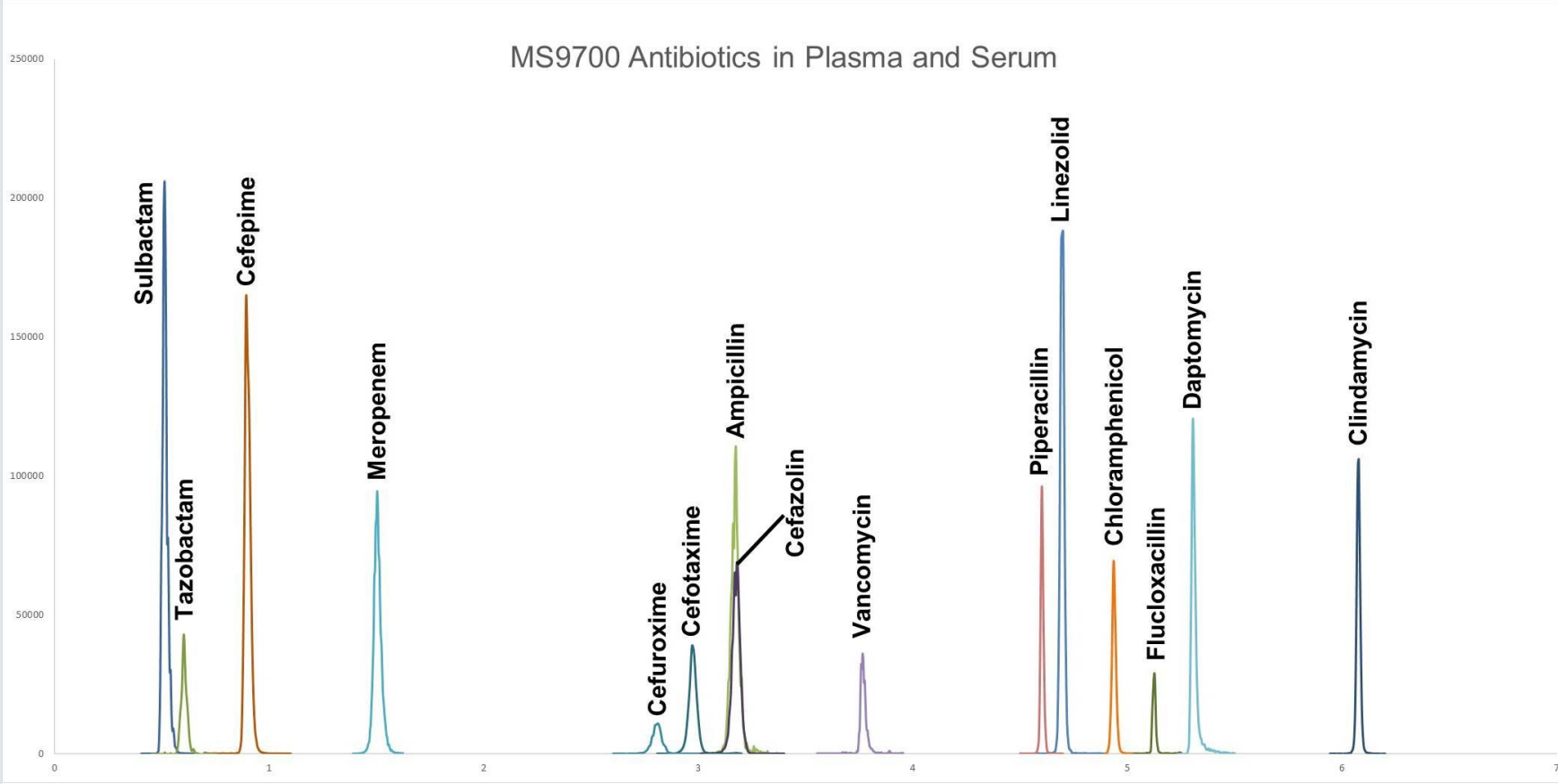
=> mix => centrifugate (10 min, 10000 x g)

Dilution	**50 µl supernatant**	**950 µl Water (< 0.5 µS/cm)**

LC-MS/MS analysis:	**Injection of 2 – 20 µl of the dilution***

Chromatogramm

Run Time: 9.5 min. Validated on a Shimadzu Nexera coupled with API TQ5500



Thank you for your attention

Děkuji za pozornost