Therapeutic Drug Monitoring

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Overview

- Definition & Purpose of TDM
- Criteria for TDM
- Therapeutic range
- Pharmacokinetics
- Pharmacogenomics (+ example)
- Other specific drug examples
- Sampling timing/requirements
- Analytical methods

TDM – Definition

The measurement of specific drugs or their metabolites at regular intervals as an aid to optimising therapy.

TDM – Why do it?

- To establish **correct dose** for each patient.
  - Individuals vary in terms of "ADME"
  - Pharmacokinetics, dynamics and genetics
- To monitor that the **dose remains. effective.**
- To **prevent/minimise toxicity.**
- To **check/support compliance** of medication.
- Better patient management and improved patient quality of life.

Criteria for TDM

1. **Narrow therapeutic index** (therapeutic range – between toxic and therapeutic effect)
2. **Long-term therapy**
3. **Good correlation between serum concentration and clinical response**
4. **Variable pharmacokinetics**
   - Intra-individual
   - Inter-individual
5. **Absence of suitable biomarker** associated with therapeutic effect or outcome
6. **Co-administered with potentially interacting drugs**

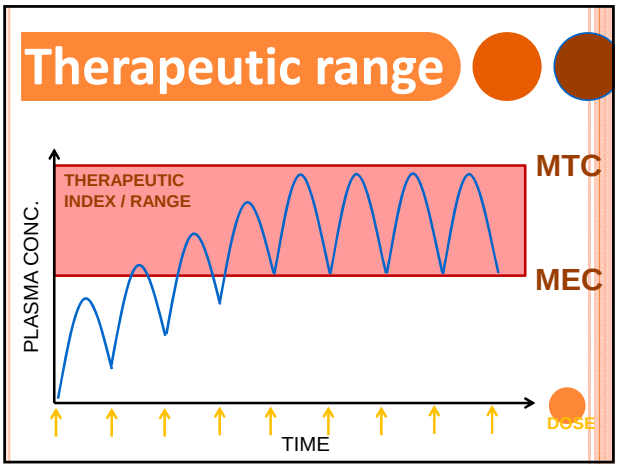
Therapeutic range

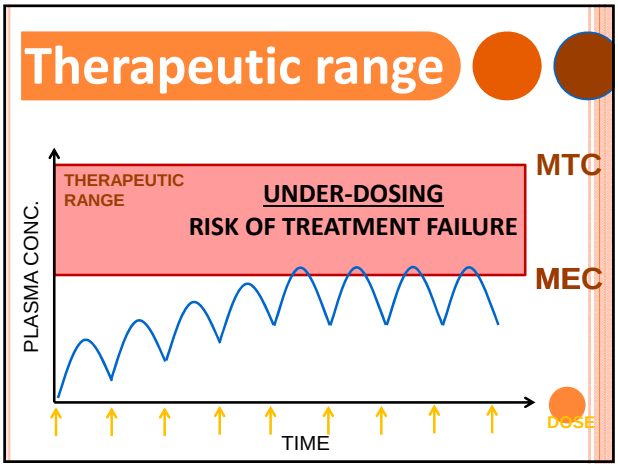
Represents the interval between:

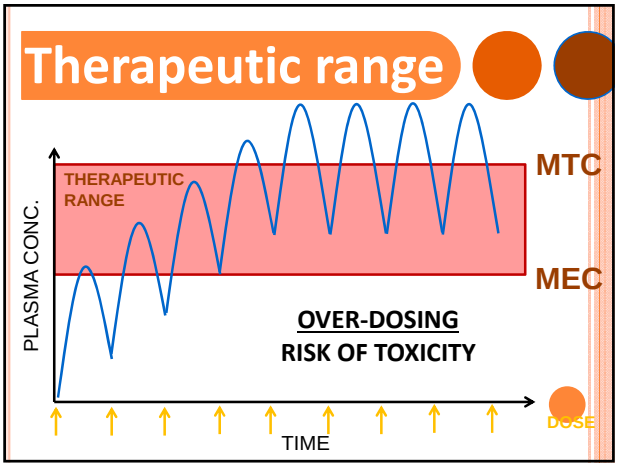
- **MEC** – minimum effective concentration
- **MTC** – maximum therapeutic concentration
  – minimum toxic concentration

In optimal dosing:

- Trough blood concentration should not fall below the MEC
- Peak blood concentration should not exceed the MTC

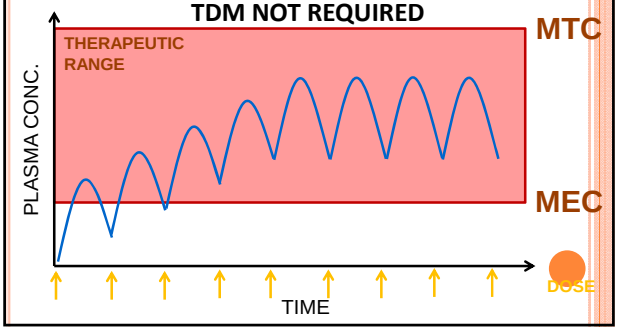

Therapeutic range
THERAPEUTIC INDEX / RANGE
MTC
MEC
PLASMA CONC.
TIME
DOSE


Therapeutic range
THERAPEUTIC RANGE
UNDER-DOSING
RISK OF TREATMENT FAILURE
MTC
MEC
PLASMA CONC.
TIME
DOSE


Therapeutic range
THERAPEUTIC RANGE
MTC
MEC
OVER-DOSING
RISK OF TOXICITY
PLASMA CONC.
TIME
DOSE

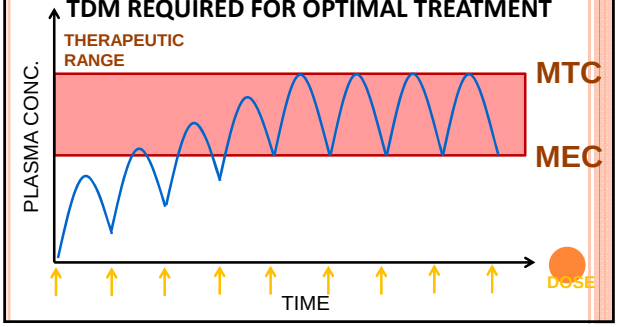
Therapeutic range

WIDE THERAPEUTIC RANGE
TDM NOT REQUIRED



Therapeutic range

NARROW THERAPEUTIC RANGE
TDM REQUIRED FOR OPTIMAL TREATMENT

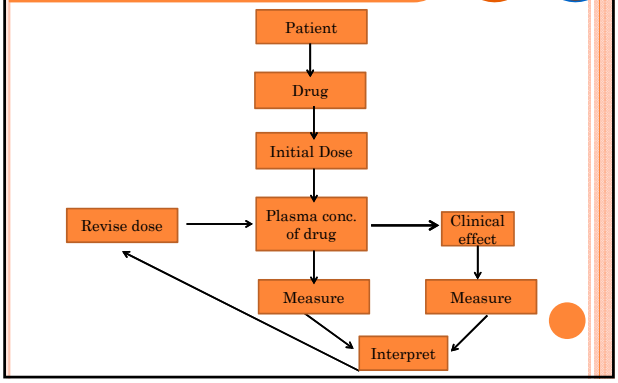


The Essentials

For effective TDM…

- **Rational indication for request** (e.g. suspected toxicity or non-compliance)
- **Accurate patient information**
- **Appropriate sample and timing** (patient should be @ 'steady state' on current dosage unless ?toxicity)
- **Accurate analysis**
- **Correct results interpretation**
- **Appropriate action**

Principles of TDM



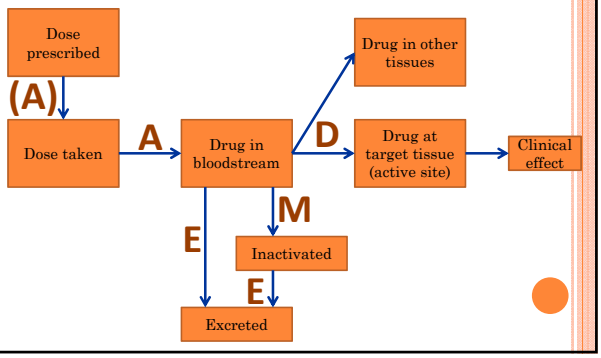
Pharmacokinetics

- Describes what the body does to drugs
- Factors affecting concentration of drug in plasma
- "ADME"
- Differs between individuals (inter-individual variation)
- Differs within an individual (intra-individual variation)

PharmacoKinetics

(A)	(Adherence)
A	Absorption
D	Distribution
M	Metabolism
E	Elimination

PharmacoKinetics



(A)

ADHERENCE

- aka "compliance"
- Whether the patient actually takes the drug they have been prescribed, or not
- Issues with chronic therapy

A

ABSORPTION

- Amount of drug taken that actually reaches the bloodstream
  - **iv** = 100%
  - **oral** = variable
- Depends on:
  - Drug formulation
  - Co-administered food / drugs
  - GI tract integrity / function
  - Genetic variability
  - First-pass metabolism ([drug] greatly reduced before reaches systemic circulation)

D

DISTRIBUTION

- Once in the bloodstream, drugs are transported around the body to the various tissues
- Drug will either prefer to stay in the bloodstream or to enter the body tissues
- Depends on:
  - Relative solubility in fat or water
  - Binding to plasma proteins
  - Binding to tissue lipids
- ↑ distribution:
  - Fat soluble
  - ↓ Plasma protein binding
  - ↑ Tissue lipid binding

M

METABOLISM

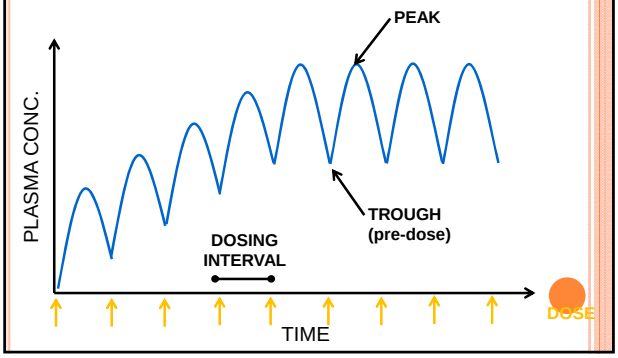
- Process by which the body alters the chemical structure of a compound
- Function:
  - Make drug more water-soluble
  - Enhance excretion
- Location:
  - Mainly in the liver (enzymes)
  - (Other tissues)
- N.B. Metabolism ≠ Inactivation
- Some drug metabolites are active

E

ELIMINATION

- Removal of drugs from the body
- Routes:
  - Urine
  - Faeces
  - Sweat
  - Breath
  - Breast milk
  - Hair
  - Nails
  - Placental transfer
- Kidney function very important
- Reduced kidney function = reduced elimination

Plasma drug levels



Plasma drug levels

STEADY STATE:

- Point of equilibrium
- Rate of administration = Rate of elimination

HALF-LIFE ($t_{1/2}$)

- Time taken to reduce plasma concentration to one-half of its initial value
- $t_{1/2}$ = **dosing interval** (drugs usually administered once every $t_{1/2}$)
- Takes 5-7 x $t_{1/2}$ to reach steady-state

Pharmacogenomics

PHARMACOGENOMICS

- The role of genetics in drug response
- Describes how genetic variation alters Pharmacokinetics
  - ADME
- Predict how well a patient will respond to a drug regime based on their genetics
- **"Personalised medicine"**

Pharmacogenomics

FAST METABOLISERS

- Metabolise drugs quickly
- May clear drugs before they have had time to work
- May require higher doses

SLOW METABOLISERS

- Metabolise drugs slowly
- Drug stays in body for longer = ↑ Efficacy
- But potential for build-up of drug > MTC
- Risk of toxicity
- May require lower doses

PGs – Example

TPMT and Thiopurine Drug Metabolism…

Thiopurine Drugs

- Azathioprine (AZA), 6-Mercaptopurine (6-MP
- Steroid-sparing immunosuppressant agents for autoimmune and chronic inflammatory diseases
- Widely used in inflammatory bowel disease (IBD) and other medical specialties.
- Efficient re: induction and maintenance of IBD remission
  - Induce remission in 50-60% patients.
  - Complete steroid withdrawal in up to 70% patients.

Past approach to dosing…

- Give a 'standard dose'
- Monitor patient clinically ± basic lab tests
  - Some respond, some don't
  - Most - no side effects
  - Some - fall in cell counts
  - Some - fatal bone marrow toxicity
  - Some experience other side effects
- **Hit and miss!**

Current approach to dosing…

- Susceptibility to some side effects determined by genetic make-up.
- Predict who is likely to experience side effects and adjust starting dose accordingly.

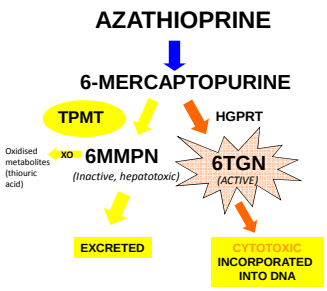
PHARMACOGENETICS

- **2011: Guidance for safe and effective prescribing of AZA**
  - All patients to be tested for Thiopurine S-Methyltransferase (TPMT) status prior to commencing treatment.

Thiopurine S-Methyl Transferase

- "TPMT"
- Cytoplasmic Transmethylase - enzyme present in many tissue types (predominantly liver & kidney).
- Catalyses formation of inactive metabolite 6-Methylmercaptopurine Nucleotides (6MMPN).
- Effectively reducing concentrations of active metabolite 6-Thioguanine Nucleotides (6TGN)
  - Therapeutic effect (cytotoxic, false bases incorporated into DNA)
  - Myelosuppression at high concentrations.

Metabolism of Thiopurine Drugs



TPMT = Thiopurine S-methyl transferase

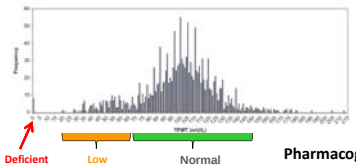
XO = Xanthine Oxidase

HGPRT = Hypoxanthine-guanine phosphoribosyl transferase

6TGN = 6-thioguanine nucleotides

6MMPN = 6-methylmercaptopurine nucleotides

Frequency of Distibution of TPMT



Pharmacogenomic Variability:

TPMT Activity	Prevalence	Treat?	Dose
Normal	89%	Yes	Standard
Low	11%	Yes	Reduced
Deficient	0.3%	No	N/A

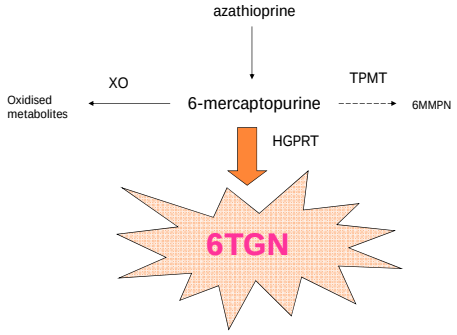
1000 outpatient study, Birmingham City Hospital
(Ann. Clin. Biochem. 2010; 47: 408-414)

Variation is due to Genetics…

- Various mutations in TPMT gene cause lower TPMT activity.
- Autosomal co-dominant pattern.

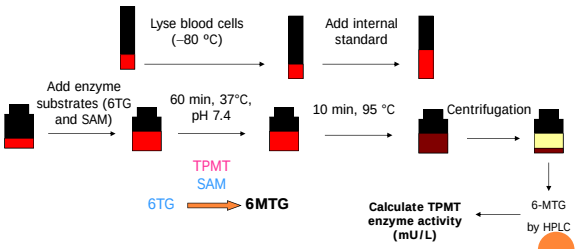
TPMT Activity (mU/L)	TPMT Status	TPMT Genotype	
<10	Deficient	*3/*3	Homozygote
20-67	Low	*1/*3, *1/*2	Heterozygote
68-150	Normal	*1/*1	Wild-type
>150	High		

Low TPMT Activity…



TPMT Method – Sample Prep

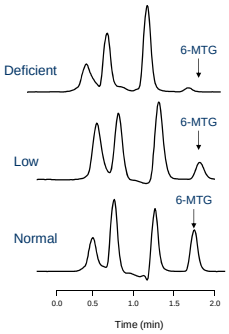
- TPMT enzyme measured in red blood cells: **EDTA whole blood**
  - Can be done on lithium heparin, but not for genotyping



6TG 6-thioguanine, **SAM** S-adenosyl methionine, **6MTG** 6-methyl thioguanine, IS = L-Tryptophan

TPMT Method – Chromatography

TPMT METHOD - CHROMATOGRAPHY



TPMT Quality Assurance

- IQC
  - Commercial IQC not available
  - Whole blood from volunteers
- Patient means
- Phenotype – genotype correlation audit
- EQA
  - Worldwide EQA scheme in progress!

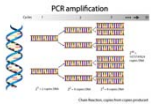
Birmingham Quality
UKNEQAS

TPMT Genotyping

- **Supports phenotyping measurement:**
  - TPMT deficient
  - Recent blood transfusion
  - Previous reaction to azathioprine
  - Change of TPMT status
  - Specific clinical details e.g. ALL (often low HCT)
  - Borderline low/normal TPMT (58 - 78 mU/L) for phenotype-genotype correlation audit

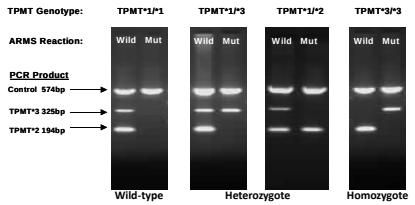
TPMT Genotyping

- **TPMT genotyping Strategy:**
  - Sample screened for common mutations: TPMT *3A/*3C and TPMT*2
  - Account for 60-95% of all mutant alleles for deficient TPMT
- **Method:**
  1. Extraction of EDTA whole blood:
     - Cell lysis
     - Protein precipitation
     - DNA column method
     - Automated application, washing and elution
  2. PCR: Multiplex amplification refractory mutation system (ARMS)
     - WT and Mutant reaction for each sample
  3. Agarose gel electrophoresis – visualisation of PCR products



TPMT Genotyping Method

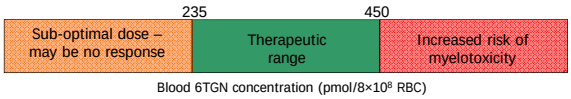
GEL VISUALISATION



Thiopurine Metabolites

- 6TGN and 6MMPN
- Blood levels don't correlate with dose taken
- Narrow therapeutic range
- Therapeutic drug monitoring and personalised therapy
  - ?Compliance
  - ?Sub-optimal dose
  - Toxicity symptoms
  - Non-responders on standard dose

6-TGN – Clinical Utility

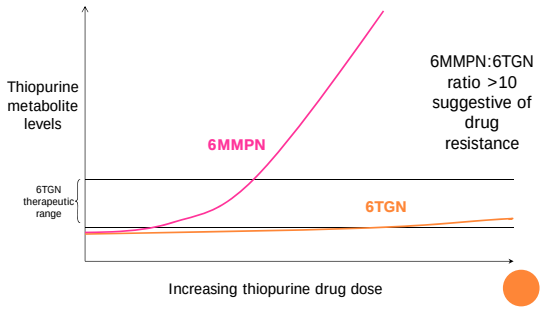


- Low 6TGN – safely increase dose (check compliance)
- High 6TGN - monitor more frequently or reduce dose
- Therapeutic range derived from IBD patients
- Recommend measure 4 weeks post commencement of thiopurine drug or change of dose

6-TGN – Clinical Utility

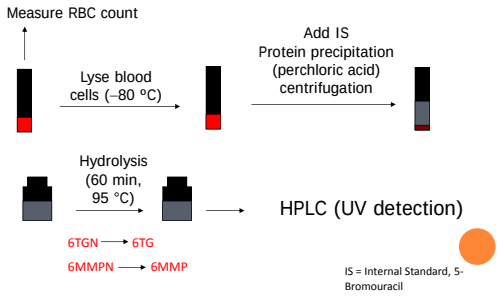
- Inactive metabolite but **increased risk of liver toxicity at high concentrations** (>5700 pmol/8 $\times 10^8$ RBC)
- Important in non-responders with normal/high TPMT activity
- As increase thiopurine drug dose see low 6TGN but 6MMPN level rises exponentially

Thiopurine Drug Resistance



Metabolites Method

- 6TGN/6MMPN measured in red blood cells: **EDTA whole blood**



Metabolites Quality Assurance

- IQC
  - No commercially IQC available
  - Pooled lysed patient samples
- EQA
  - No established EQA scheme
  - Sample swap with New Zealand Lab
- Run "blank" QC sample

Further Examples: Core Drugs

Drug Name	Clinical Use/Indication for testing
Lithium	• Treatment of manic depressive psychosis/bipolar disorder • Can be acutely toxic (causing renal impairment), diabetes insipidus = recognised consequence of therapy
Digoxin	• Treatment of chronic heart failure, increases myocardial contractility • Monitor if ?Toxic/stop drug or poor response • Monitor K^+ concs closely (toxicity exacerbated in hypok$^+$) • Beware of possible 'Digoxin-like immunoreactive substance' interference (e.g. 'Digibind' for treatment of toxicity)
Phenytoin	• Anticonvulsant for control of seizures • Particularly useful to measure for once daily dosing (e.g. alcohol-related epilepsy, in elderly), symptoms of neurotoxicity • No correlation of effect with dose but [plasma] correlate well with effect

Further Examples: Core Drugs

Drug Name	Clinical Use/Indication for testing
Carbamazepine	• Widely used anticonvulsant, used in bipolar affective disorder, mania and depression as mood stabiliser • Fewer side effects than phenytoin/phenobarbital but neurotoxic effects (blurred vision, dizziness, ataxia) related to peak plasma concs – can be minimised by altering regime – therefore measurement guides dose
Valproate	• First line anticonvulsant (along with pheny/carba), used in bipolar effective disorder (due to minimal sedative action and absence of CNS side effects) • No hard evidence for target range so routine monitoring not recommended but useful for ?compliance (pyschiatric use)
Theophylline	• Bronchodilator - facilitates relaxation of smooth muscle and prevents bronchoconstriction (e.g. in asthma, chronic obstructive pulmonary disease) • Frequent side effects – more serious as [plasma] increases • Poor correlation between dose and [plasma] also justifies TDM • Useful for initial dose optimisation & ?toxicity

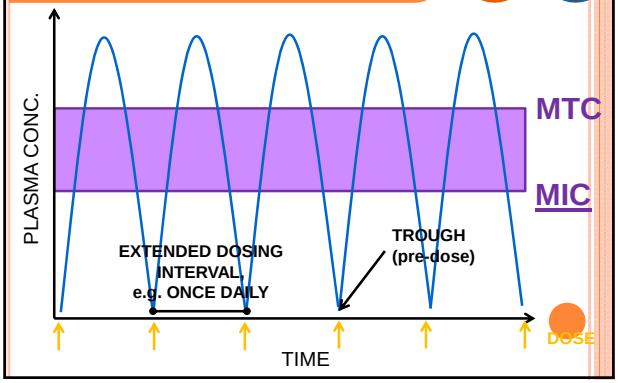
Antibiotics

E.g. Gentamicin (an aminoglycaside)

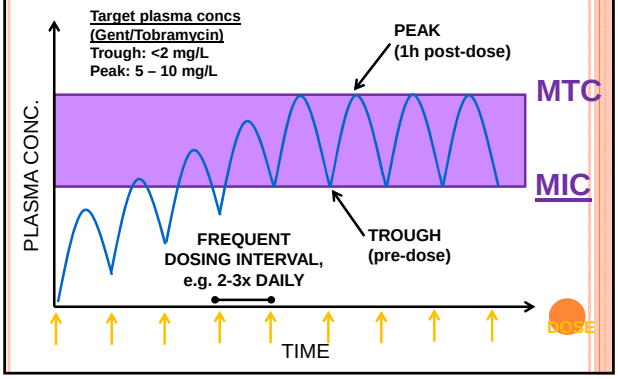
- Used in treatment of severe systemic infection – interfere with protein synthesis in susceptible microorganisms.
- Monitoring essential in infants, elderly, obesity, CF, if high doses used or impaired renal function.
- Aminoglycasides generally have short plasma half life (~2-3 hours) except in poor renal function.
- Different dosing regimes:
  - **Extended** – e.g. once daily
  - **Frequent** – e.g. 2-3 x daily

MIC – minimum inhibitory concentration

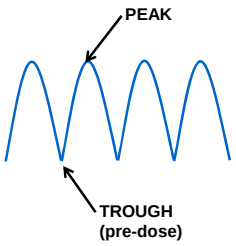
Antibiotics



Antibiotics



Sample timing



PEAK
- Rarely used
- Only for certain drugs in specific circumstances

TROUGH
- Recommended sampling time for most drugs
- Sample collected immediately before next dose
- Least intra- and inter-individual variability
- "Reference ranges" apply to trough measurements

Sample timing: Examples

- **6-Thioguanine Nucleotide (6TGN)**
  - Half life = several days therefore no need to take sample at specific time
  - Steady state reached 2-4 weeks after starting treatment/changing dose – suggest collect sample at 4 weeks
- **Lithium**
  - Elimination half life ~10-35 hours
  - Collect sample 12 hours post dose
- **Carbamazepine**
  - Shorter half life ~8-24 hours
  - Steady state trough sample (before next dose) preferable

Sample types

PLASMA / SERUM:
- Mostly serum (ideally plain, no gel)
- Do not add to gel serum specimens if >2 hours old
- Lithium - NOT LITHIUM HEPARIN PLASMA!!!

WHOLE BLOOD:
- e.g. For drugs found in RBCs, e.g. ciclosporin, 6TGN
- EDTA

BLOODSPOT:
- Home-sampling

Analytical Methods

METHODS FOR TDM

- Spectrophotometry / colorimetry — E.g. Lithium
- Element analysis:
  - ISE
  - AAS
  - ICP-MS — E.g. Lithium
- Immunoassay/Turbidimetry:
  - EMIT
  - FPIA
  - PETINIA — E.g. Carbamazepine, Digoxin, Gentamicin
- Chromatography:
  - HPLC (-UV / -DAD)
  - LC-MS/MS / LC-MS QToF
  - GC-MS — E.g. Immunosuppressants, Thiopurine metabolites

Immunoassay

PROS

- Readily automated
- Rapid results
- ↓ TAT, ↑ Throughput
- Use existing routine chemistry analysers

CONS

- Limited to repertoire provided by manufacturers
- Not available for all (esp. new) drugs
- ↑ Interference (↓ Specificity)

Chromatography

PROS

- ↑ Sensitivity (MS-MS, Fluoresence detection)
- ↑ Specificity
- Simultaneous analysis of multiple compounds
- Can work-up in-house methods

CONS

- Require specialist equipment (£££)
- Require technical expertise
- ↑ TAT, ↓ Throughput

Further Reading

- SOPs and kit inserts for relevant methods
- Text books:
  - Therapeutic Drug Monitoring and Laboratory Medicine (Mike Hallworth, Ian Watson, ACB Venture Publications 2008)

Thanks for listening

Any questions??