

Porphyrin methodology

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Cardiff Porphyria Service

Introduction

- The right choice
- General principles for sample handling
- Methods
 - Aminolaevulinic acid
 - Porphobilinogen
 - Total urine porphyrin
 - Plasma porphyrin screening

Is this investigation appropriate?

- Acute vs. cutaneous - protocols
- Patient age
 - Childhood onset (EPP, CEP, ALAD, homozygous porphyrias)
 - Adult onset (AIP, VP, HC)
- Red herrings (Tyrosinaemia, Pb poisoning, secondary coproporphyrinuria)

Sample choice

- Photosensitivity (EPP) EDTA blood
- Skin fragility (early onset) Urine + EDTA
- Skin fragility (VP, HC, PCT) Urine + EDTA + faeces
- Acute (Child) Urine
- Acute (Adult) Urine + EDTA

Sample handling

Urine and faecal samples should be stored frozen unless they are to be assayed immediately.

Do not freeze whole blood samples.

A random urine sample,
protected from light, is the
preferred specimen for urinary
porphyrin analysis.

Arguments against 24 hr urine collections

- Unnecessary delay
- Incomplete collections
- Bacterial action
- Inconvenience to patients
- Expense of transport
- Exposure to light

Protection from light

- Pre-analytically
- Throughout analysis

Urine samples must NOT be
centrifuged

Care should be taken when interpreting the results from very dilute urine samples

High risk samples

Clinical details

Genetic consent

Methods - generally

Methods for porphyrin screening should fulfil the following criteria :

- Robust methodology
- Internal quality control
- External quality assurance
- Realistic turn-around times

Methods – acute presentation

- ALA
- PBG – screening vs quantitation

All laboratories should be able to offer a rapid, sensitive and proven method for urinary porphobilinogen analysis.

PBG methodology

- Acute attack = increased urine PBG
- Requirement to detect ALL urines with clinically significant PBG
- Detection limit
- Interference
- Speed

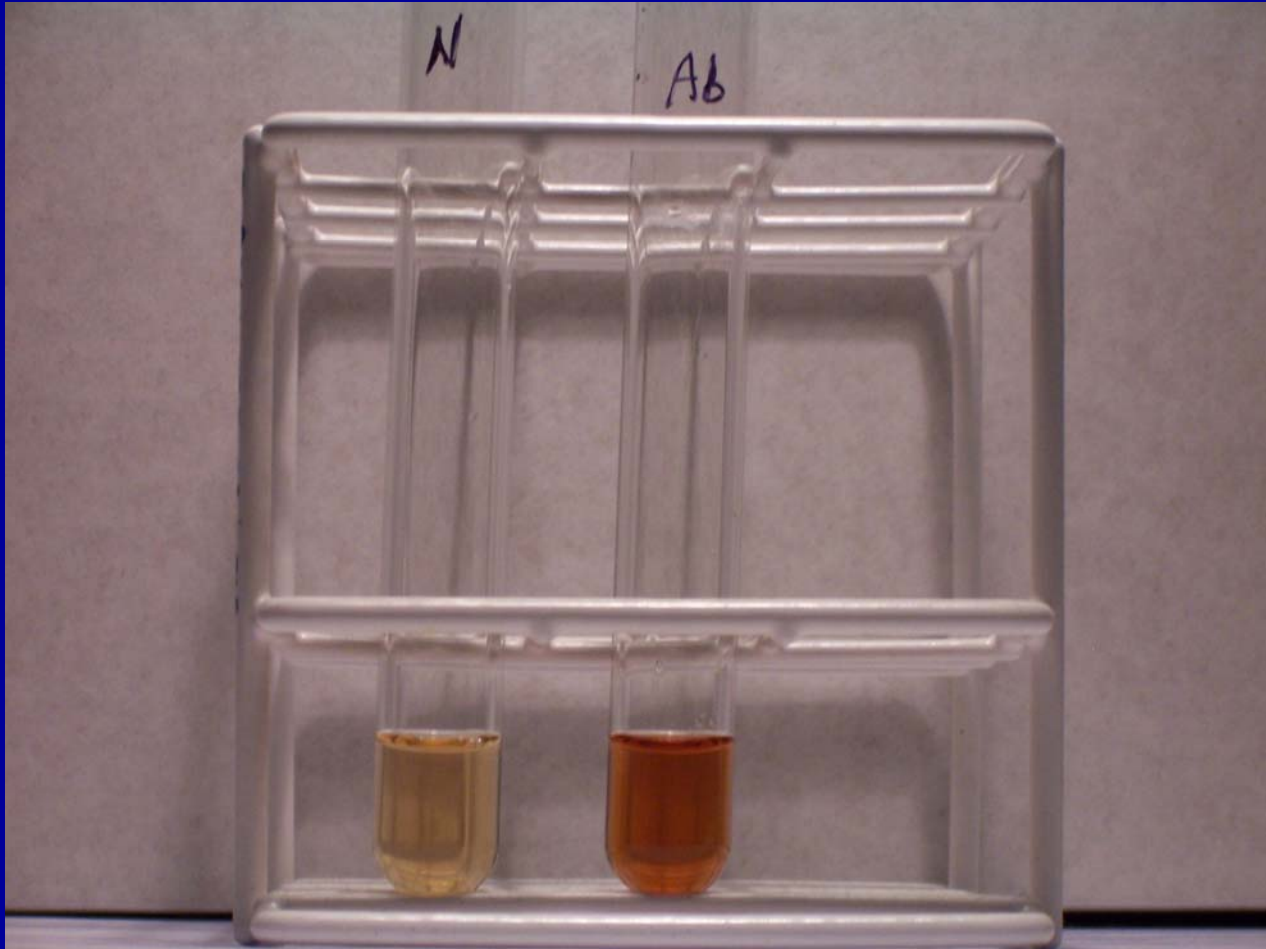
PBG methodology (cont)

- Watson-Schwartz
- Trace kit
- Mauzerall & Granick (modified)

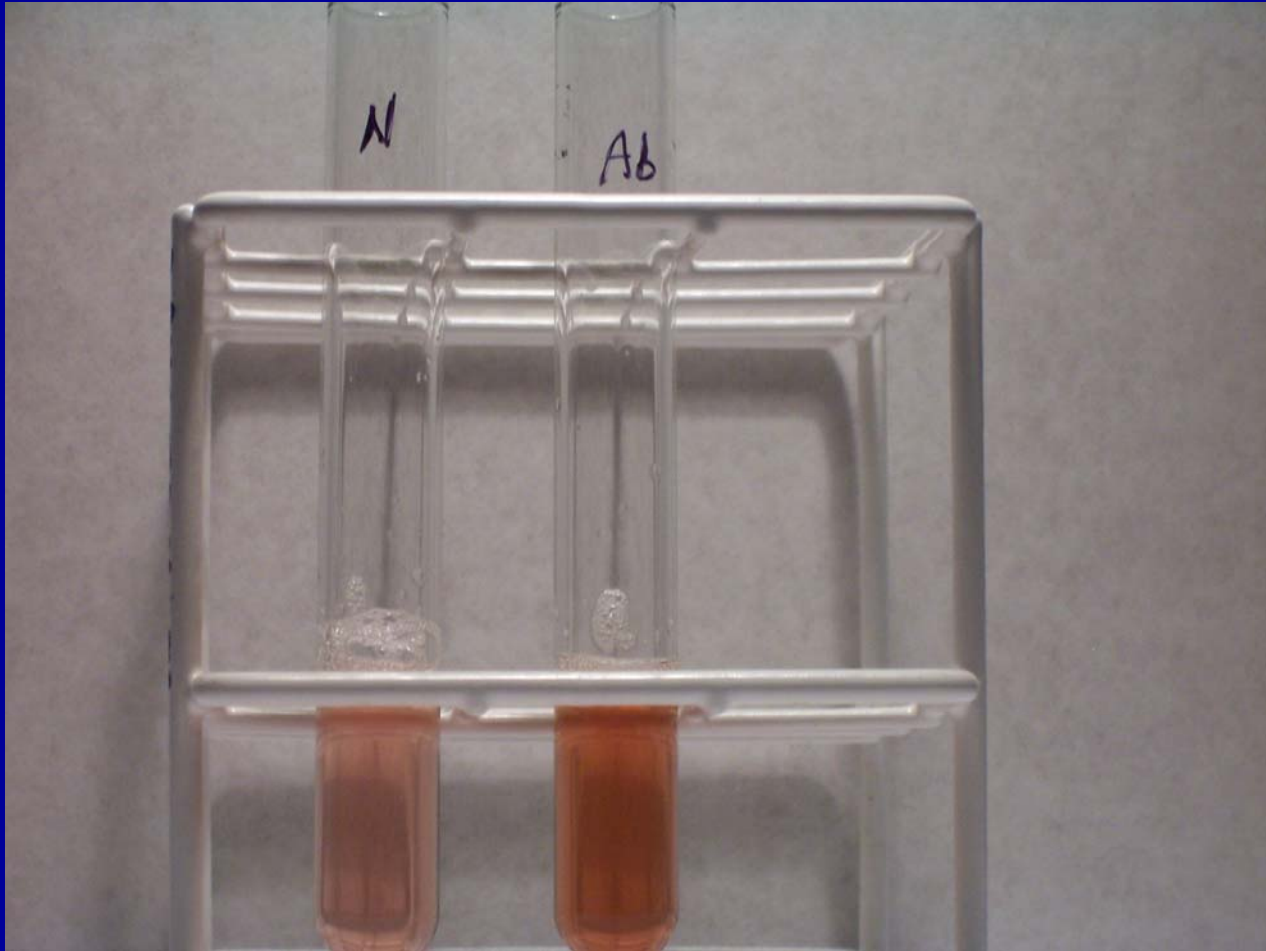
Watson - Schwartz

- Qualitative
- Quickest
- Urine + Ehrlich's (DMAB)
- Organic solvent
- False +ve vs false -ve
- Validated protocols

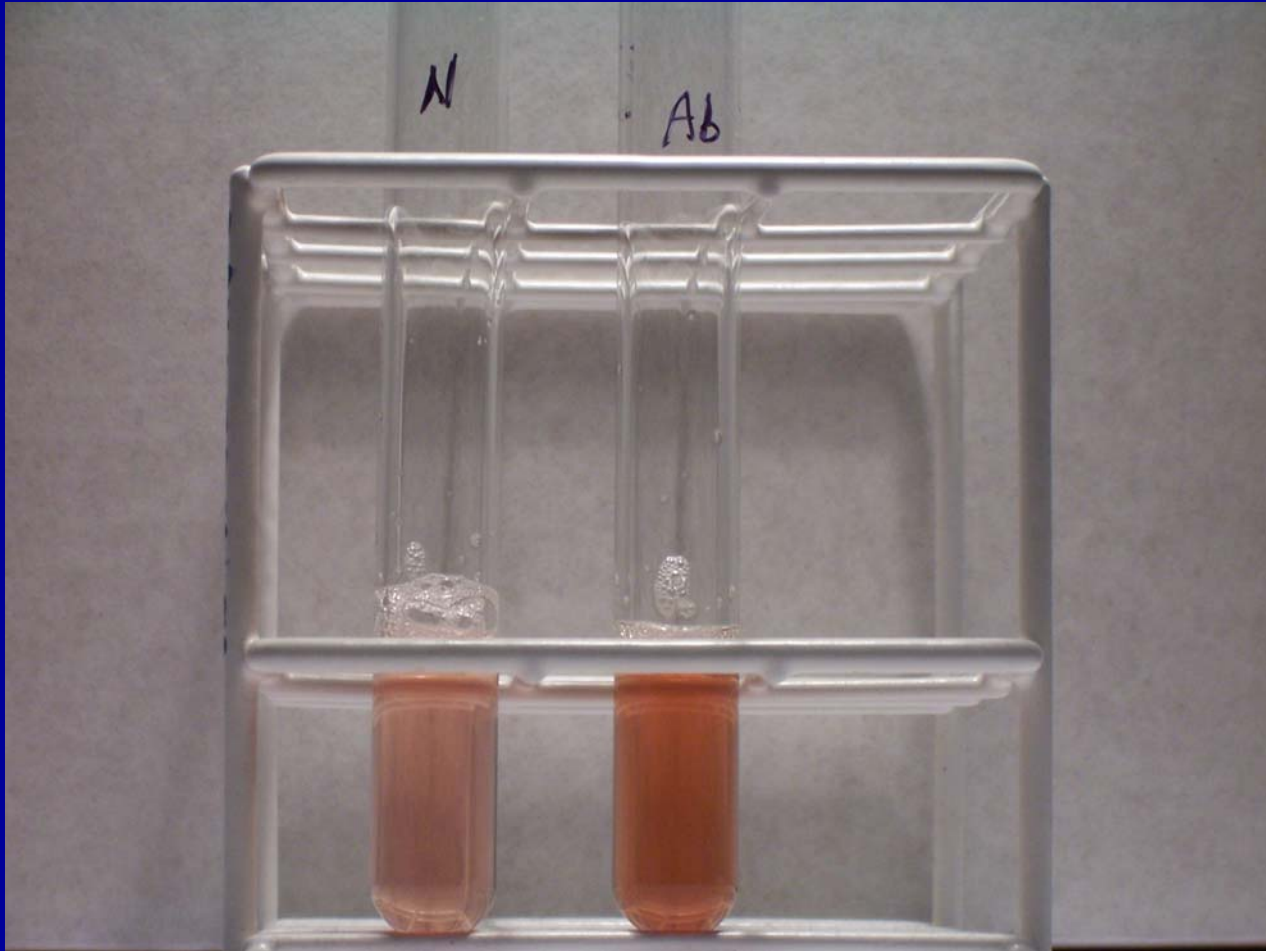
1ml QC or urine



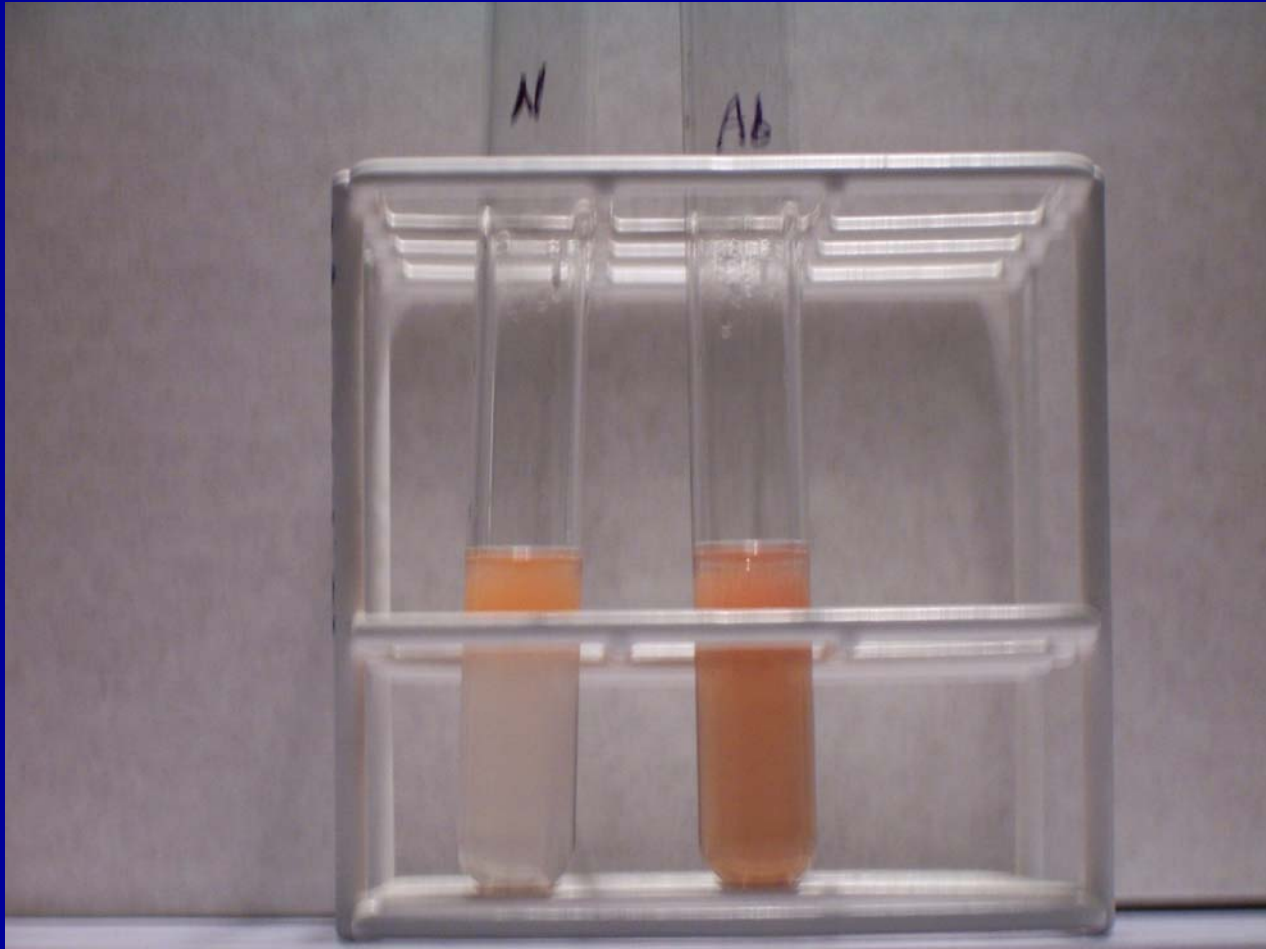
+ 1ml Ehrlichs reagent



+ 2ml saturated sodium acetate



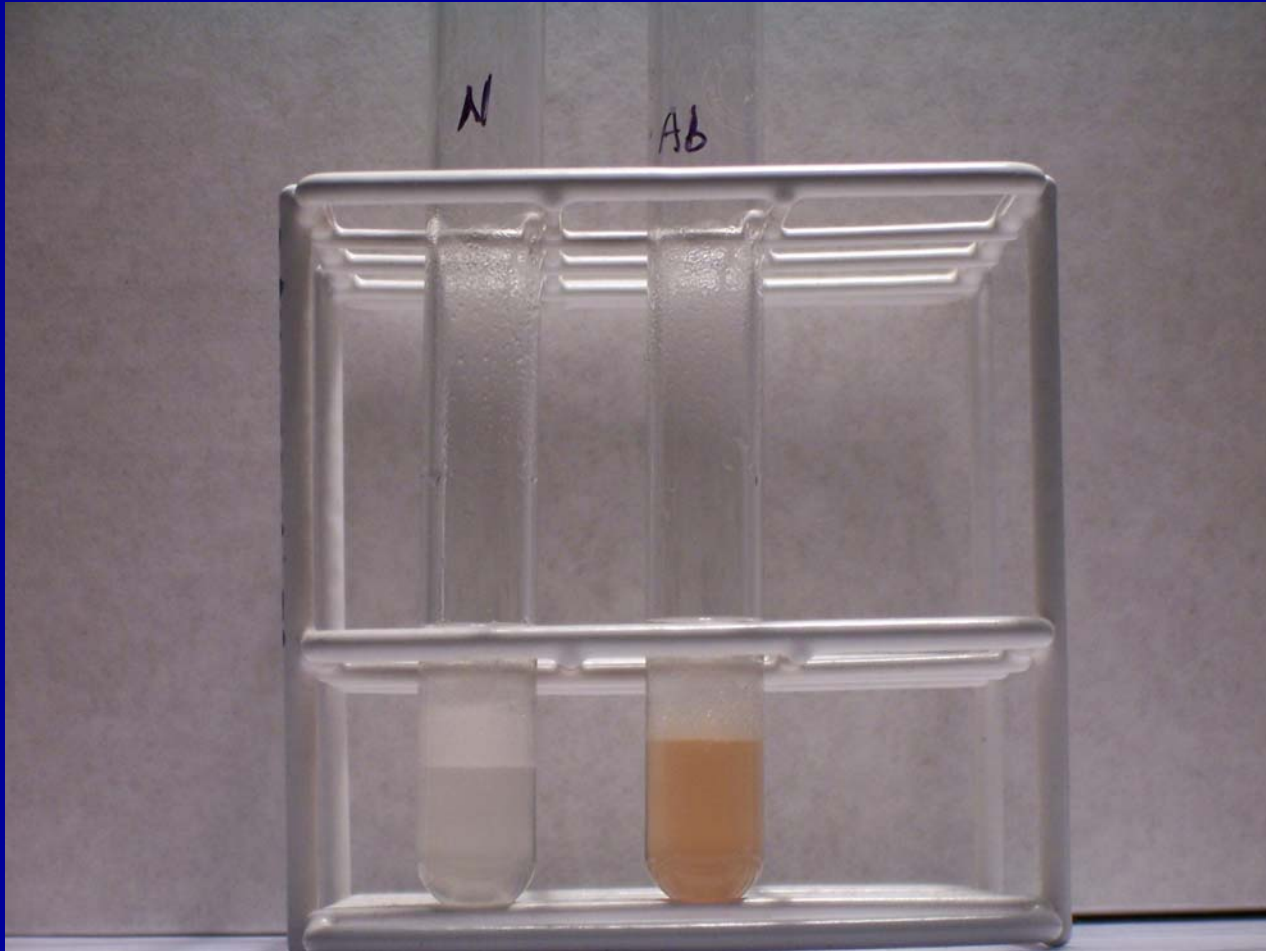
+1ml amyl alcohol



Re-extract with amyl alcohol



Clear upper layer



Trace kit

- Resin filled syringes + filters
- Comparison with artificial standards
- Quick
- Symptomatic initial screening
- ? Asymptomatic screening - unsuitable
- ? More sensitive & specific

Mauzerall & Granick

- Time consuming
- Gold standard (reliable & specific)
- Few interferences
- Follow-up to +ve screen or continuing clinical suspicion.

Basic ingredients

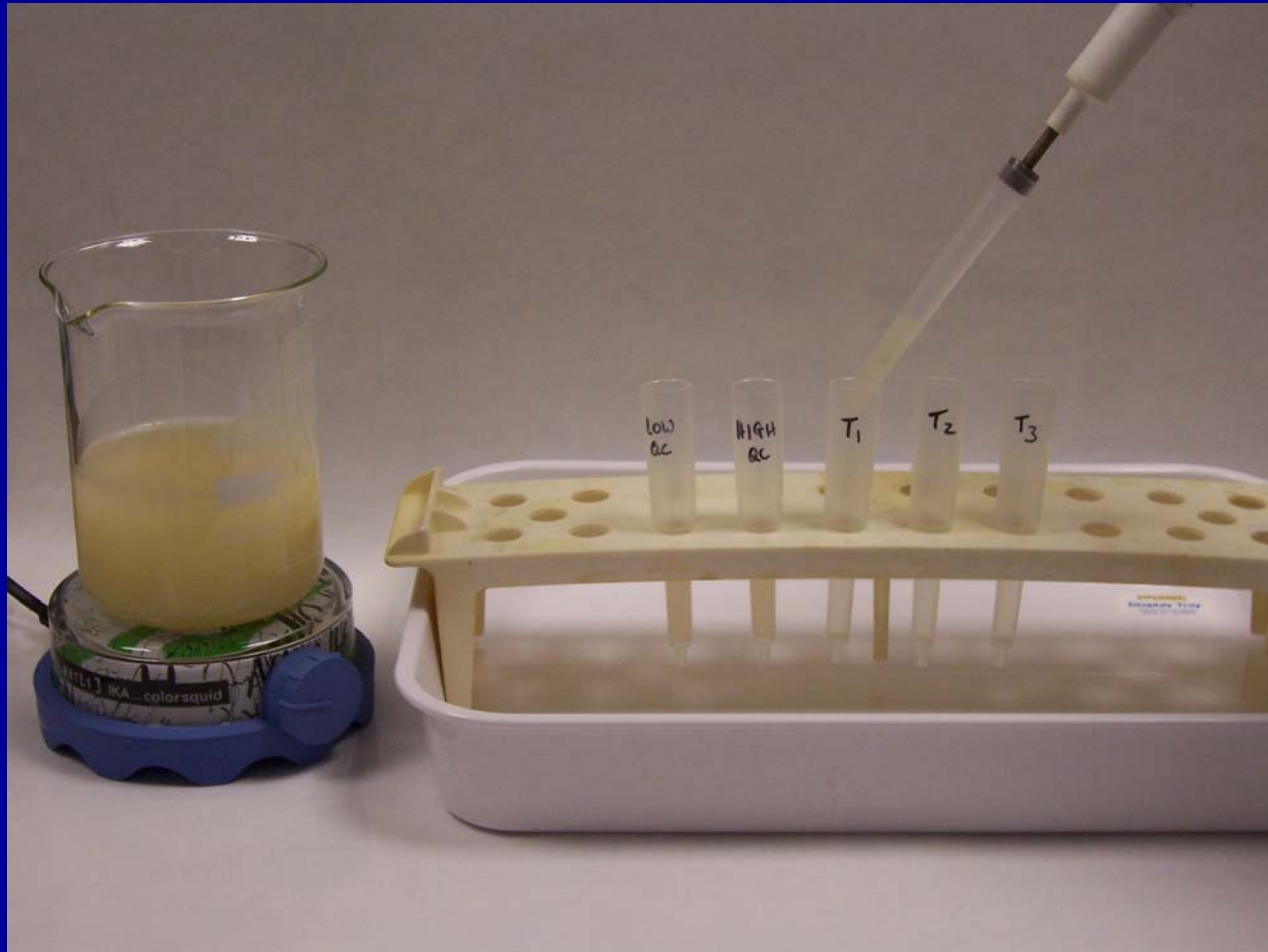


100g
Resin

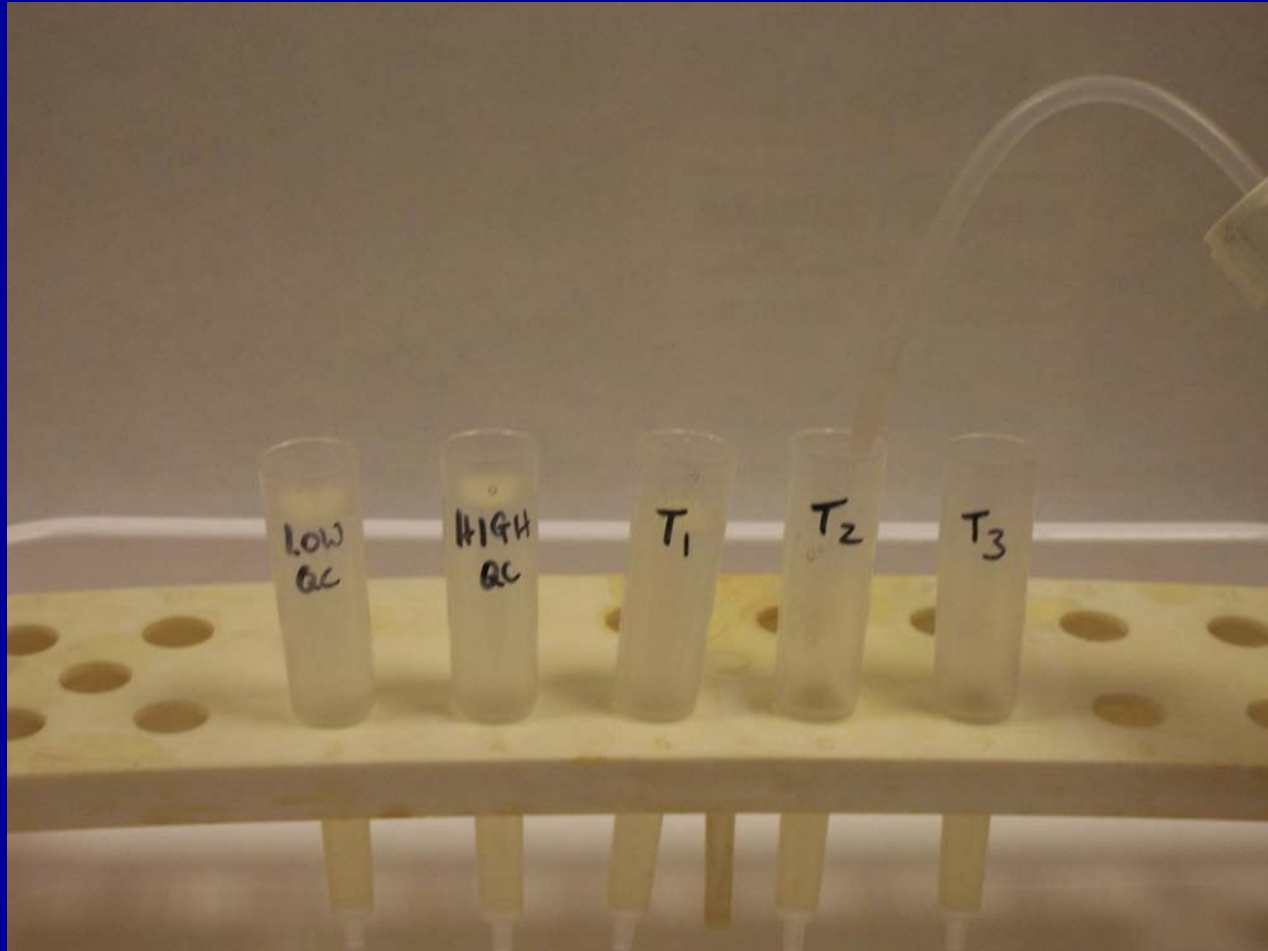
200ml
H₂O

Working
resin

Loading 2ml resin



Column volume resin wash (H₂O)



Ehrlich's reagent



DMAB

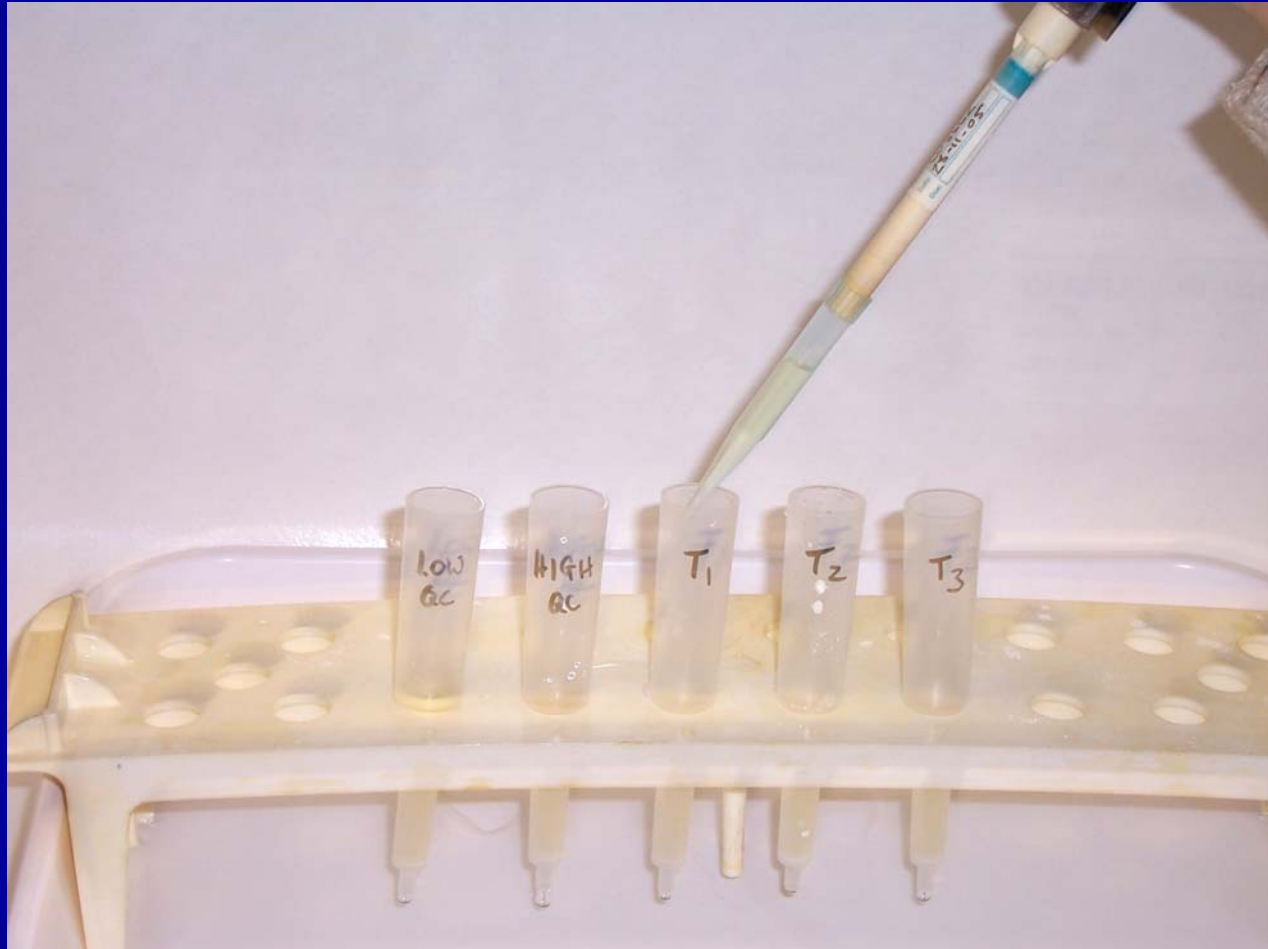
Glacial acetic
acid

70% perchloric
acid

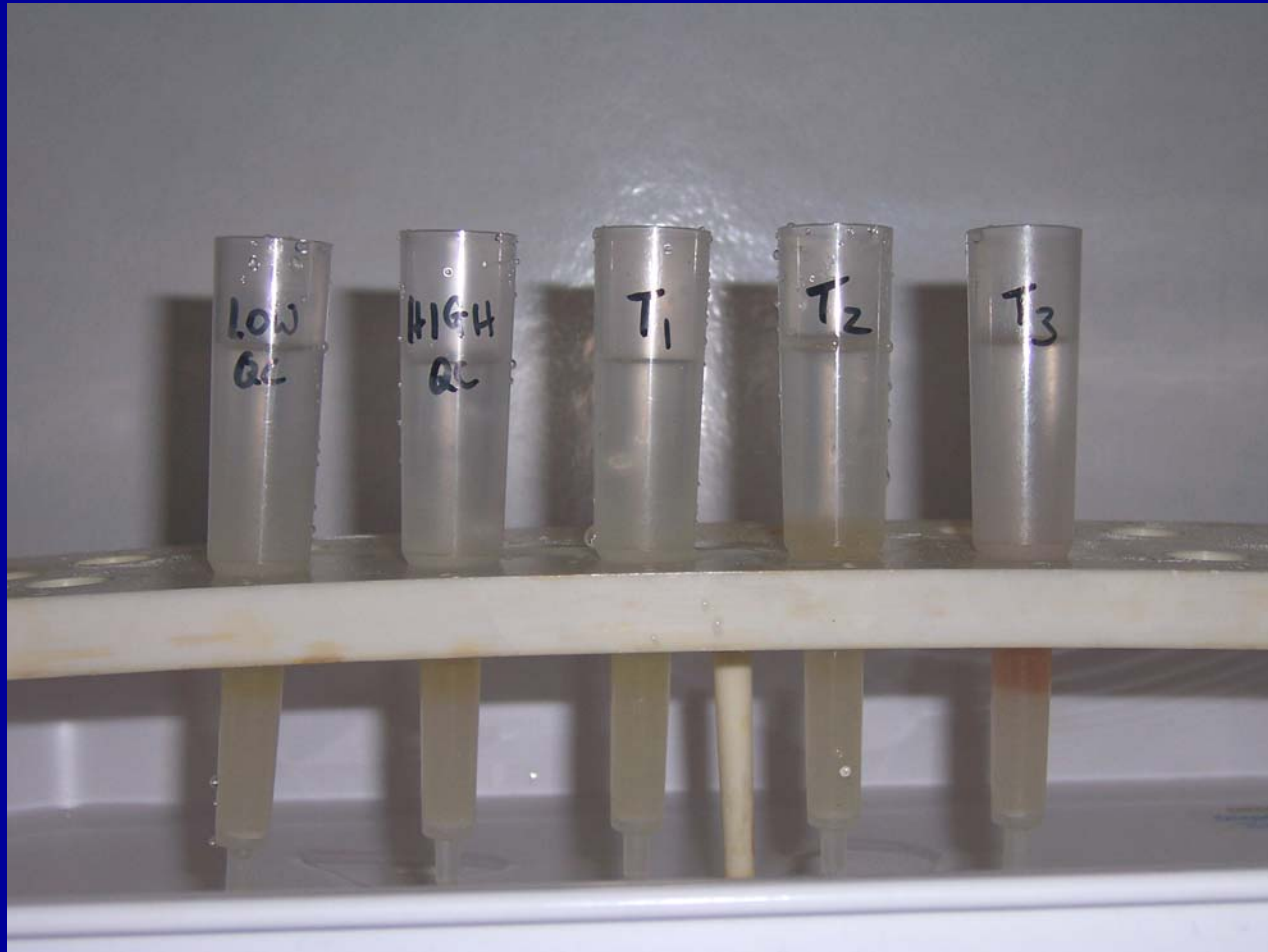
Commercial QC and samples



1ml QC or urine sample



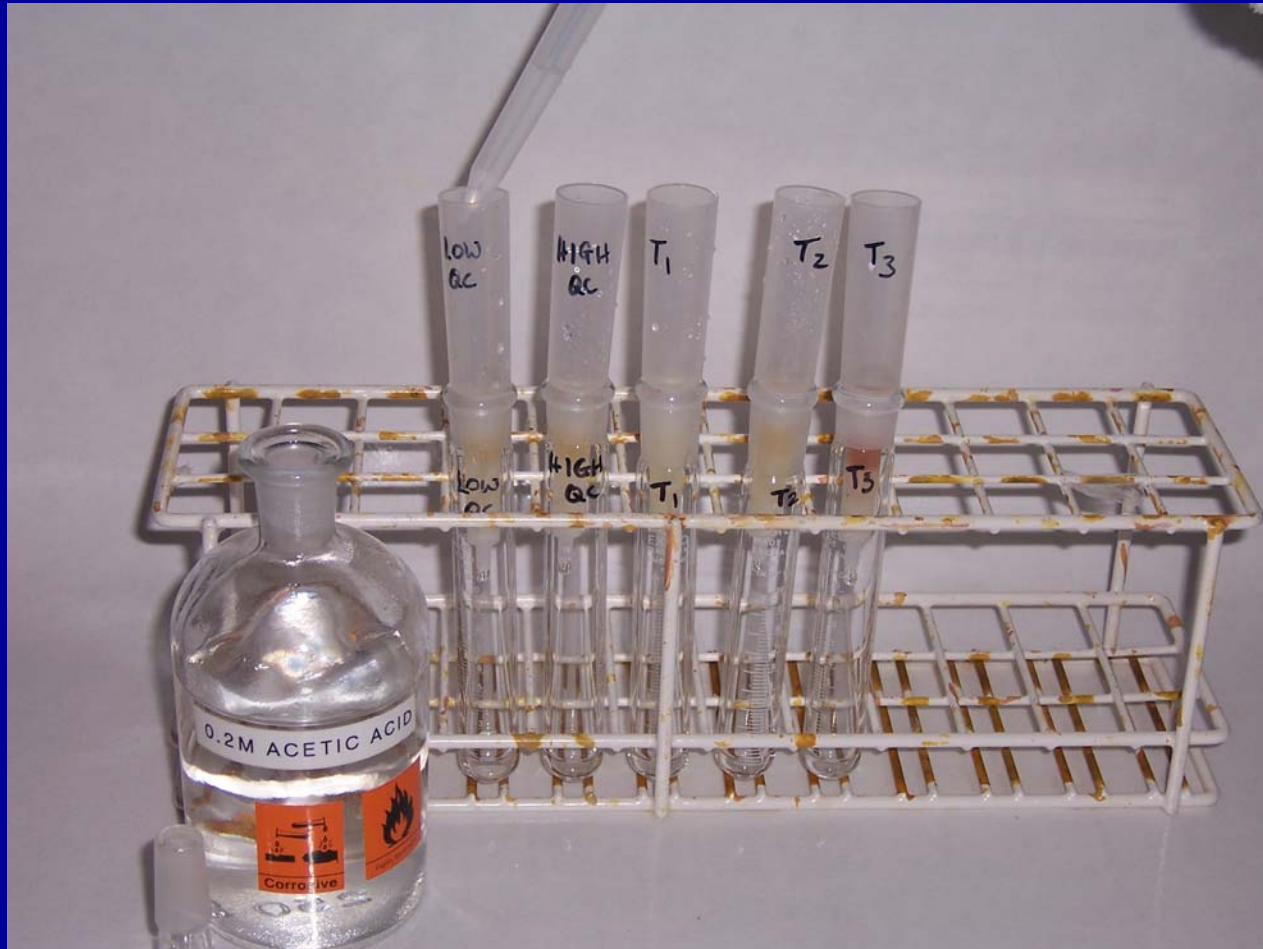
H₂O column wash



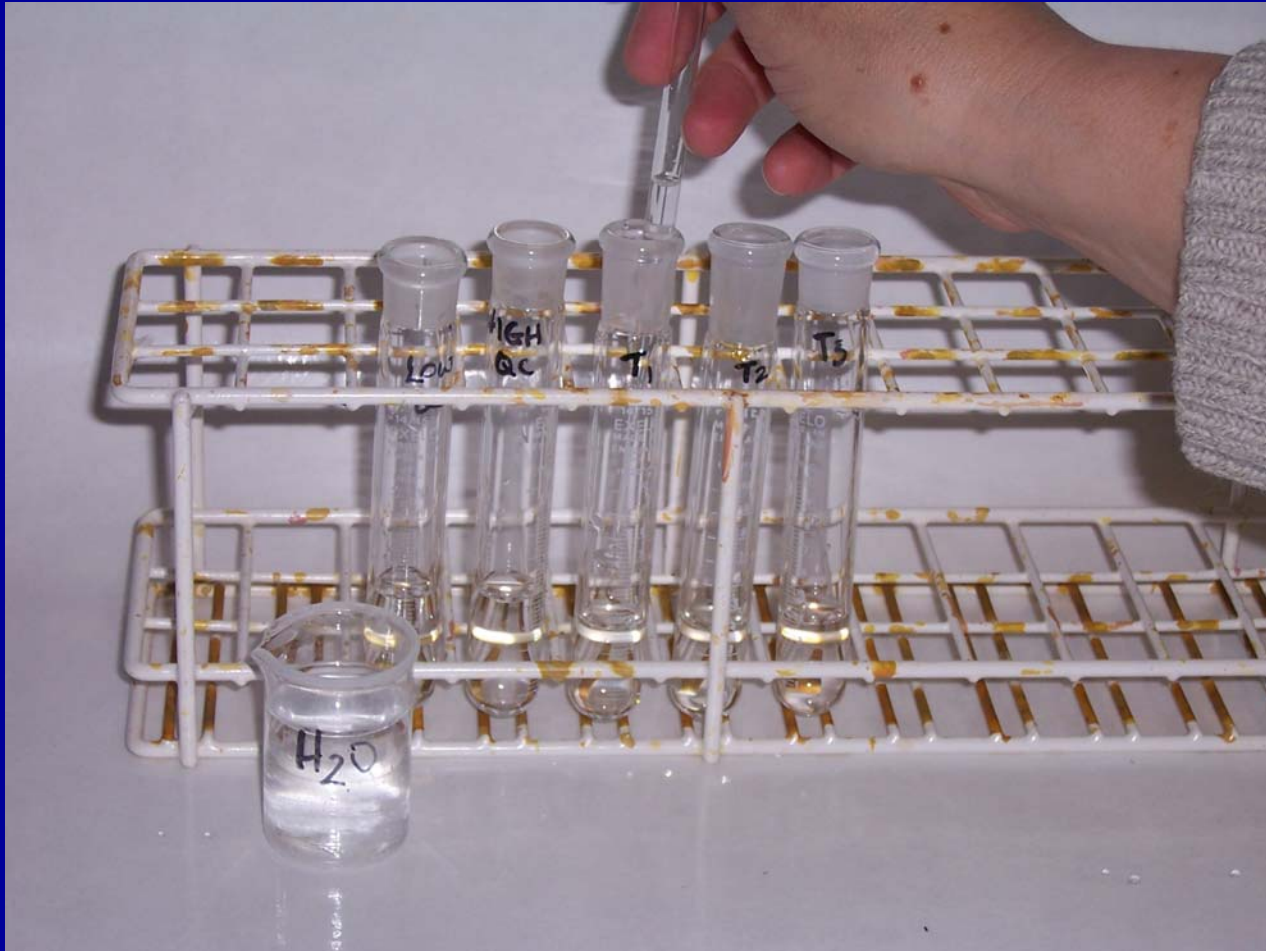
Elute PBG – 1st stage



Elute PBG – 2nd stage



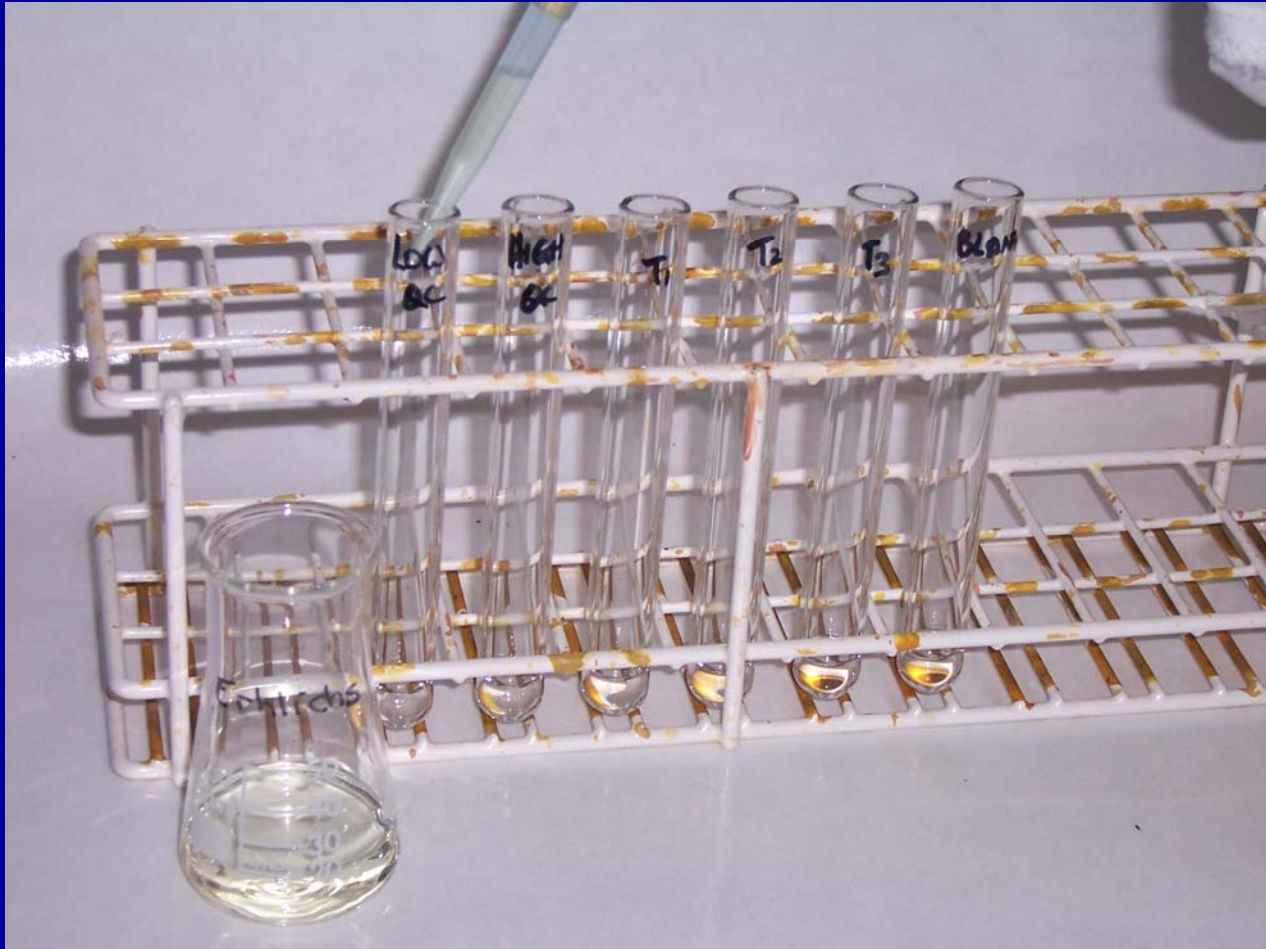
Volume adjustment



Mix well



1ml eluate + 1ml Ehrlichs



Mix well



15 minute incubation



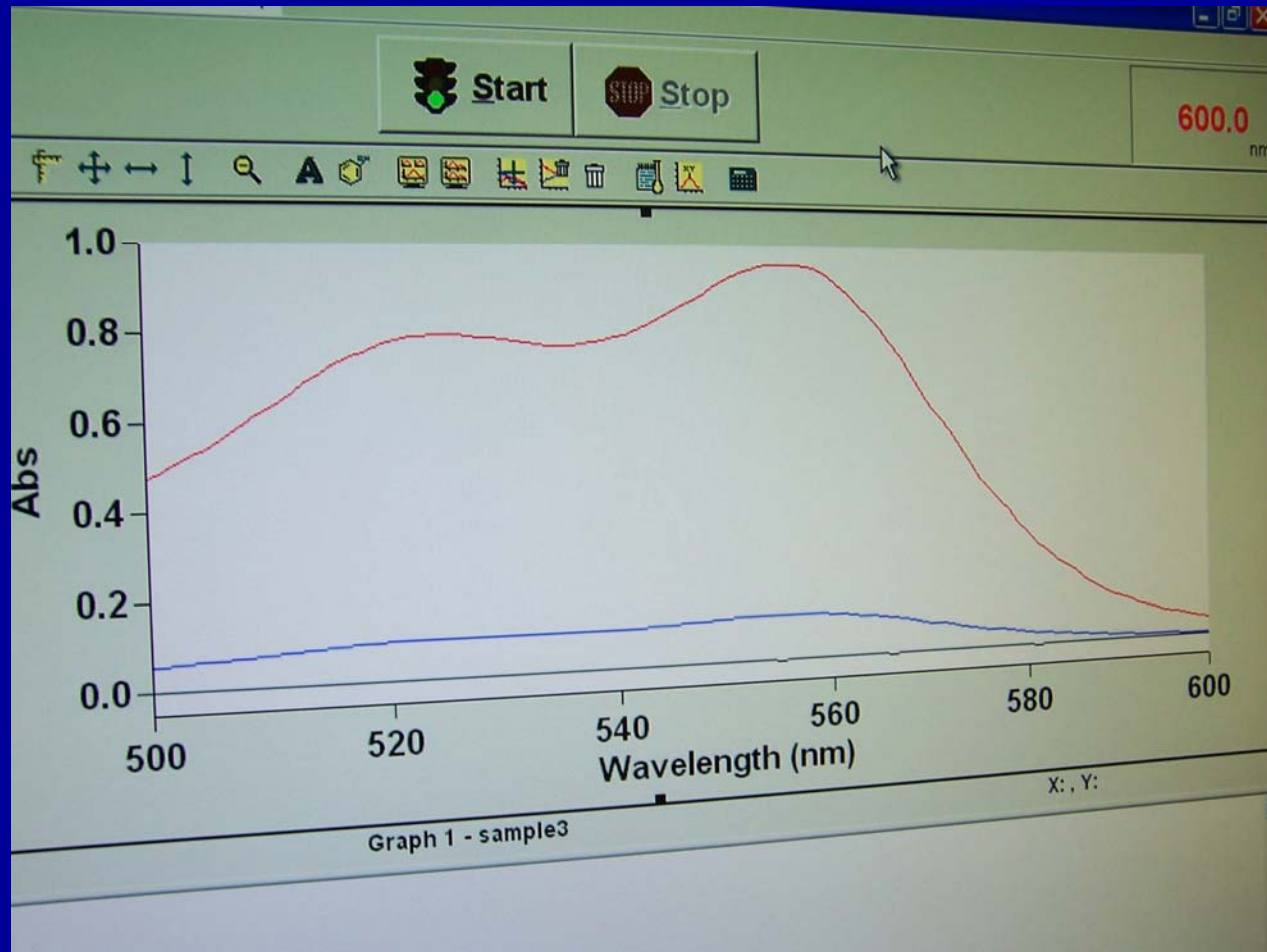
Incubation complete



Spectrophotometer (553nm)



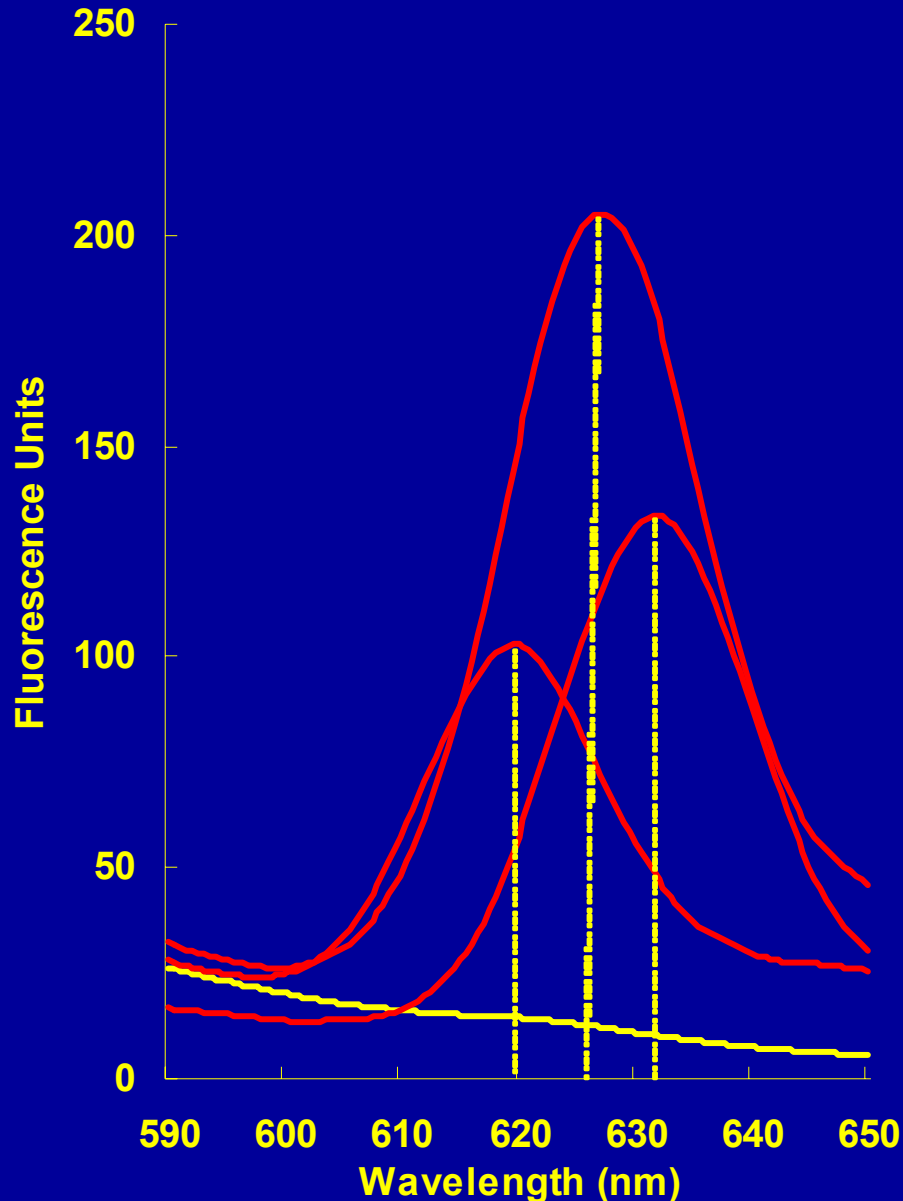
Increased PBG vs. Normal PBG



Methods - Photosensitivity

- Plasma fluorescence emission spectroscopy
- Calibration of fluorimeters for porphyrin analysis is instrument specific
- A red-sensitive photomultiplier is essential for porphyrin analysis

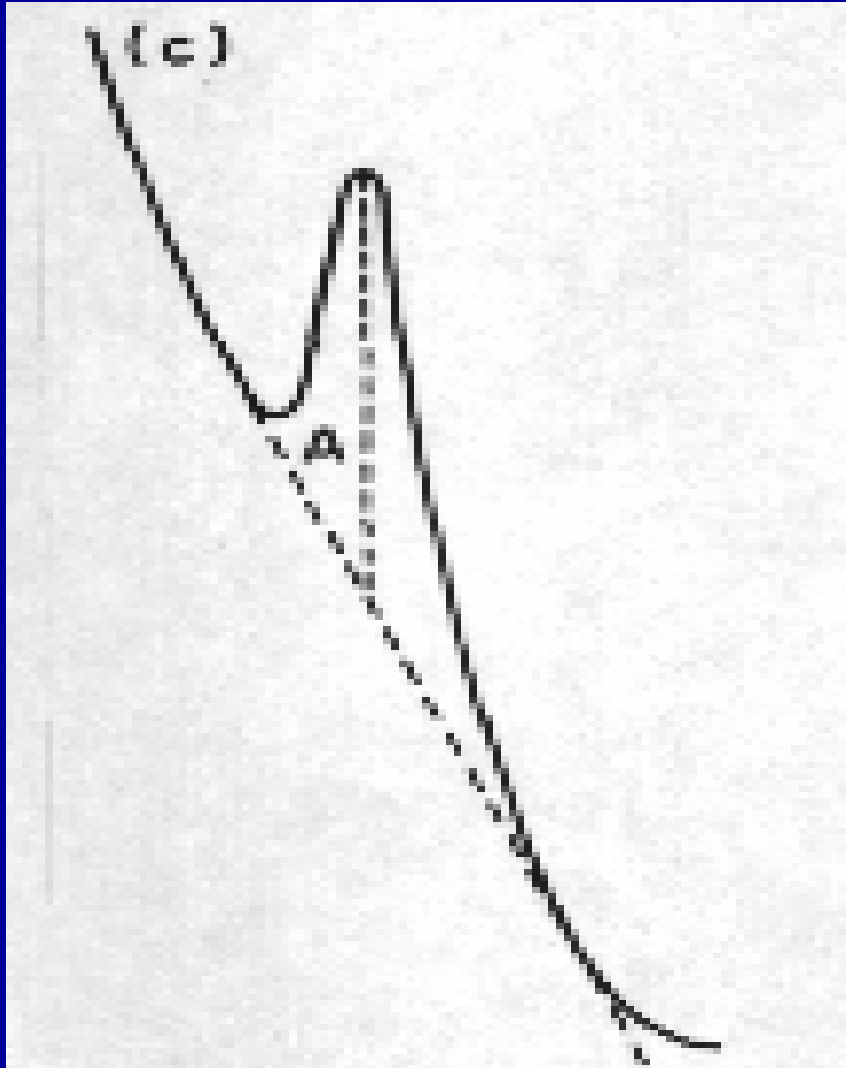
Plasma porphyrin fluorescence



Methods – skin fragility

- The soret band
- Porphyrin carboxylic acids and their methyl esters in organic solvents have a characteristic absorbance peak at 400-410nm
- The wavelength and relative intensity vary according to the β -substituents present.
- Absorbance at soret band maximum in acid solution widely used to determine conc.

Absorption spectrum of an acidified urine sample



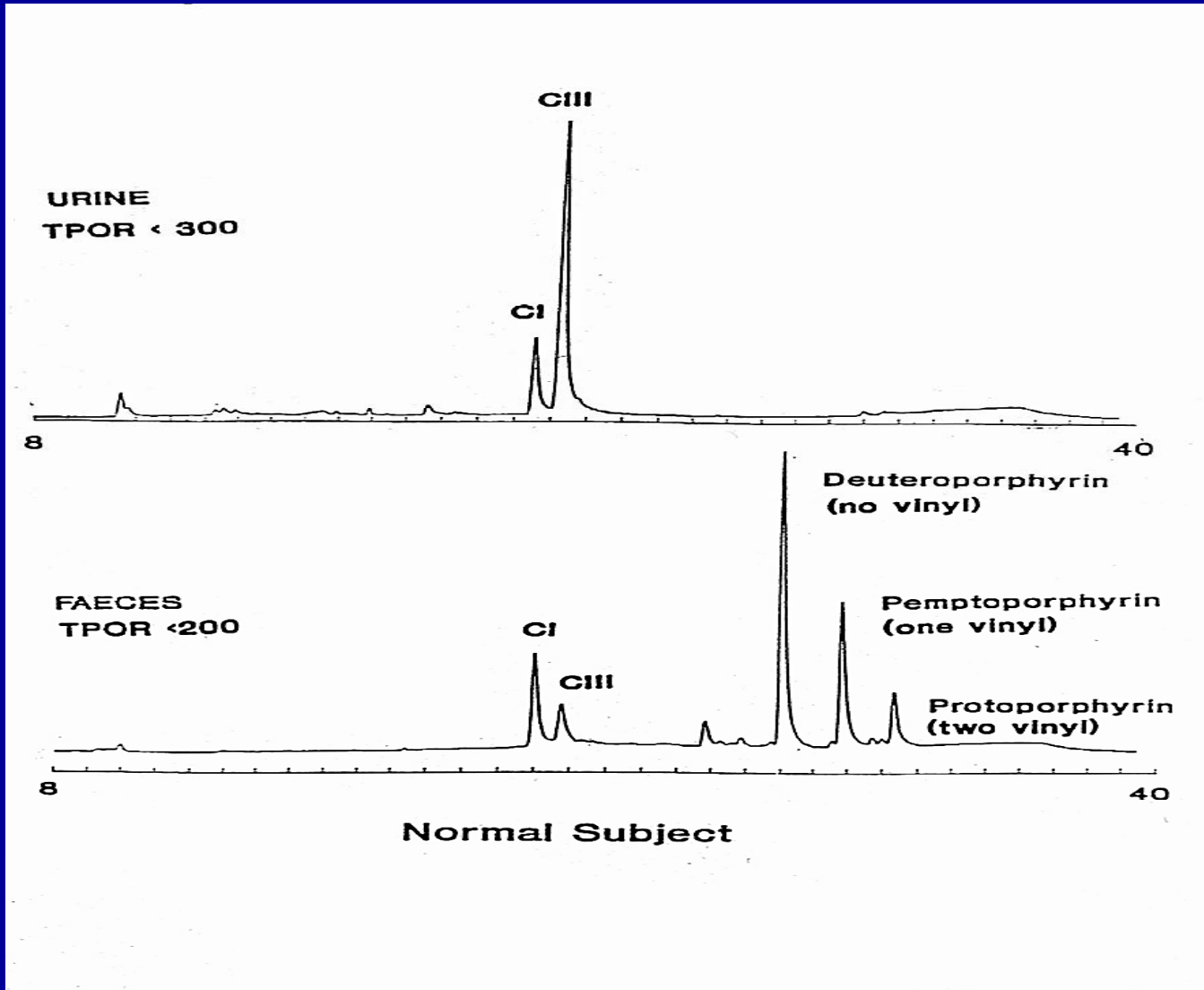
Methods - fractionation

- HPLC
- C18 column with guard
- Gradient elution
- 50ul sample volume
- 25min run time/sample
- Fluorimetric detector (red-sensitive PMT)

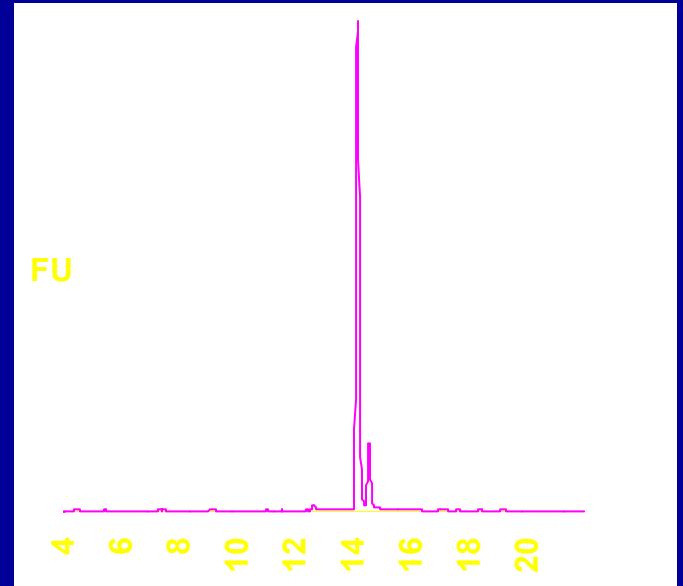
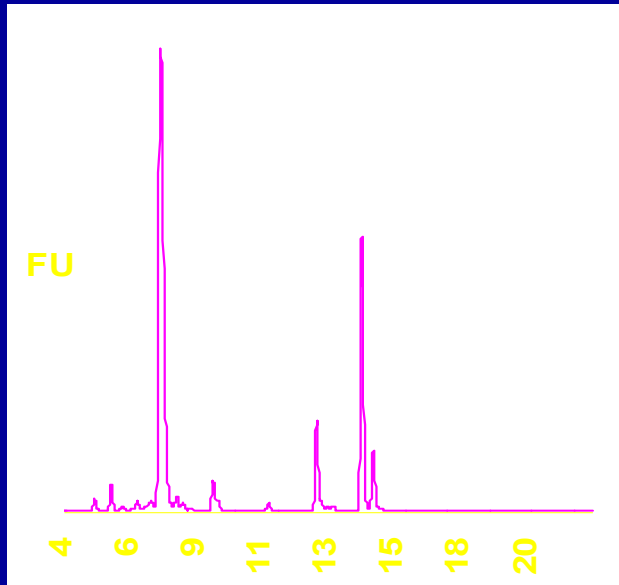
Advantages of HPLC

- Ease of separation + rapid
- Can use products from quantitative assays
- Good resolution
- Quantitative
- Less labour intensive
- BUT - costs more

Unaffected patient



CEP



Enzymes - to measure or not to measure

- Cytosolic (PBGD) - OK
- Mitochondrial (ALA synthase, COPPOX, PPOX, Ferrochelatase,) - small sample, lymphocytes, fibroblasts + low activities. Technically difficult
- Overlapping ranges

Tandem MS

- Complete isomer separation (providing all have different molecular weights)
- Sensitivity (pmol)
- Speed

Summary

- Minimum service
- Collaboration
- Encourage appropriate investigation
- Maintain analytical and interpretative expertise
- Use satisfactory methods – sensitive and specific

Summary continued

- Use appropriate IQC
- Participate in available EQA schemes
- Seek advice from specialist centres.