

MetBioNet BMS Training Group Workshop – Bristol

Tackling All Techniques

Manual Techniques

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Manual Techniques- A Broad Topic

Electrophoresis

Chromatography

Test tube based assays

Spot tests

Enzyme assays

The list goes on.....



Example of electrophoresis

Mucopolysaccharides (MPS) / Glycosaminoglycans (GAGs)

Long chains of sugar molecules essential for building bones, cartilage, skin, tendons and other tissues in the body.

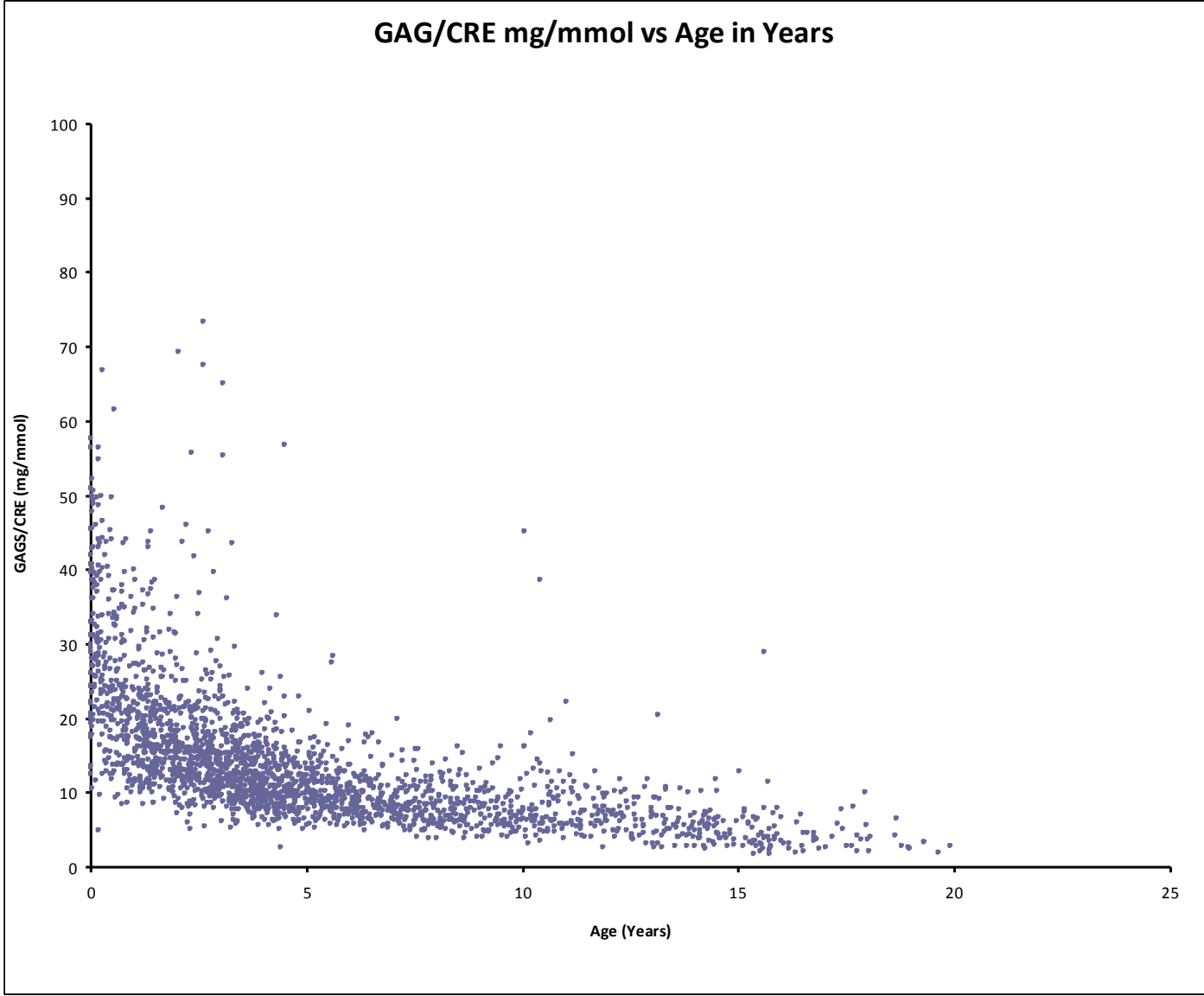
GAGs continuously synthesised and degraded during normal metabolism.

Lack of a specific catabolic enzyme leads to....a specific MPS disorder
and

Accumulation of GAGs in various tissues and organs

GAGs detected in patient urine

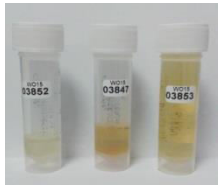




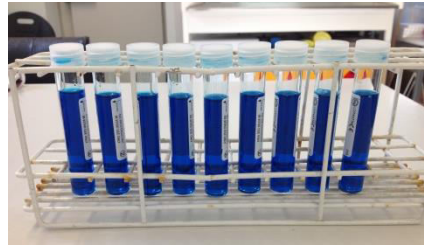
Graph Showing Normal GAG/Creatinine vs Age Willink laboratory



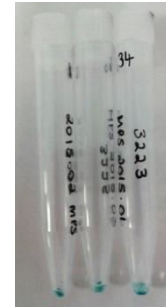
Qualitative measurement of GAGs



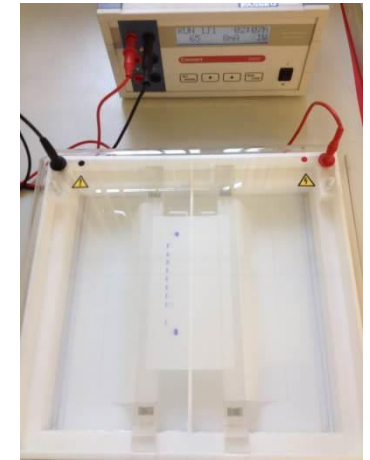
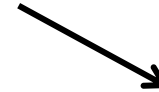
Urine samples



Extract each sample with Alcian blue



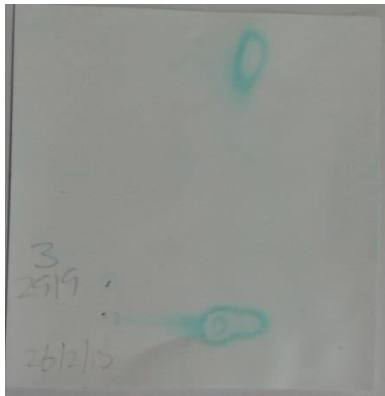
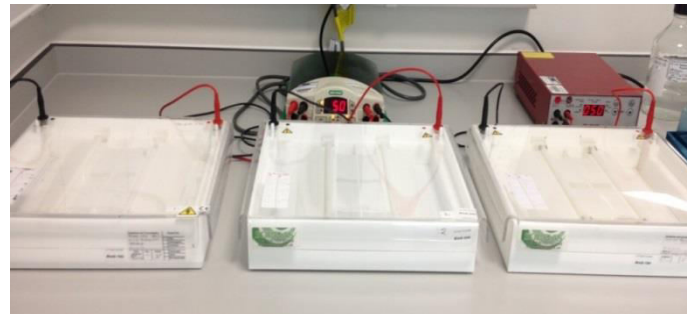
Isolated GAGs



1D Electrophoresis



Second dimension
Reference marker added

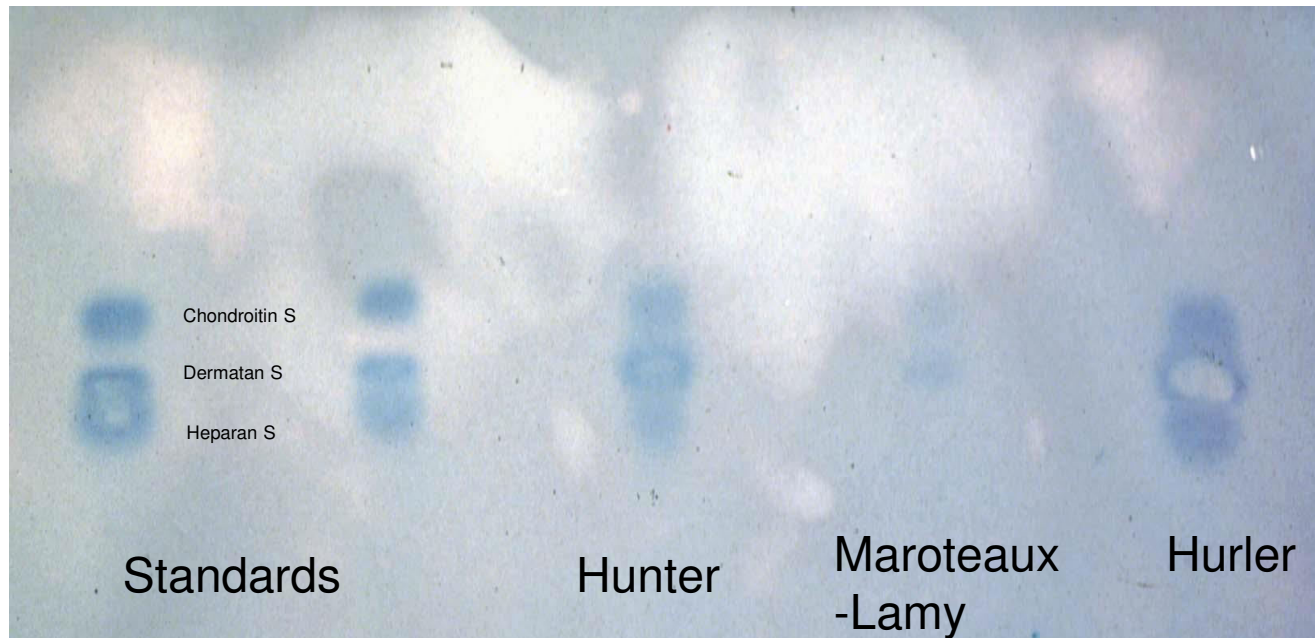
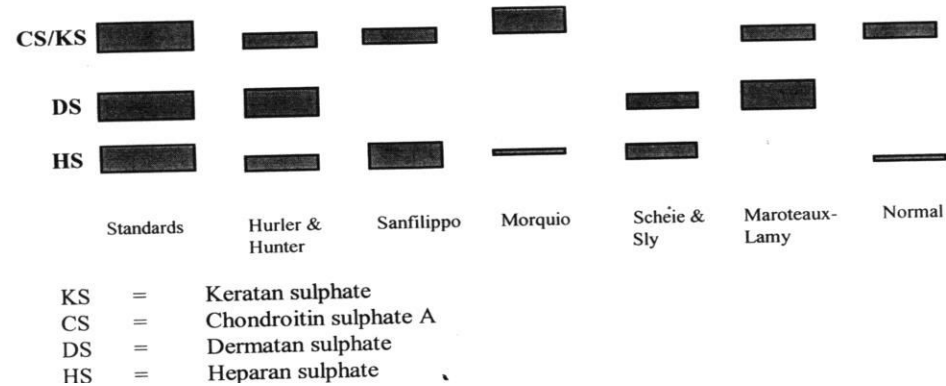


Electrophoretogram

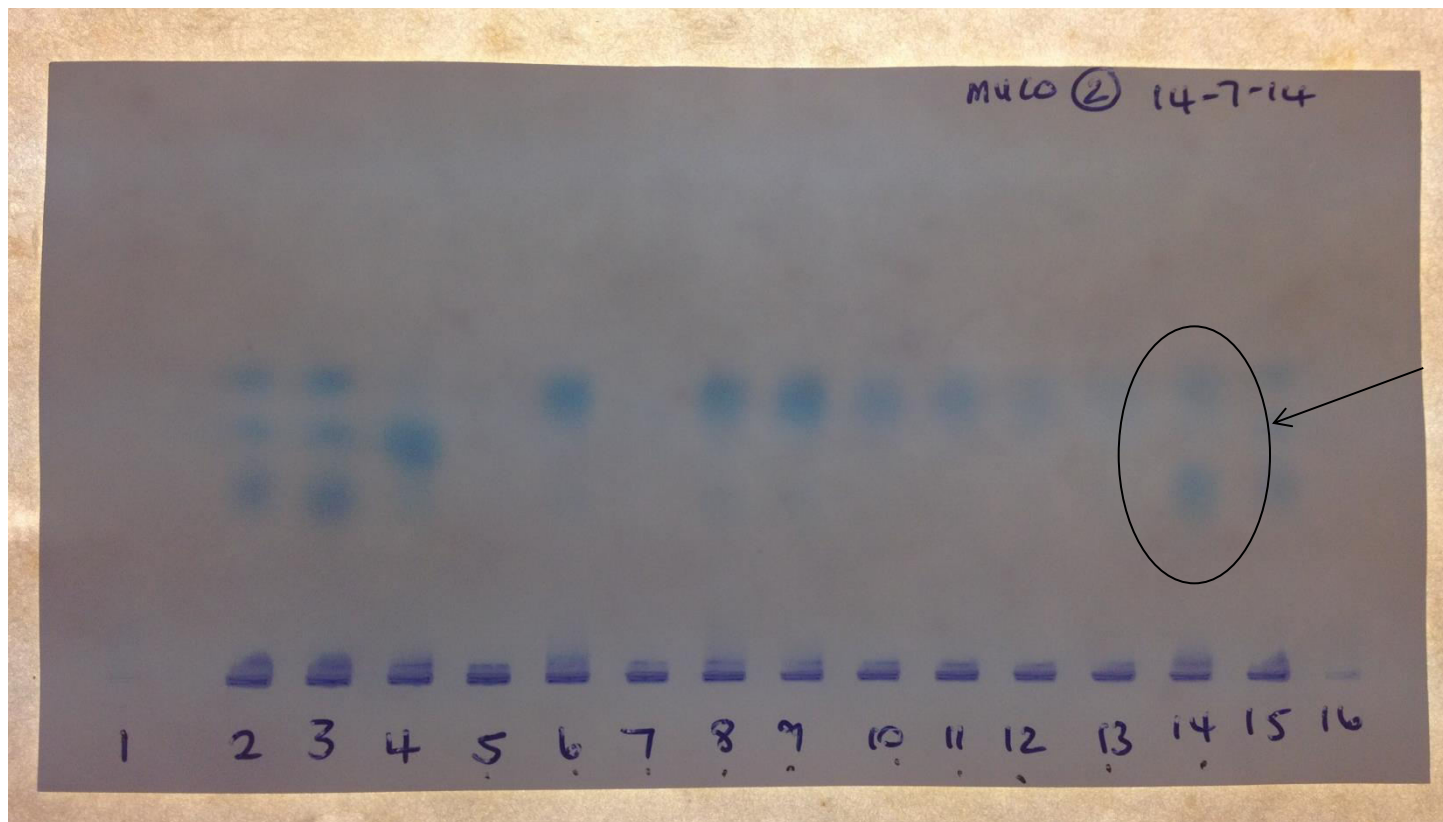


1D Electrophoresis of extracted urinary GAGs

Diagram of expected band patterns :

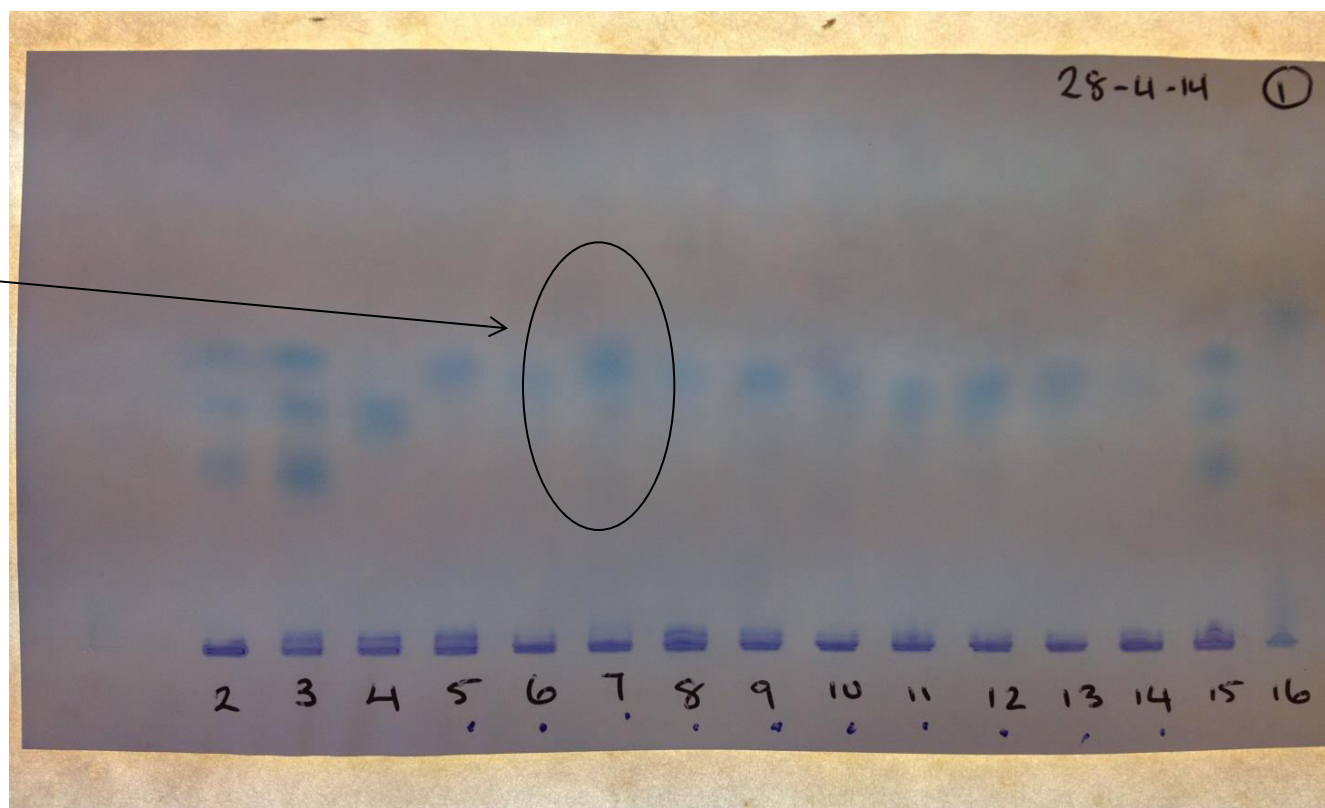


1D electrophoresis

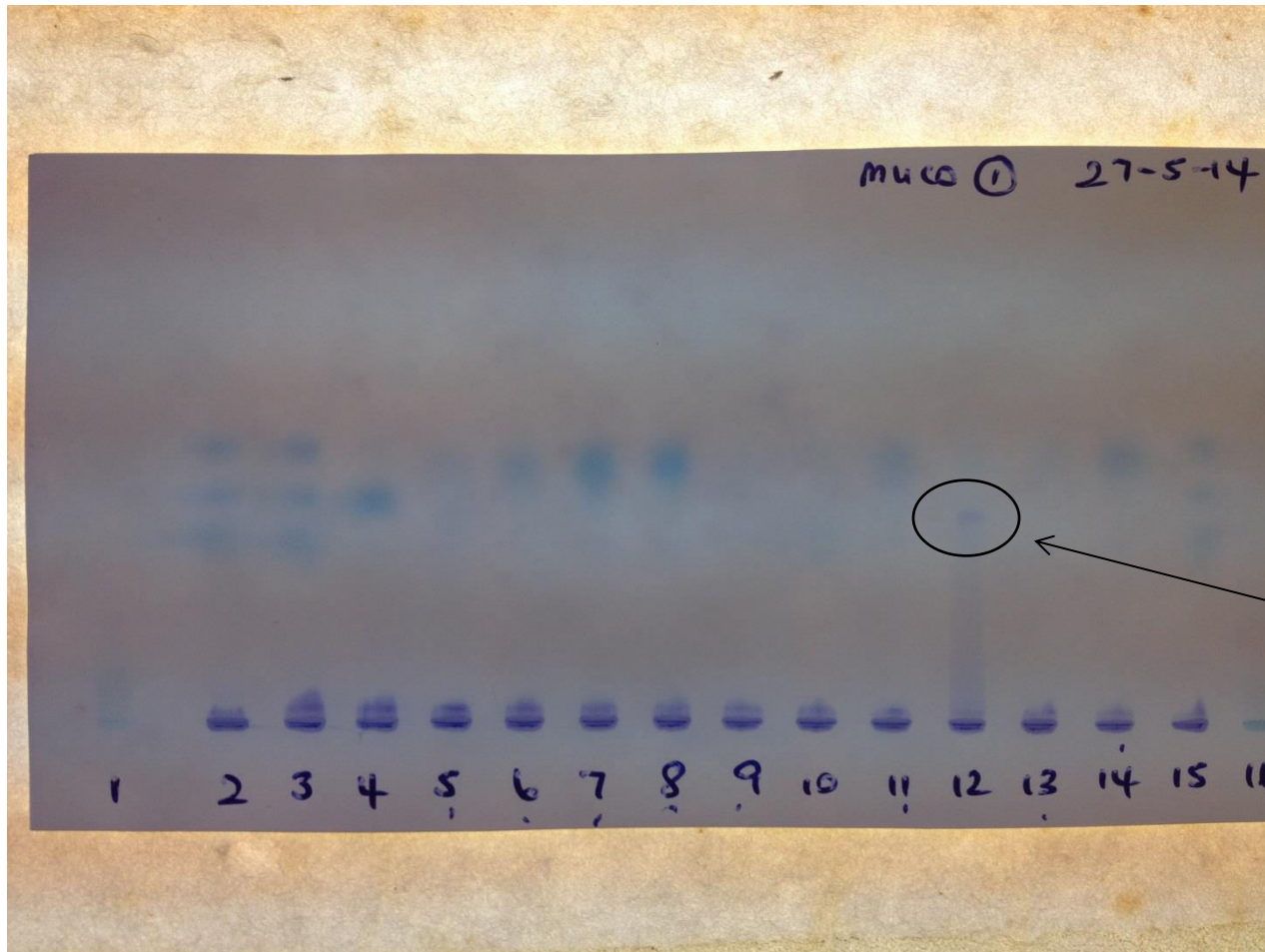


1D electrophoresis

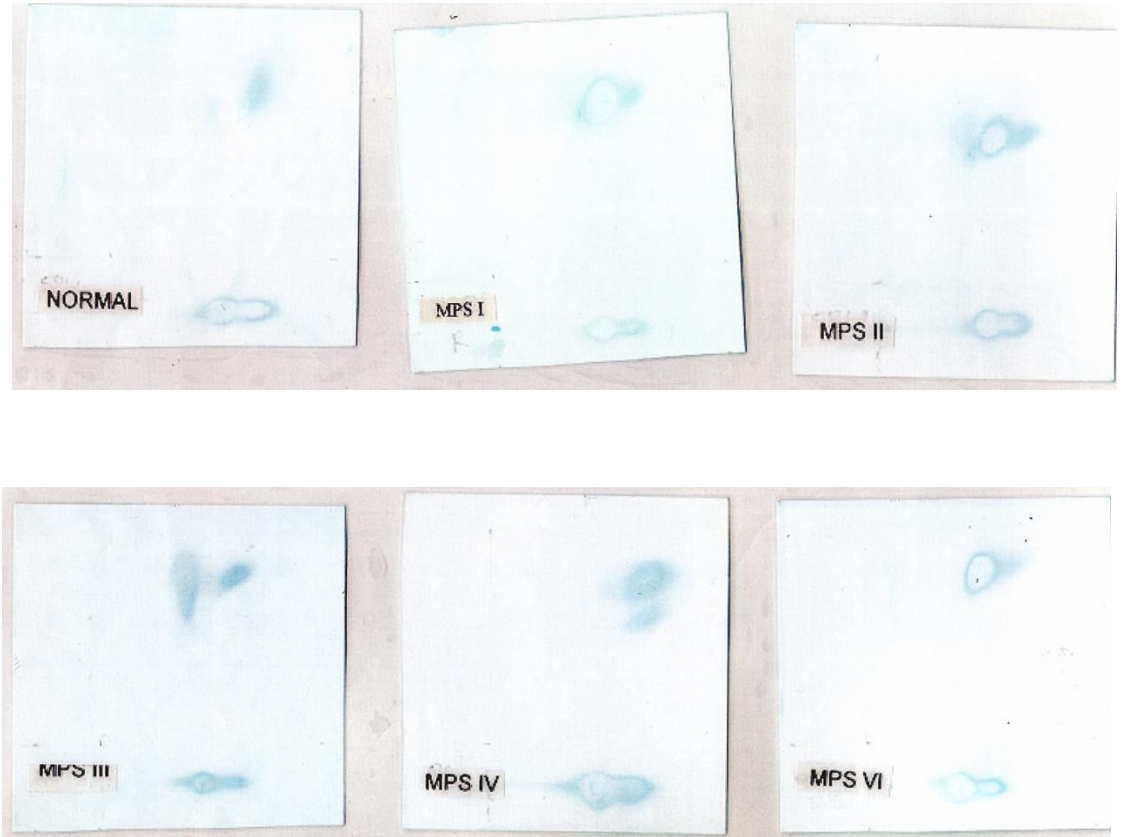
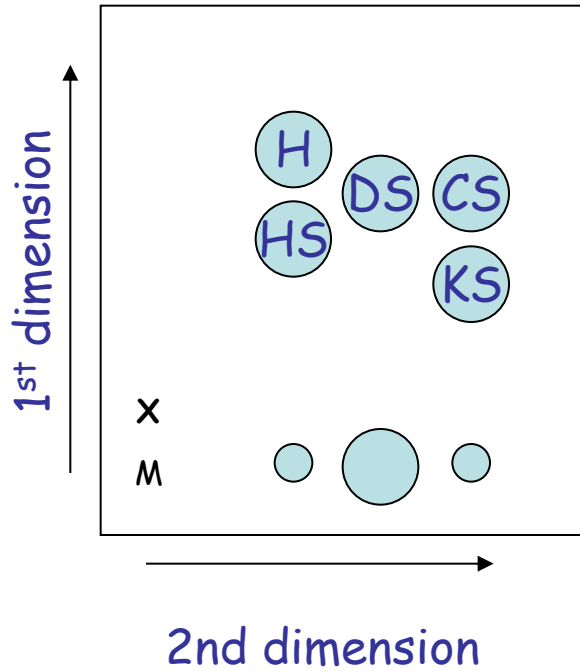
MPS IV



1D electrophoresis

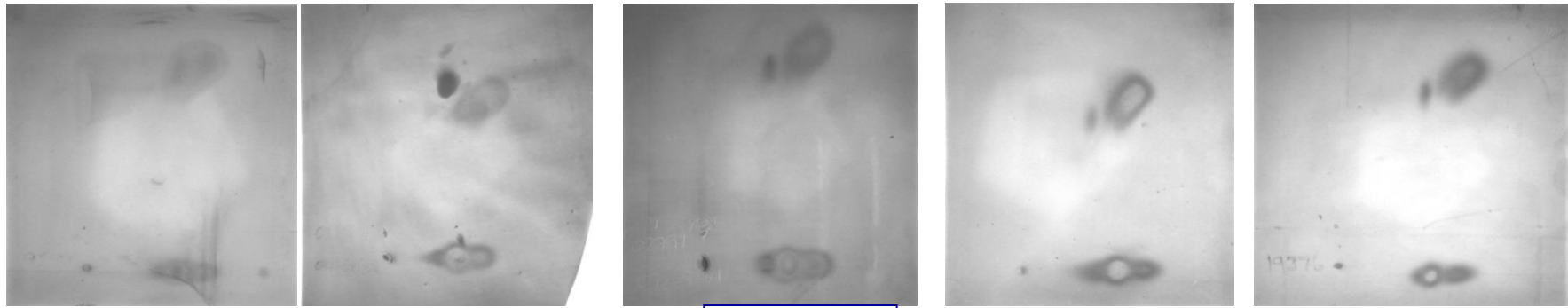


2D electrophoresis Interpretation of results



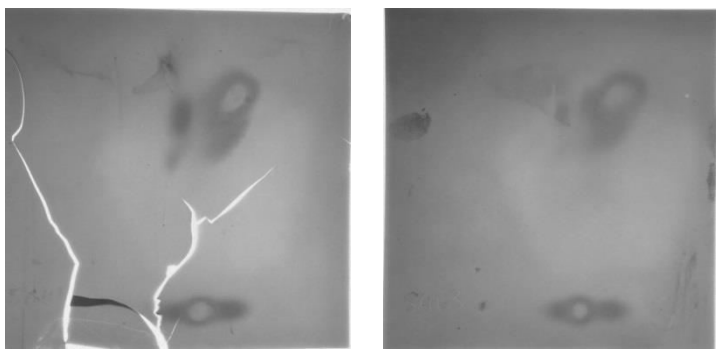
Examples of pattern variation

5 Examples of MPS VII: Note the variation in size of the dermatan Sulphate spot



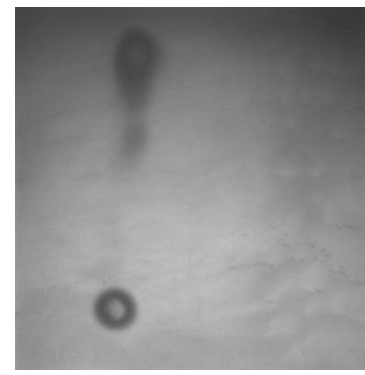
HS DS CS

Filters are fragile.....



(2 Examples of MPS IV)

Sometimes it fails.....



1D ran well, 2D failed to run



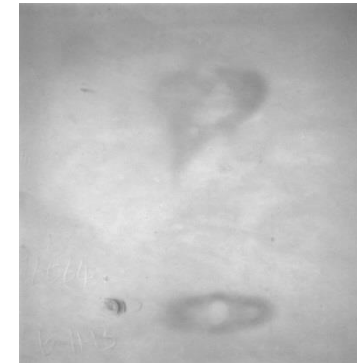
Examples of problems



Left filter is under loaded



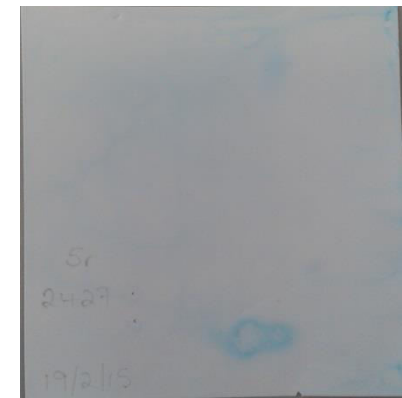
Spot outside area
? Hyaluronic acid



Pattern looked like MPS II
Enzymology showed MPS I



Possible Heparin therapy

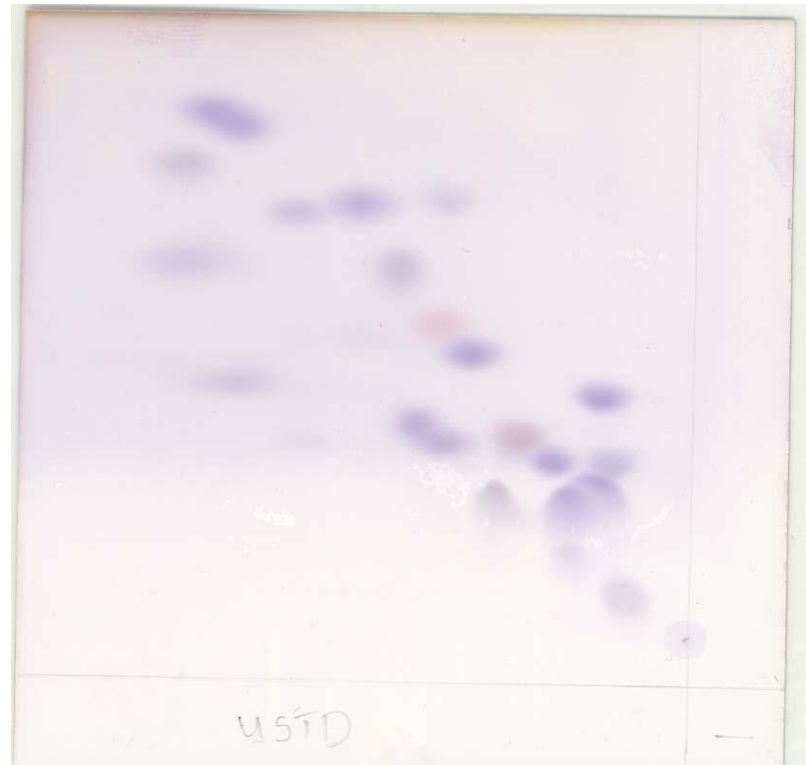


Faint spot
weak urine



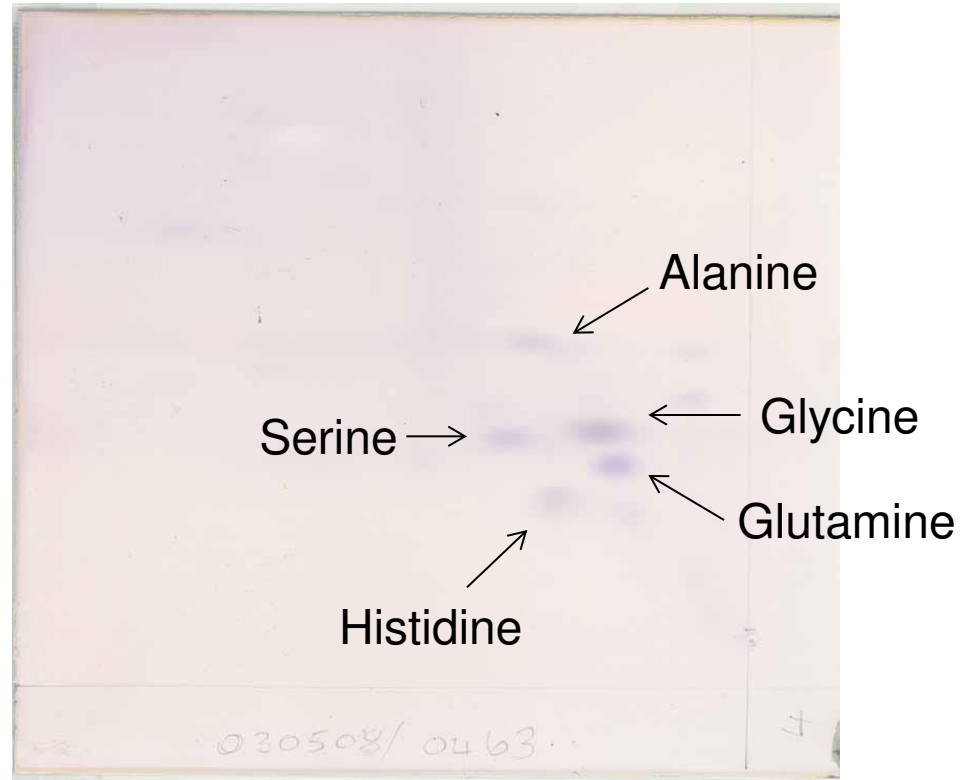
2-Dimensional Amino Acids

Urine Standard



2-Dimensional Amino Acids

Normal urine

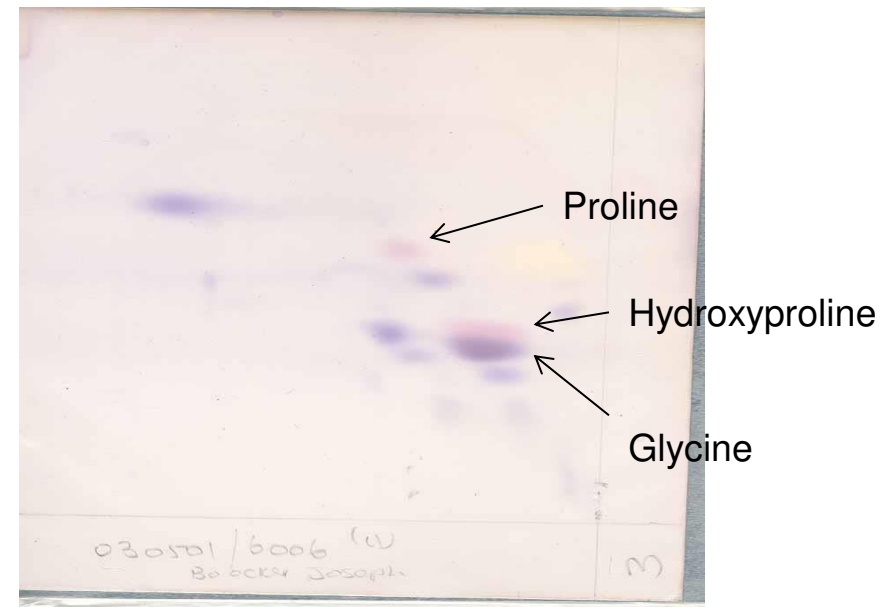


2-Dimensional Amino Acids

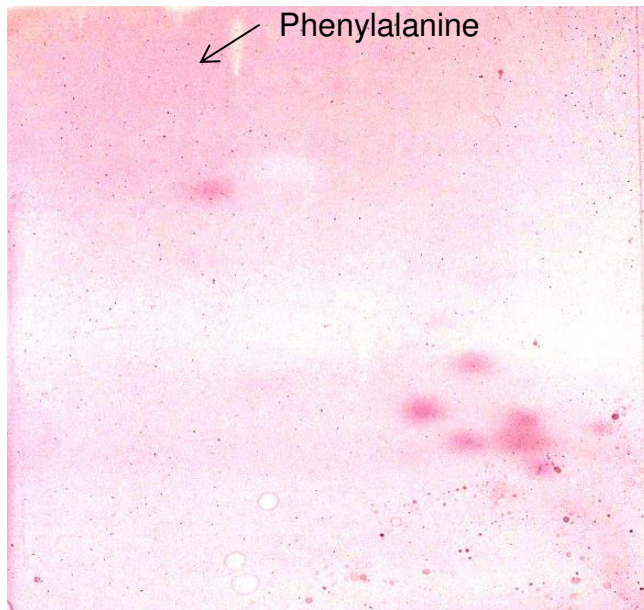
Generalised aminoaciduria



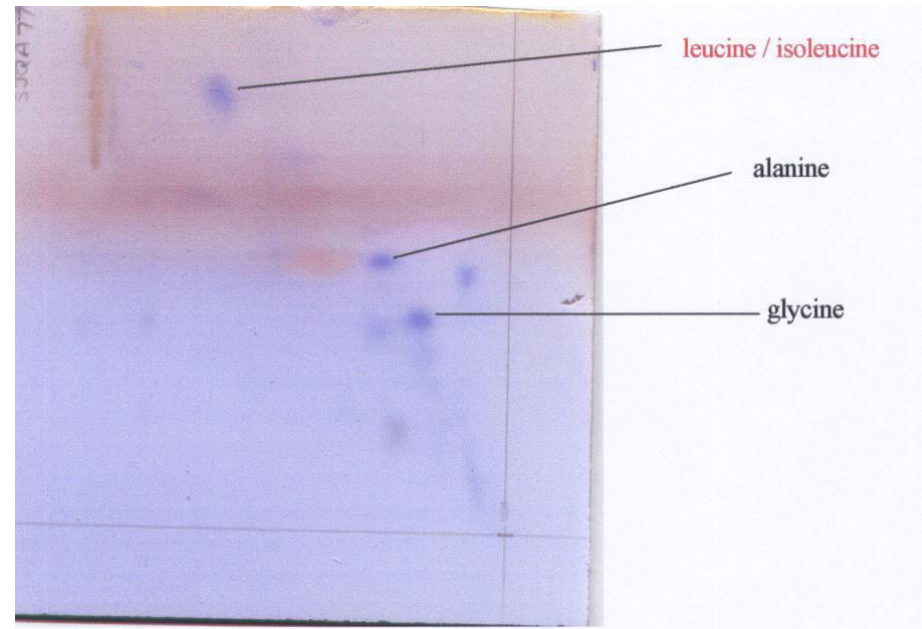
Iminoglycinuria



2-Dimensional Amino Acids



PKU

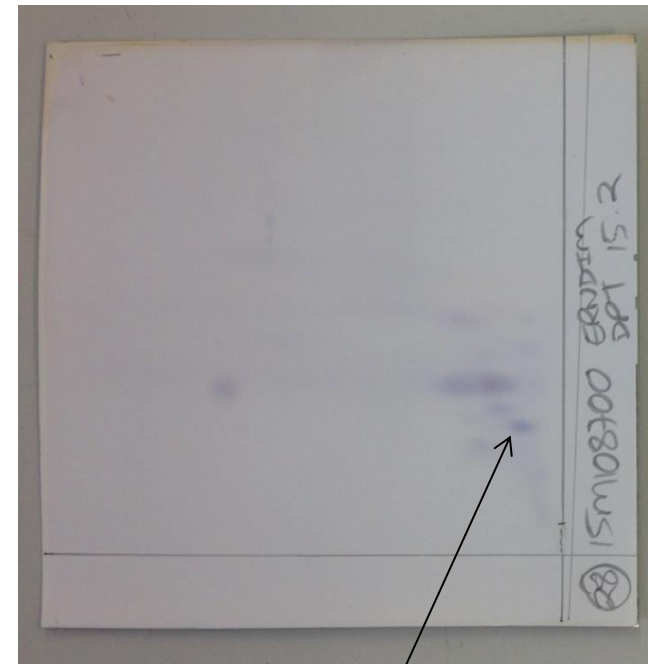
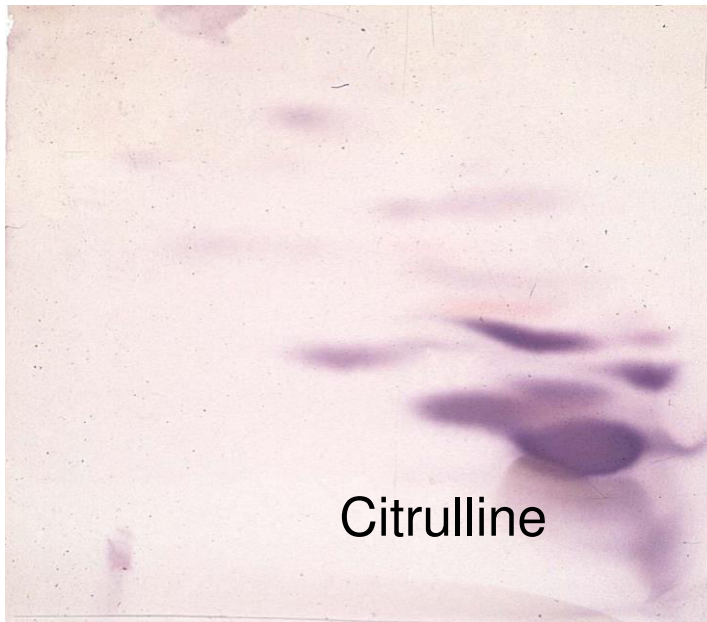


MSUD



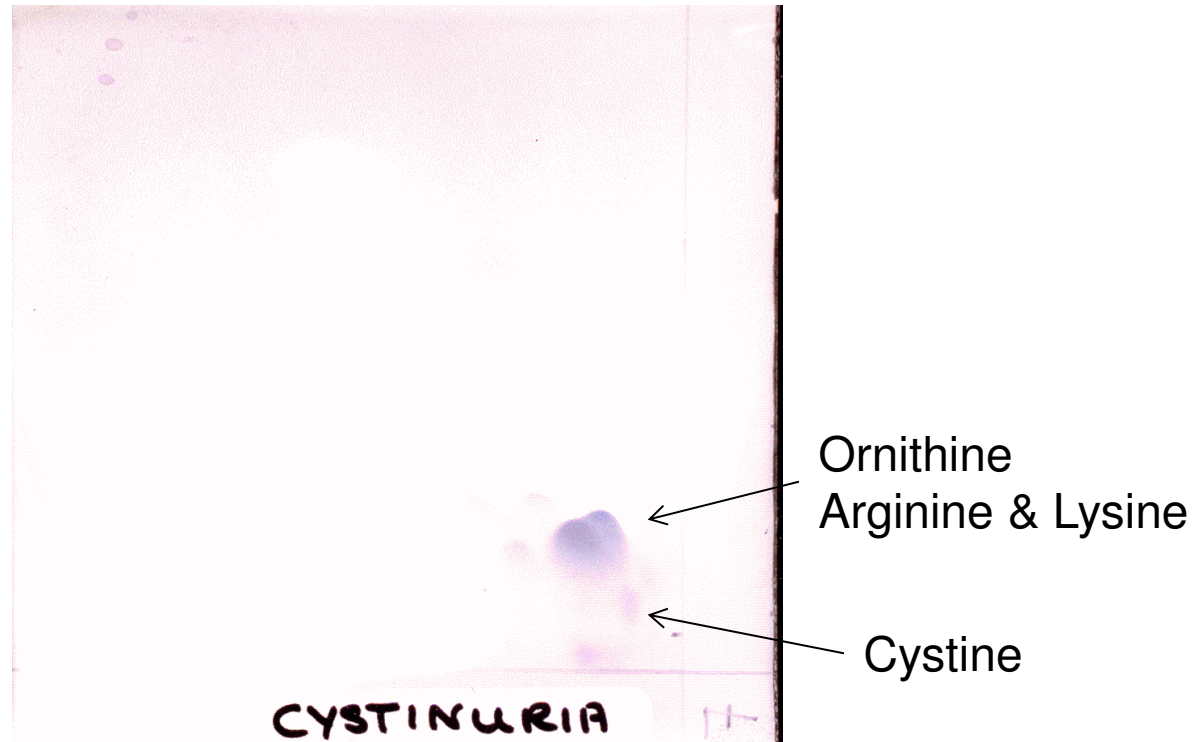
2-Dimensional Amino Acids

Citrullinaemia

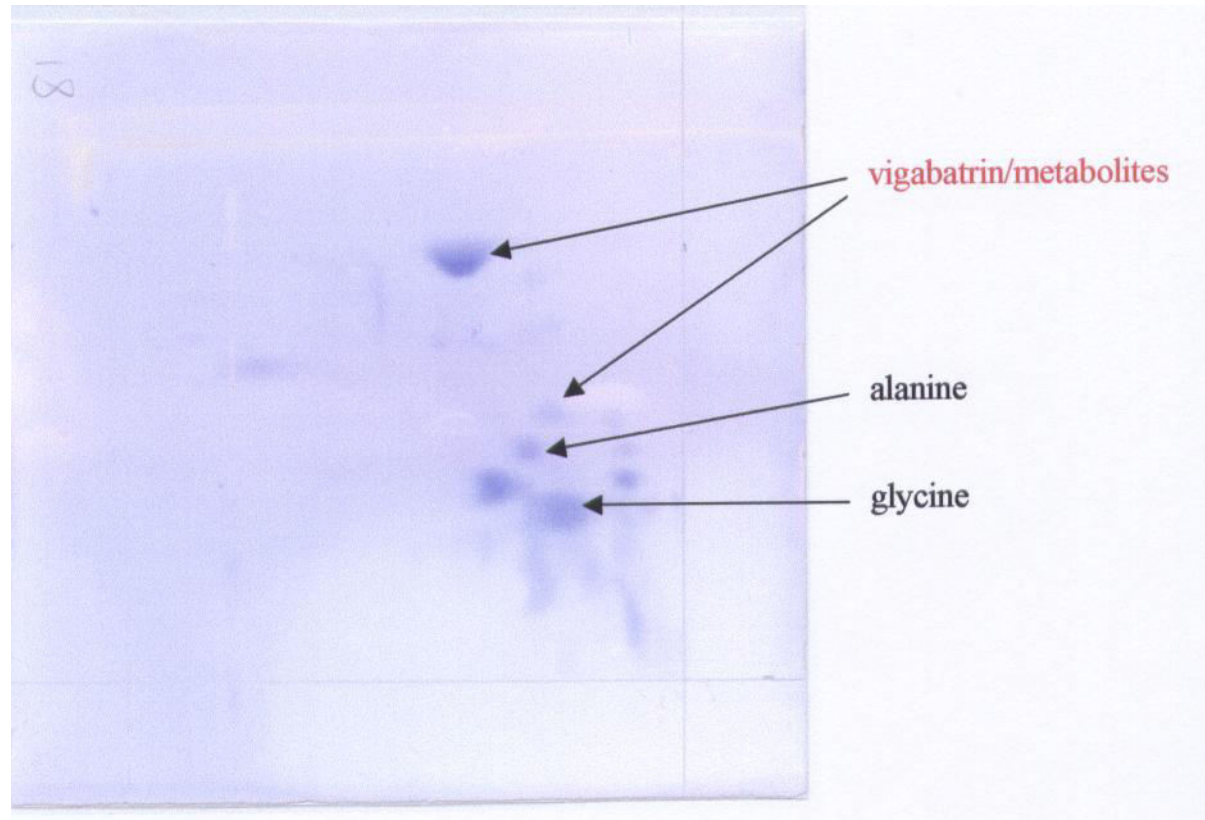


2-Dimensional Amino Acids

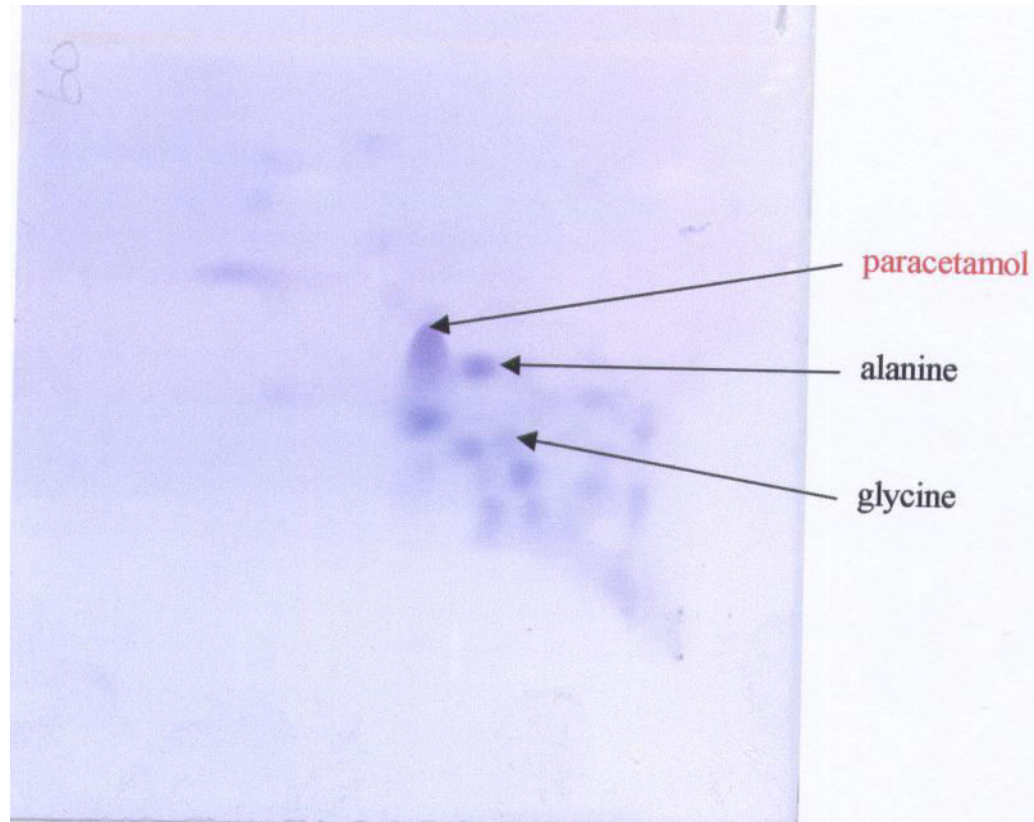
Cystinuria



Vigabatrin spot on TLC

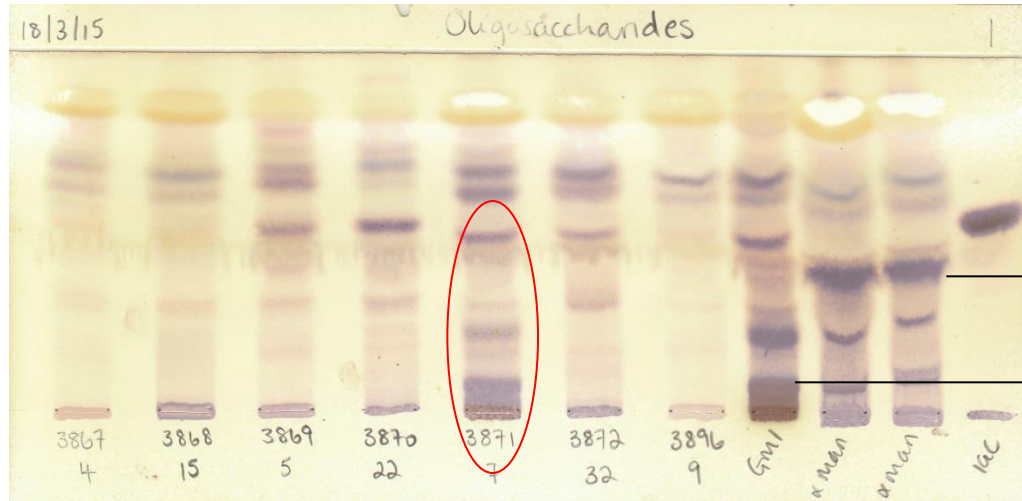


Paracetamol spot on TLC



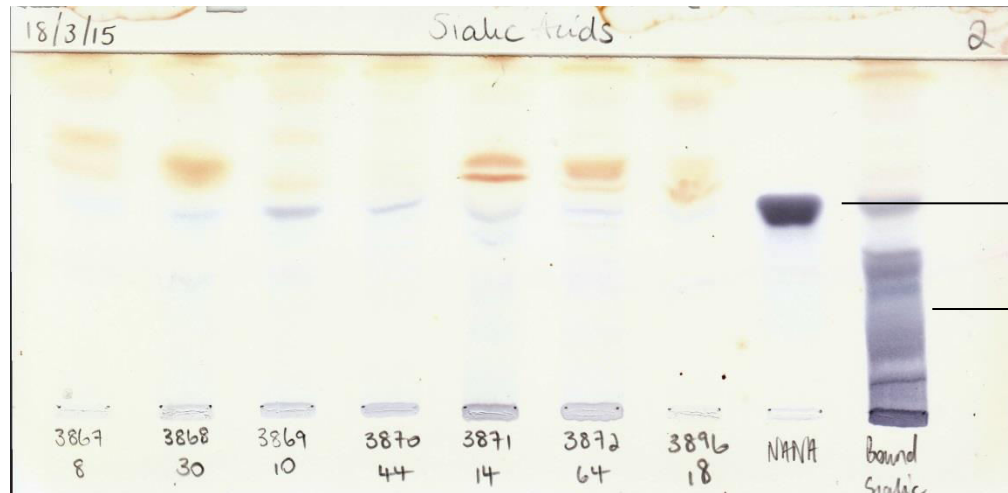
Examples of Thin Layer Chromatography

Oligosaccharide plate



Lactose
 alpha-mannosidosis
 (Reference urine)
 GM1
 (Reference urine)

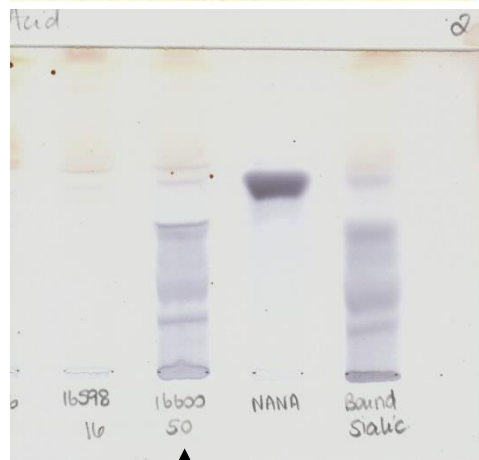
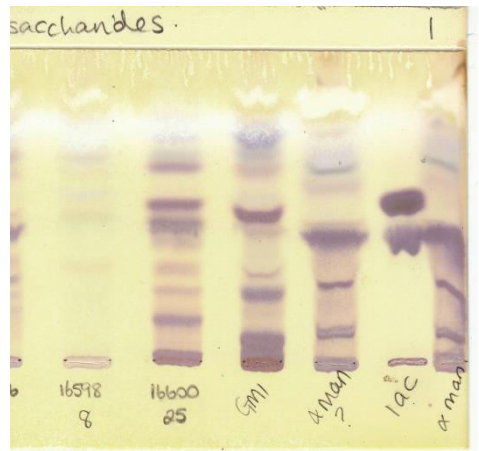
Sialic acid plate



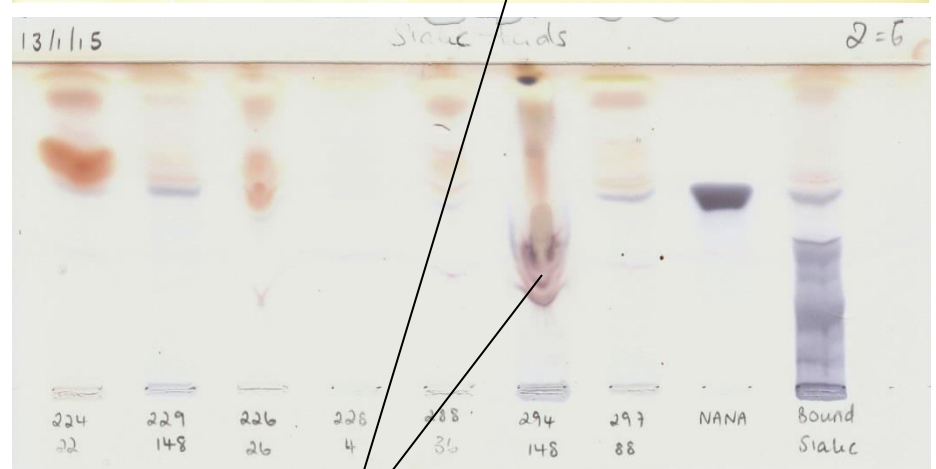
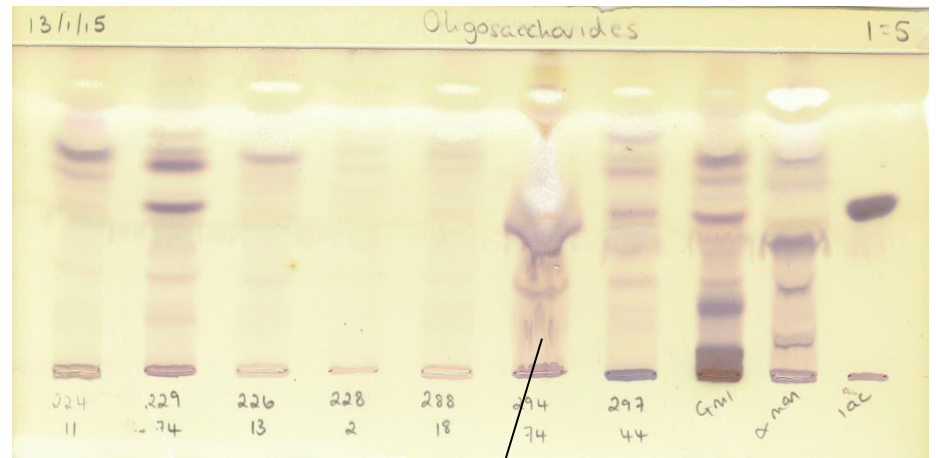
Free sialic acid
 Bound sialic acid
 (Reference urine)



Examples of Thin Layer Chromatography



Bound sialic acid
(Neuraminidase deficiency)

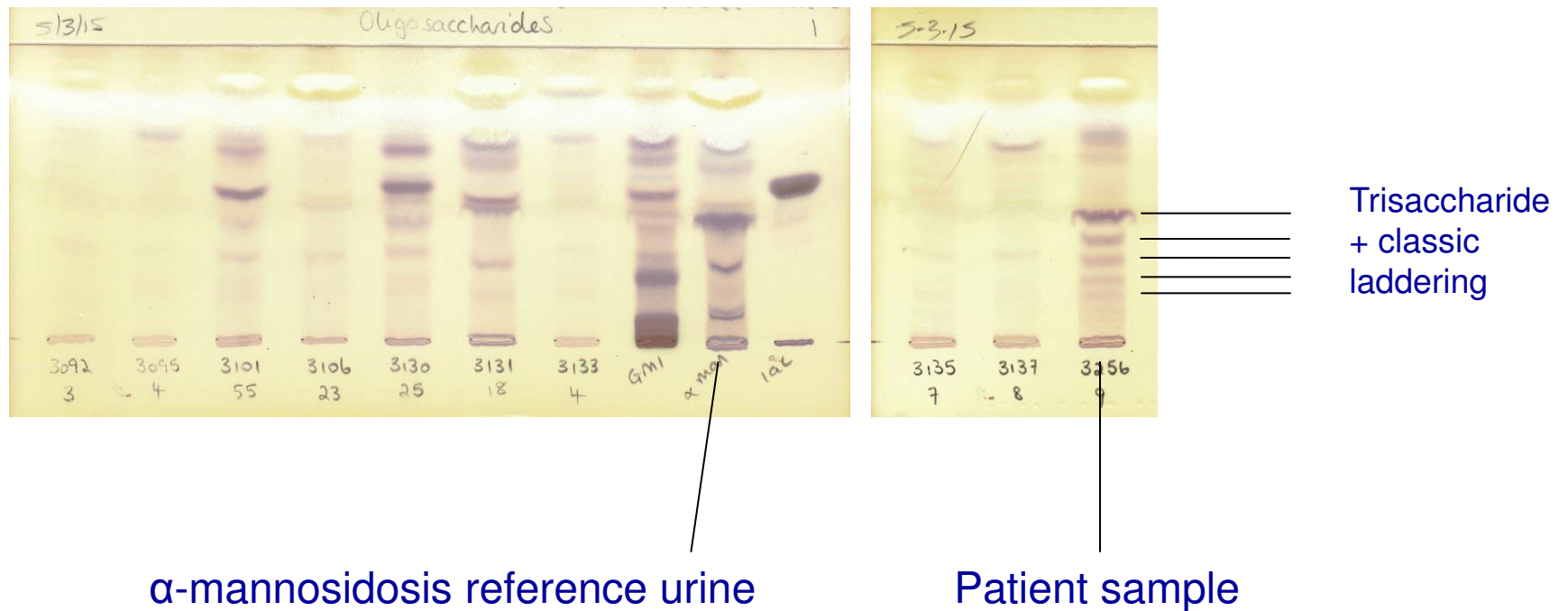


Pattern distorted
? Urinary salts

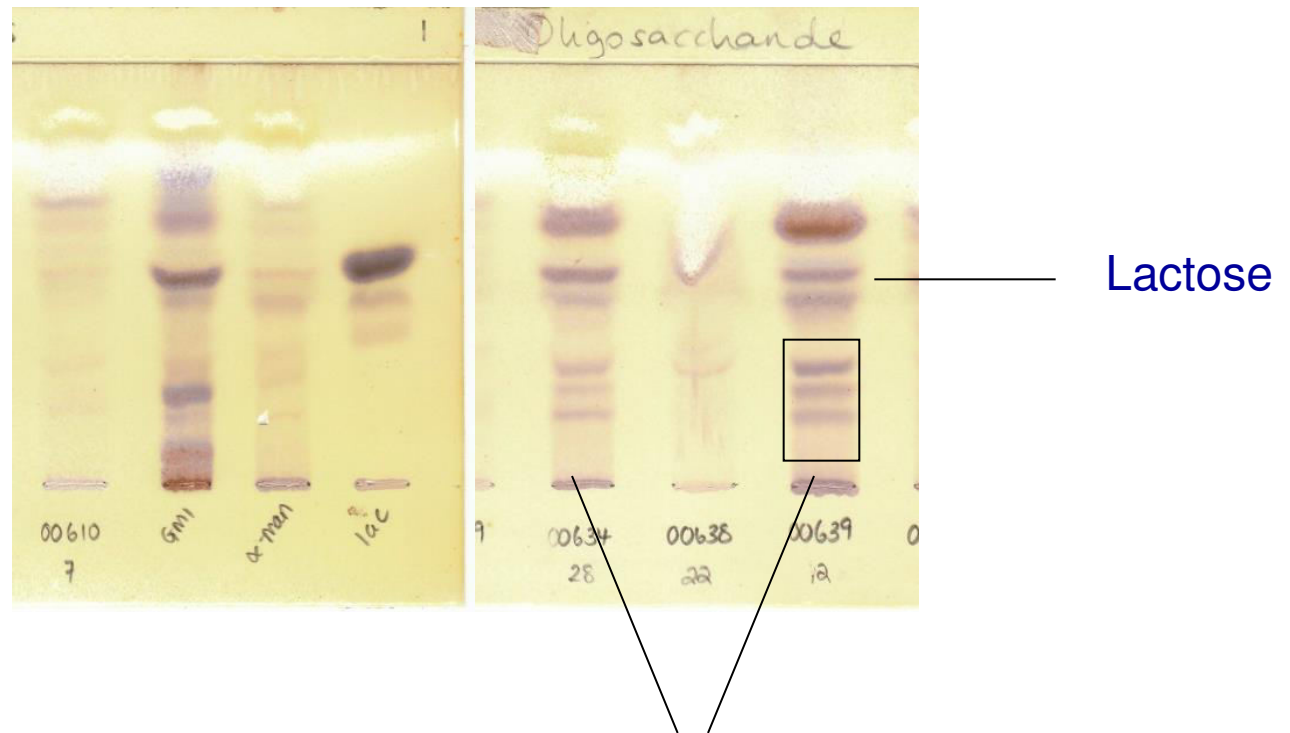


Oligosaccharide plates showing α -mannosidosis

Note the heavy trisaccharide band Laddering pattern is not always seen



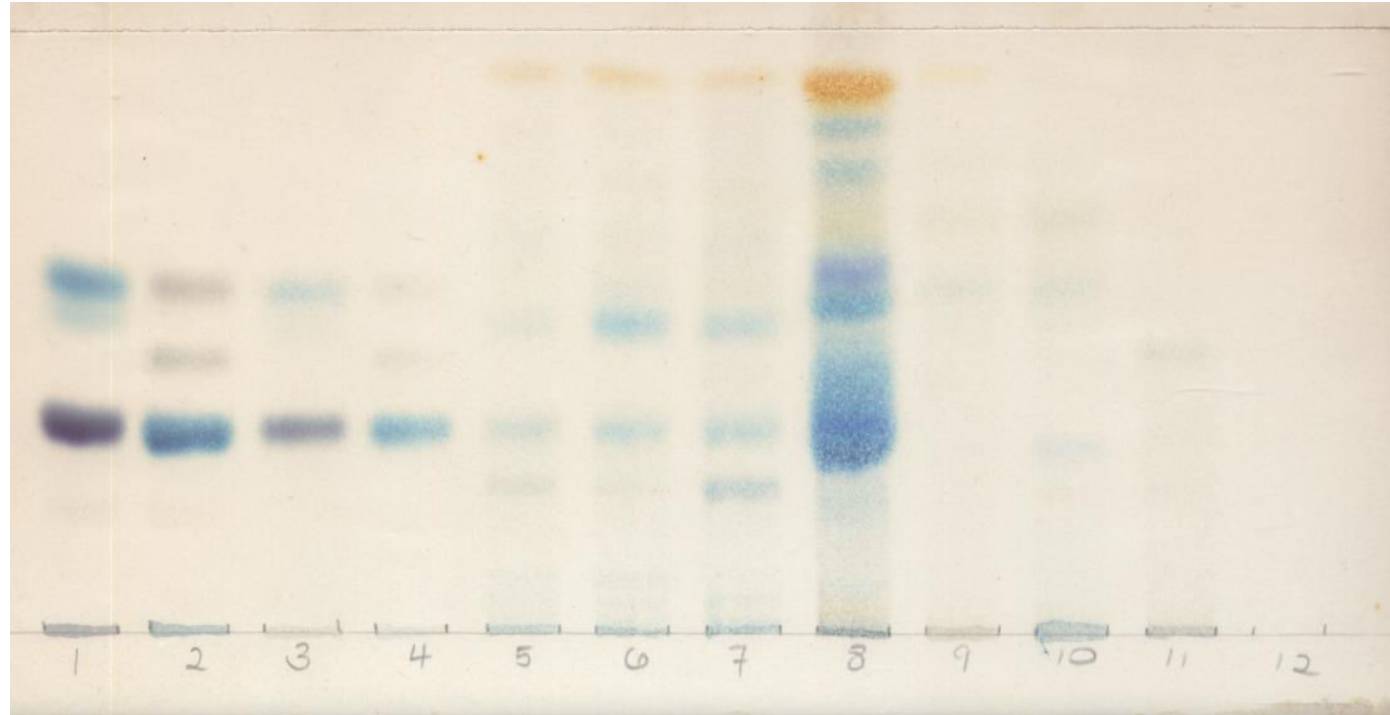
Examples of Thin Layer Chromatography TLC



Oligosaccharides showing pattern often seen in young / jaundiced babies

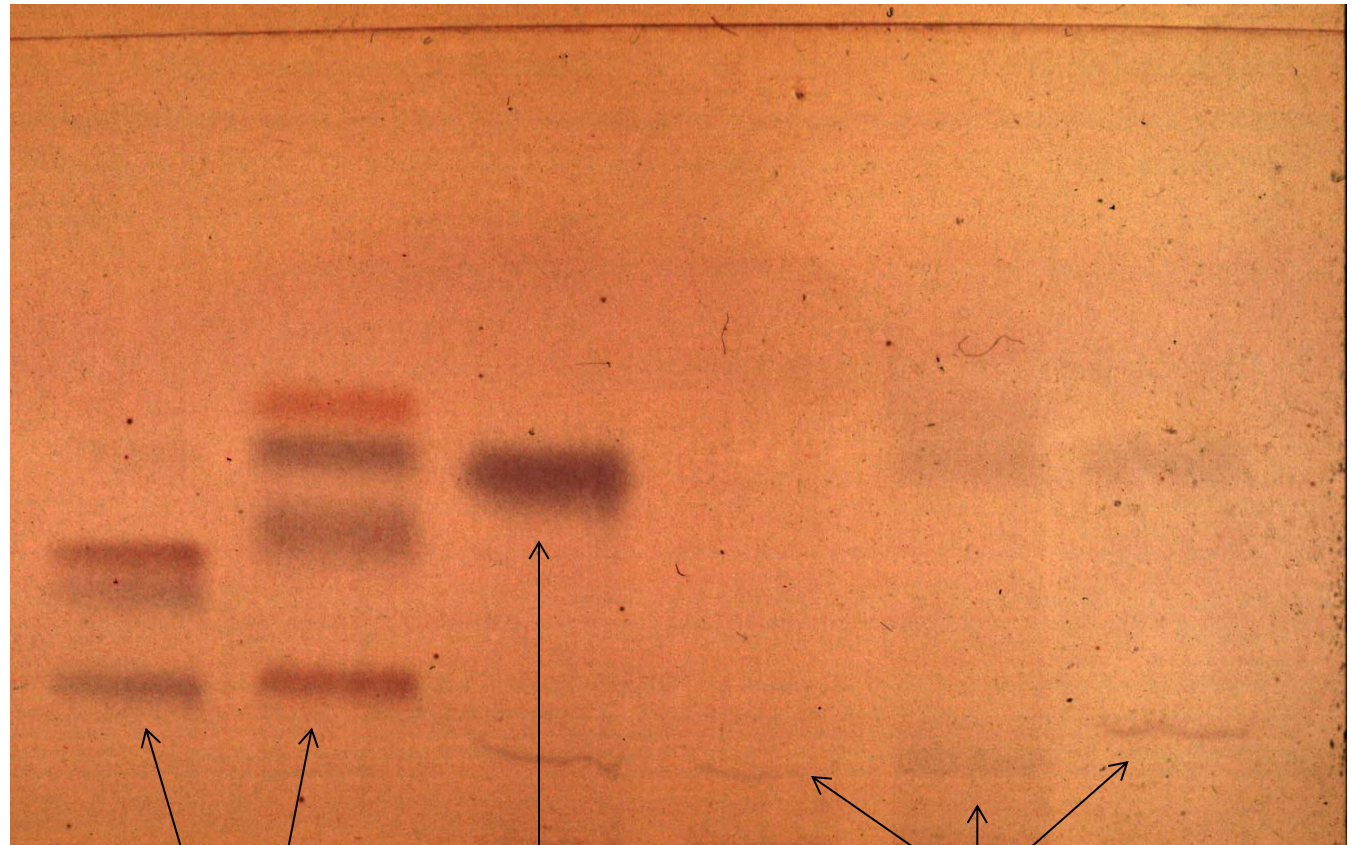
Examples of Sugar Chromatography

Glucose	Fructose
Galactose	Sucrose
Lactulose	Lactose



Examples of Sugar Chromatography

Fructose
Galactose
Sucrose
Glucose
Maltose
Lactose
Lactulose



Standards

Galactosaemia

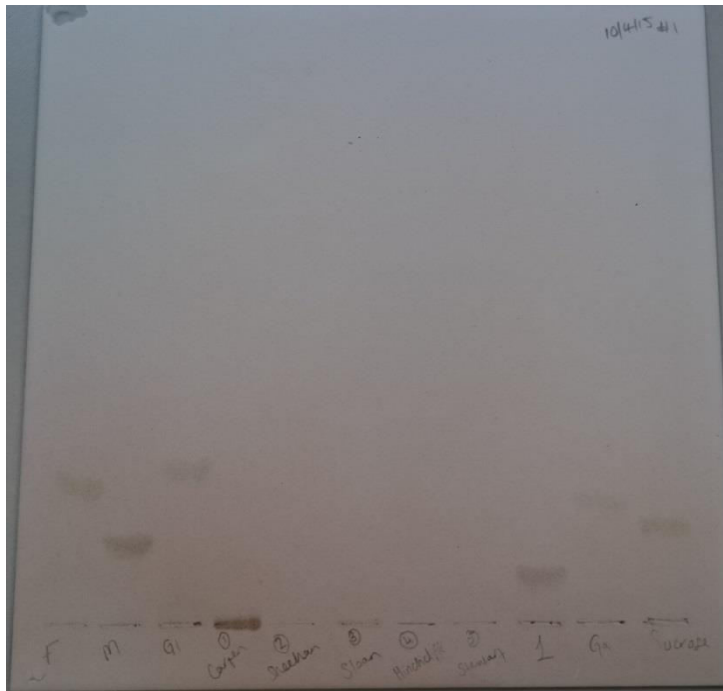
Normals



Example of faecal sugar chromatography

Problems

Patient samples too dilute



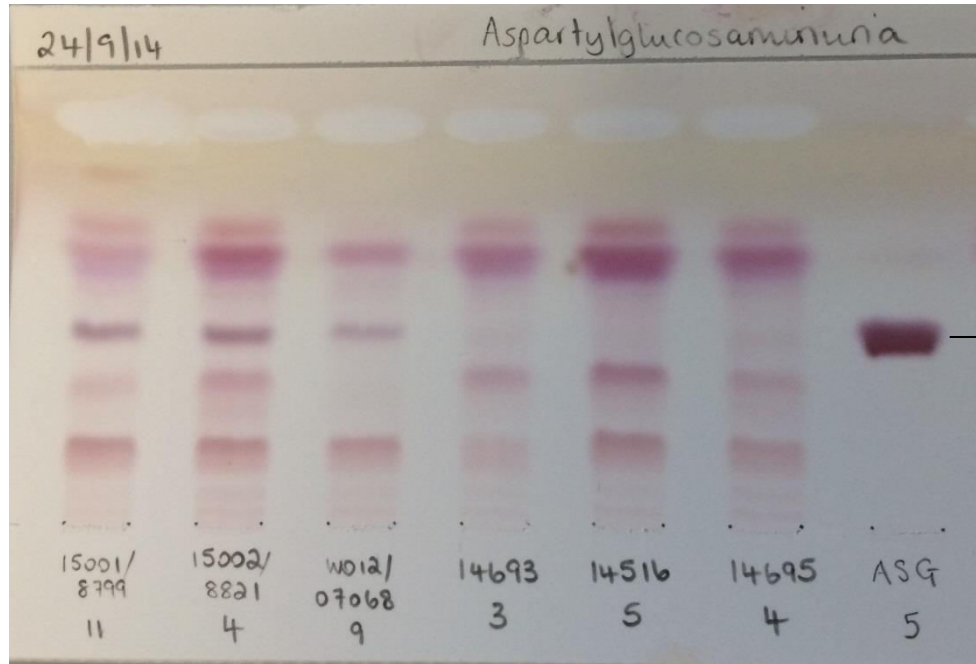
Standards distorted Plate scratched



Images from Deborah Riley Alder Hey Children's Hospital Liverpool

Examples of Thin Layer Chromatography

Aspartylglucosaminuria



— Asg band

Test

Positive
control

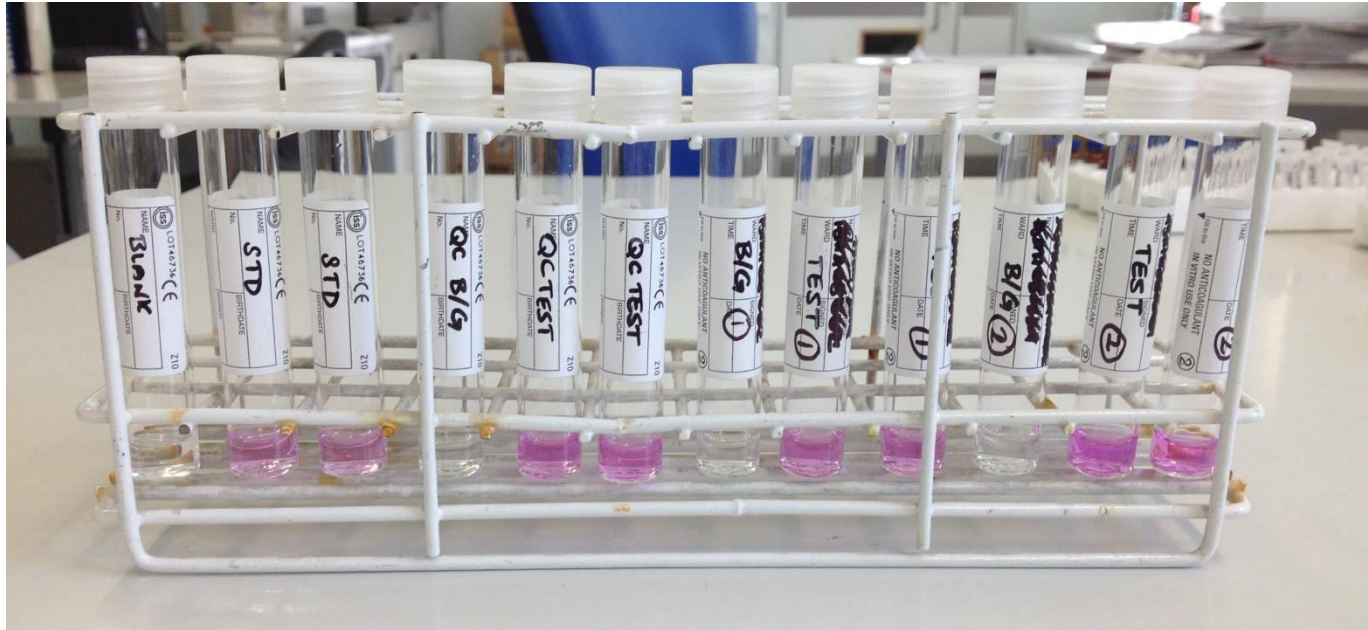
Normal

Plates fade quickly

CAMAG TLC Robot



Example of a test tube based assay



Blank

Standard

QC

Patient 1

Patient 2

Biotinidase assay – Colourimetric assay.

Thank You

June Petty - Willink Biochemical Genetics Unit
Deborah Riley - Alder Hey Children's Hospital Liverpool
Colleagues at Nottingham University Hospitals