

MetBioNet BMS Training Group Workshop – Manchester

Tackling All Techniques

Manual Techniques

June Petty
St Mary's Hospital Manchester

20th April 2015



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.

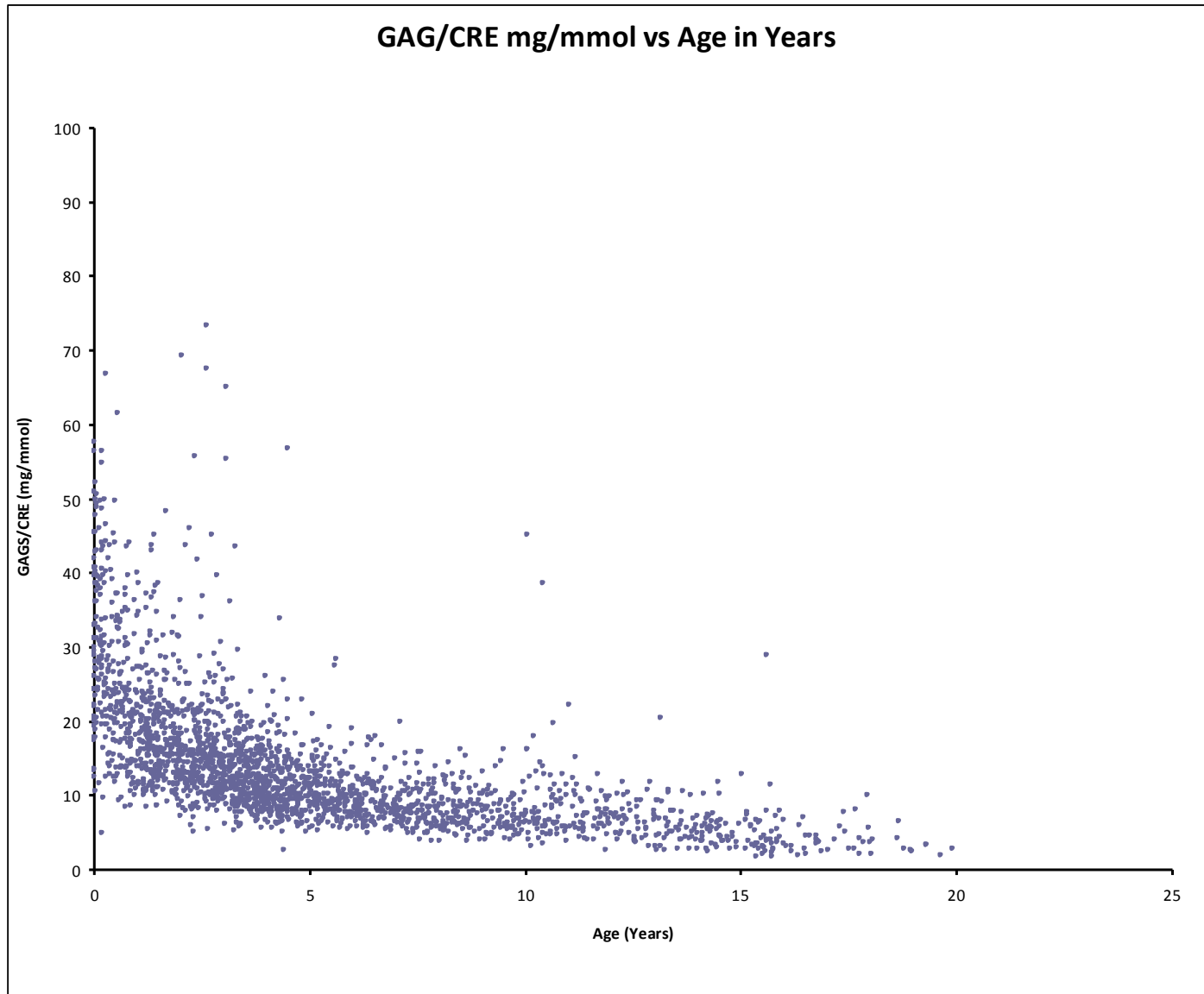
GAGs continuously synthesised and degraded during normal metabolism.

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

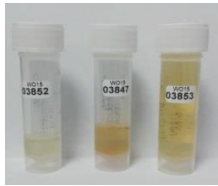




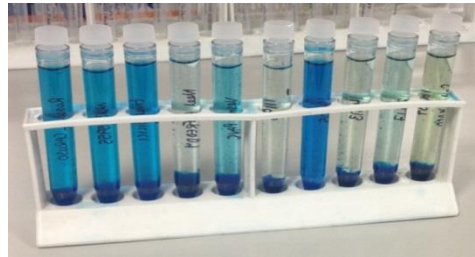
Graph Showing Normal GAG/Creatinine vs Age Willink laboratory



Qualitative measurement of GAGs (2 dimensional electrophoresis)



Urine samples



Extract each sample with Alcian blue

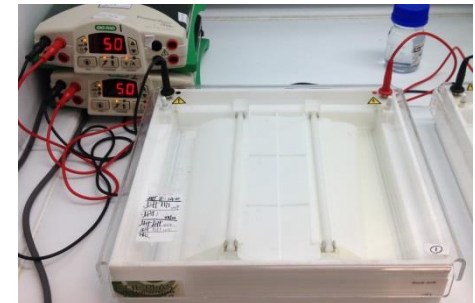


Isolated GAGs

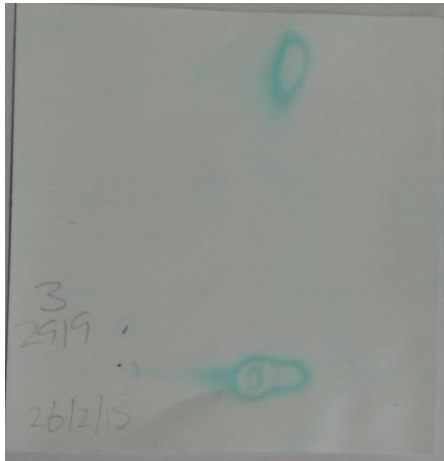
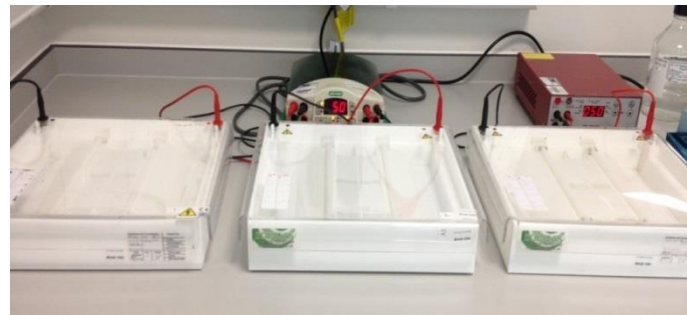


2D Electrophoresis

First dimension
one patient sample per filter



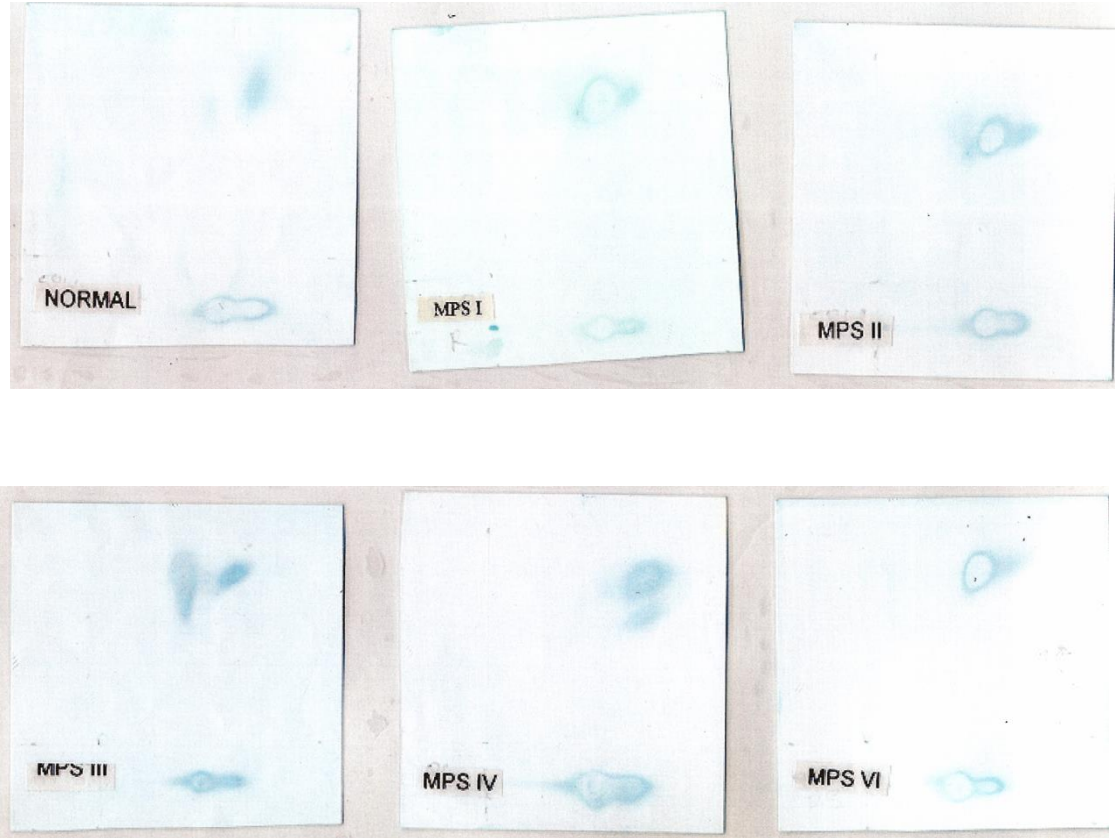
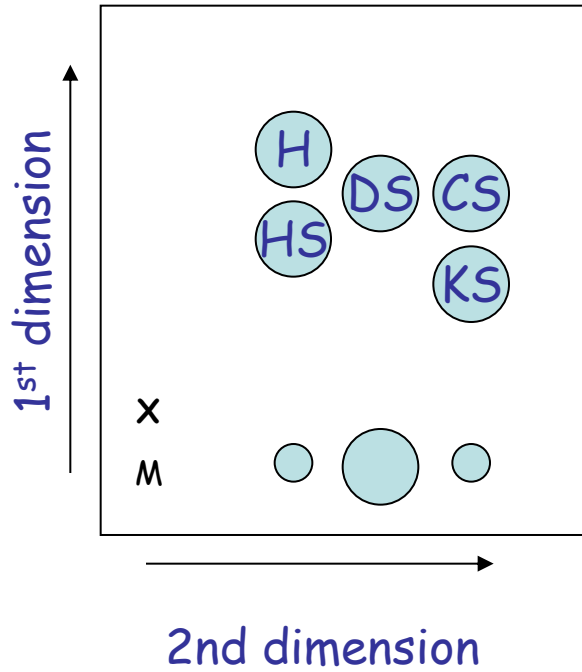
Second dimension
Reference marker added



Electrophoretogram



2D electrophoresis Interpretation of results



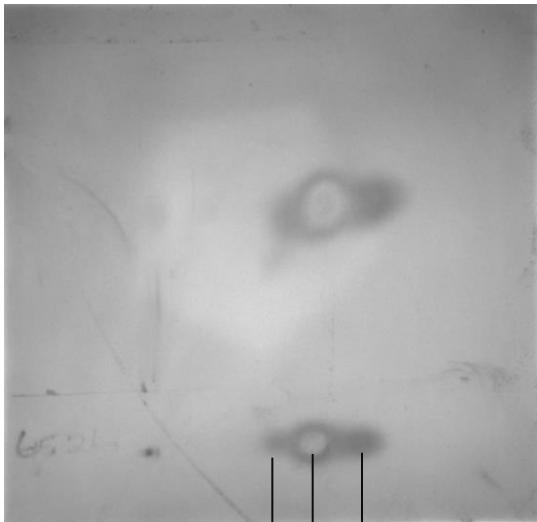
What does this result mean?

GAG/Creatinine ratio was within normal range
Some dermatan sulphate seen

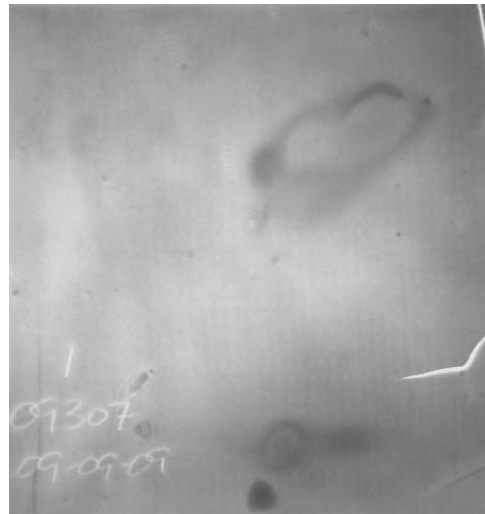
MPS I

???

MPS VI

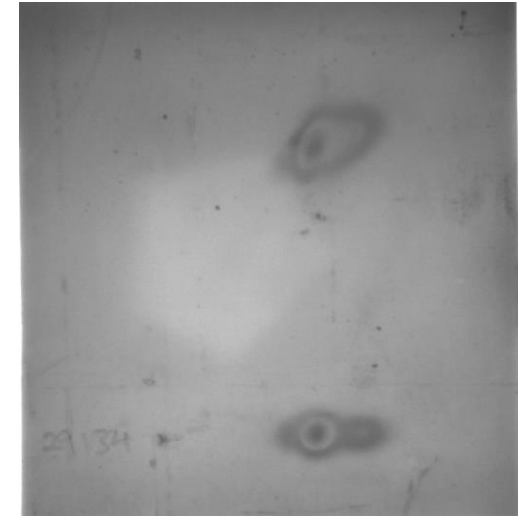


Markers : HS DS CS



DS spot smaller than expected for
undiagnosed patient

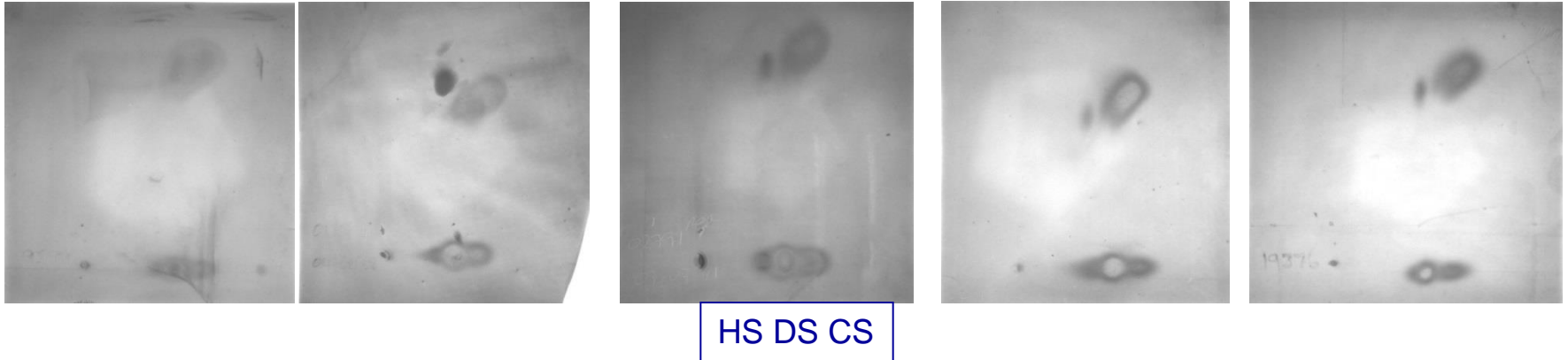
Attenuated MPS VI patient
normal GAG/Cre ratio



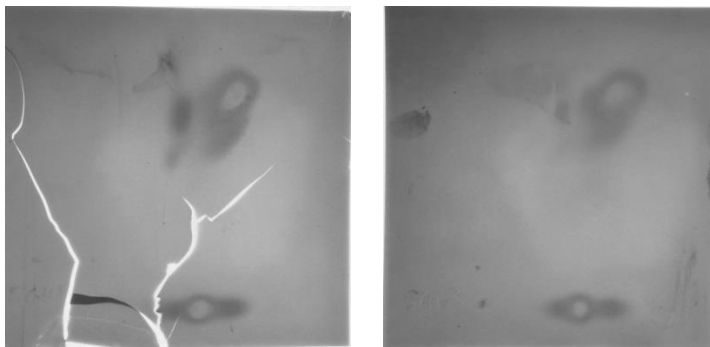
Images of electrophoretograms taken using UVP gel doc imager and white light filter

Examples of pattern variation

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

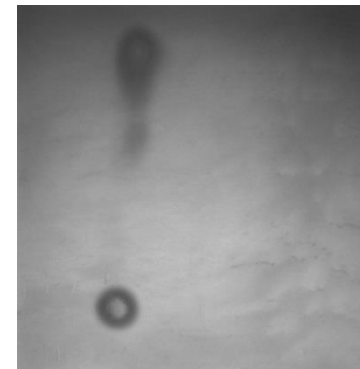


Filters are fragile.....



(2 Examples of MPS IV)

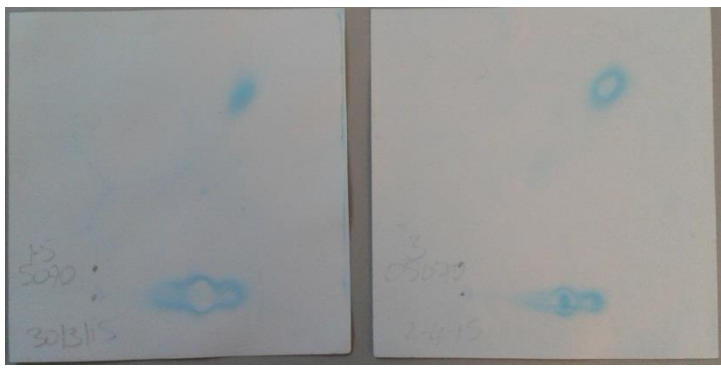
Sometimes it fails.....



1D ran well, 2D failed to run



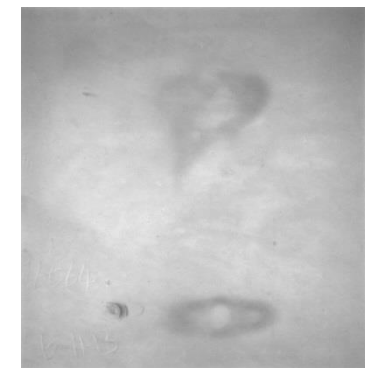
Examples of problems



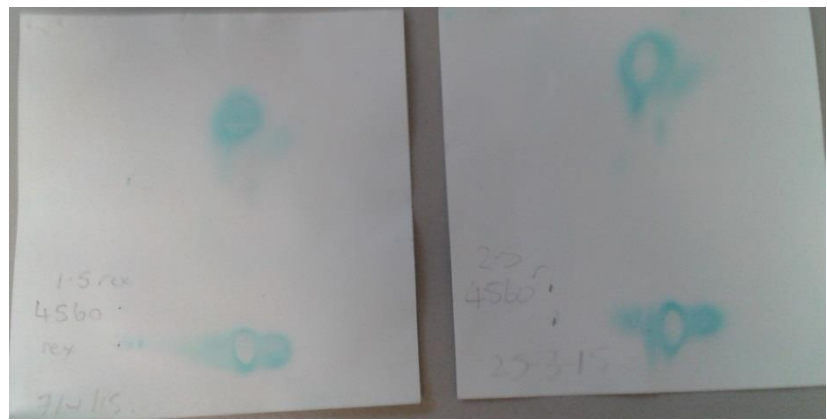
Left filter is under loaded



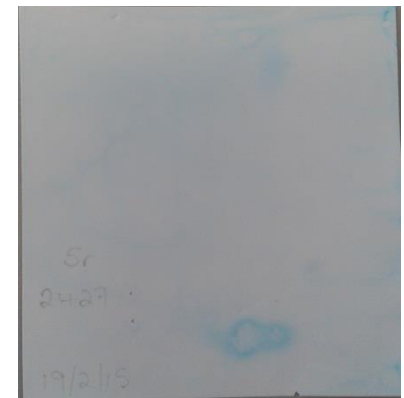
Spot outside area
? Hyaluronic acid



Pattern looked like MPS II
Enzymology showed MPS I



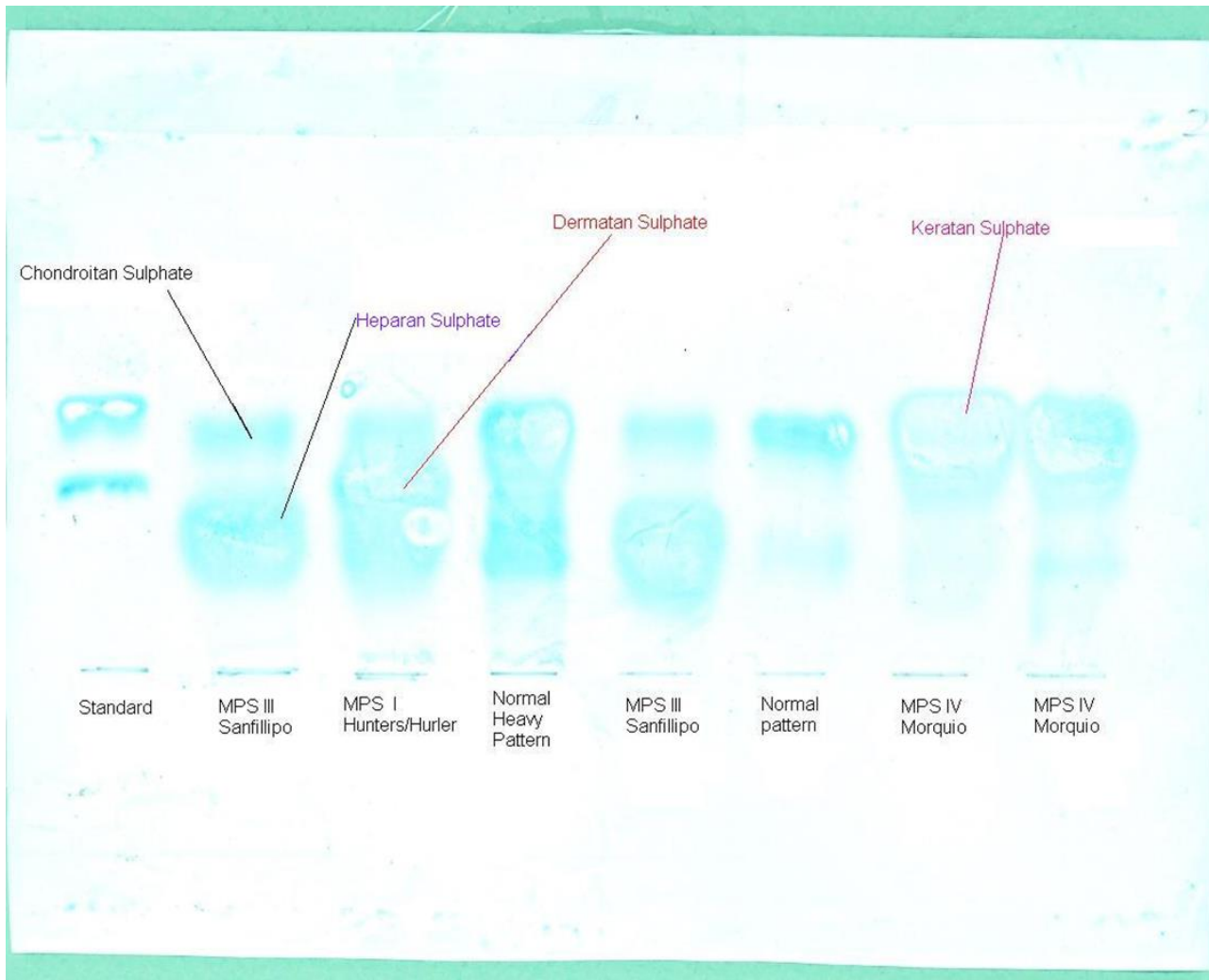
Possible Heparin therapy



Faint spot
weak urine



1D Electrophoresis of extracted urinary GAGs

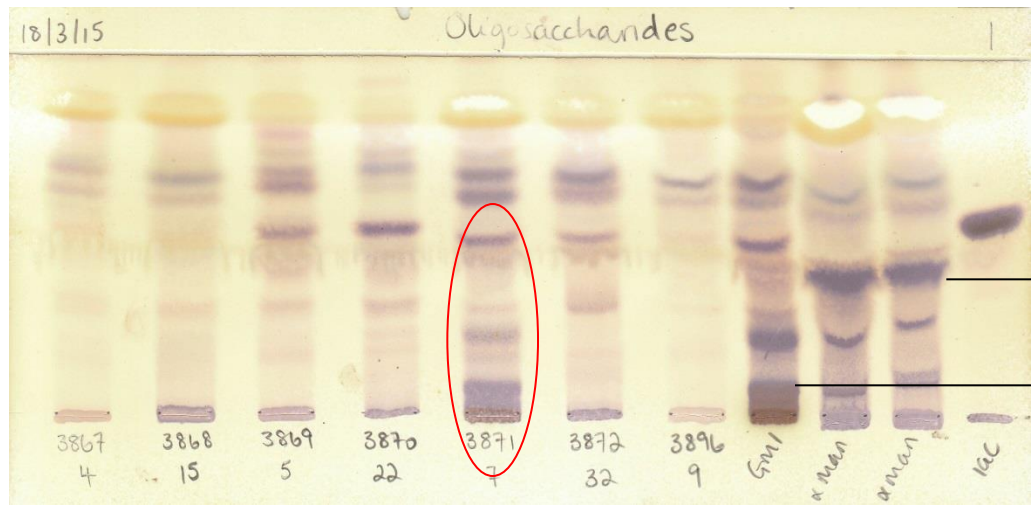


Slide provided by Louise Allen Birmingham Children's hospital



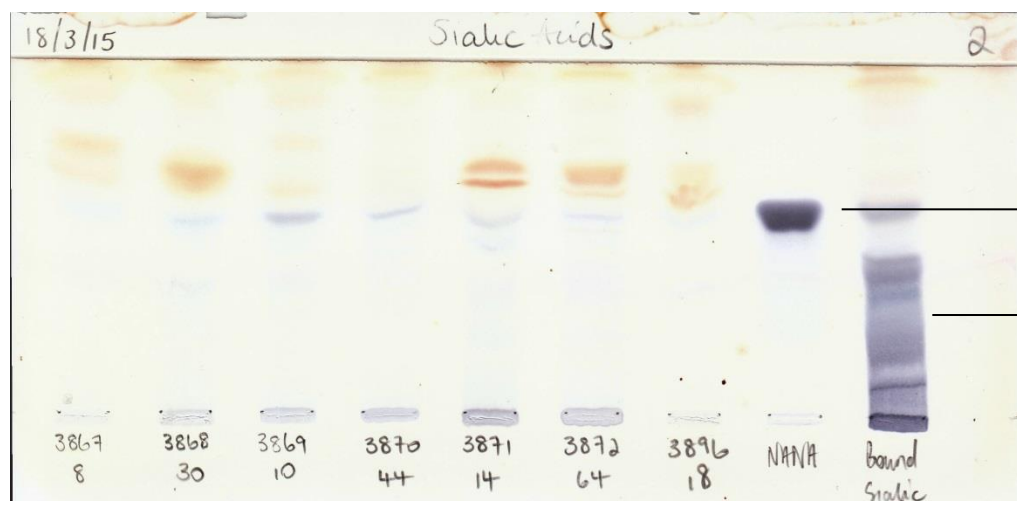
Examples of Thin Layer Chromatography

Oligosaccharide plate



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

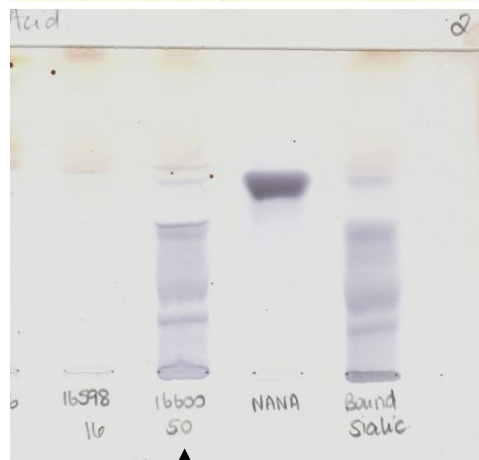
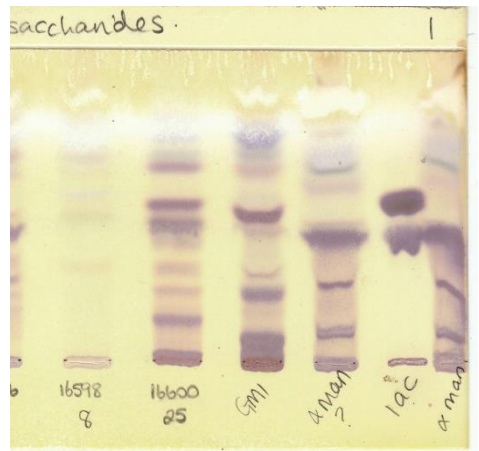
Sialic acid plate



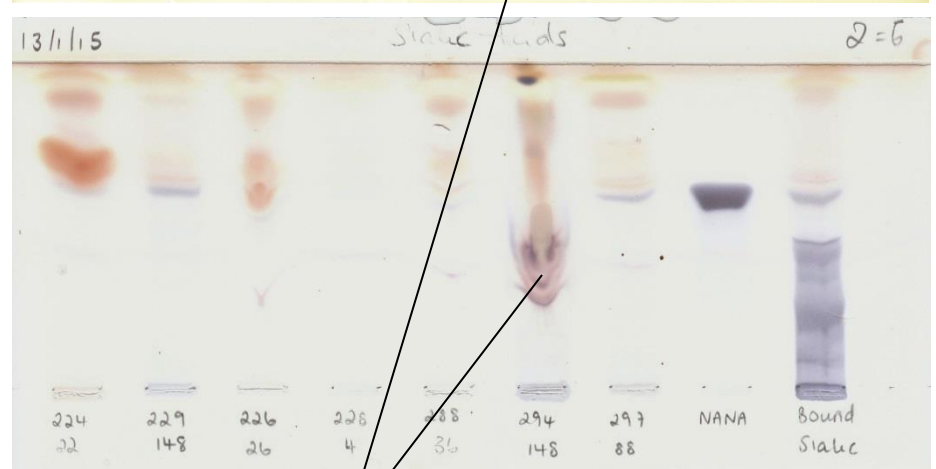
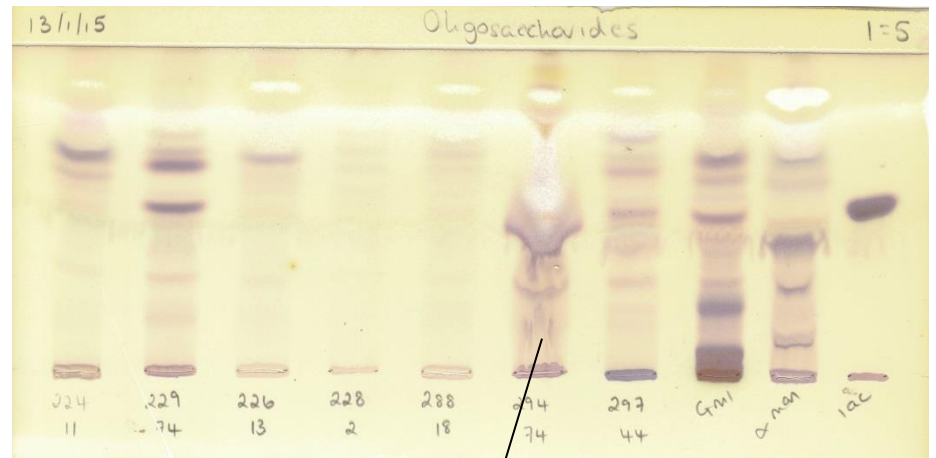
Free sialic acid
 Bound sialic acid
 (Reference urine)



Examples of Thin Layer Chromatography



Bound sialic acid
(Neuraminidase deficiency)

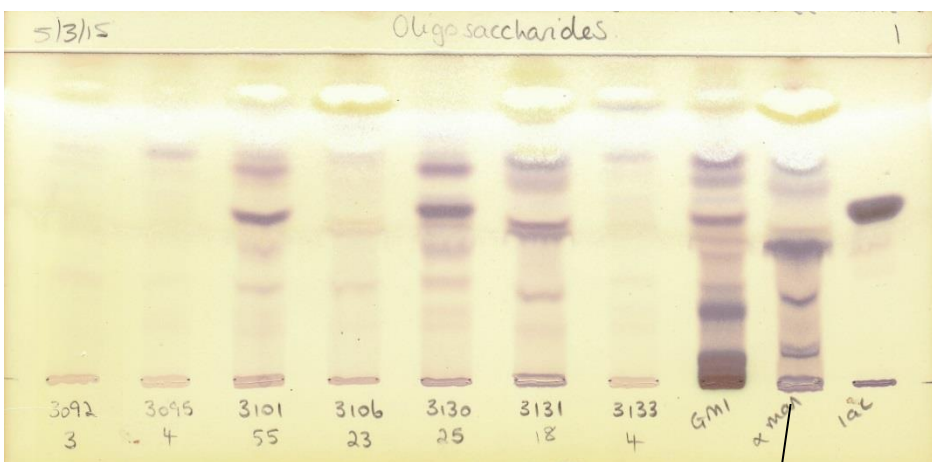


Pattern distorted
? Urinary salts

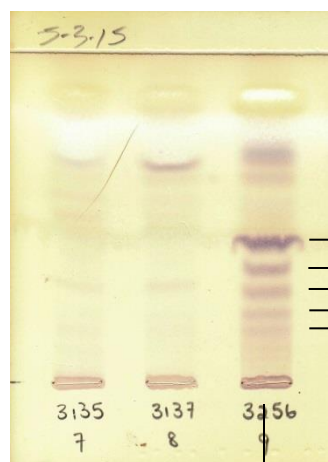


Oligosaccharide plates showing α -mannosidosis

Note the heavy trisaccharide band Laddering pattern is not always seen



α -mannosidosis reference urine

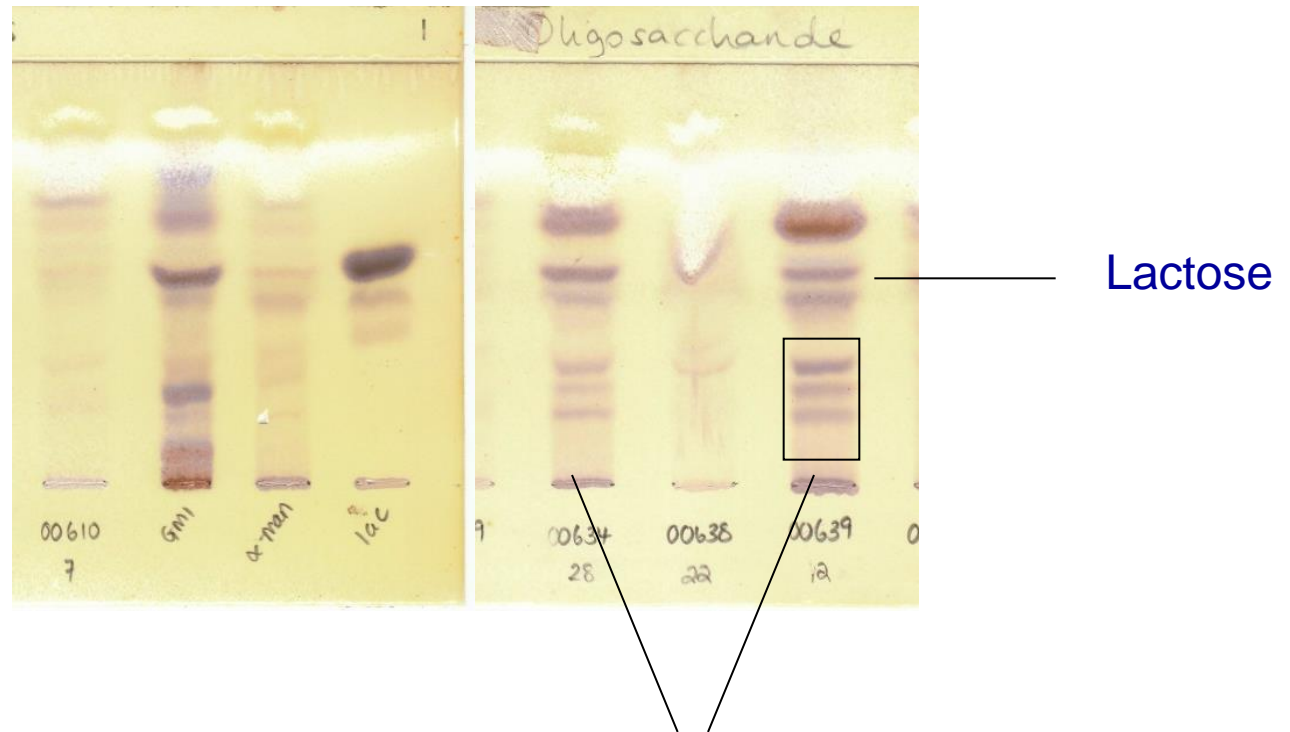


Trisaccharide + classic laddering

Patient sample



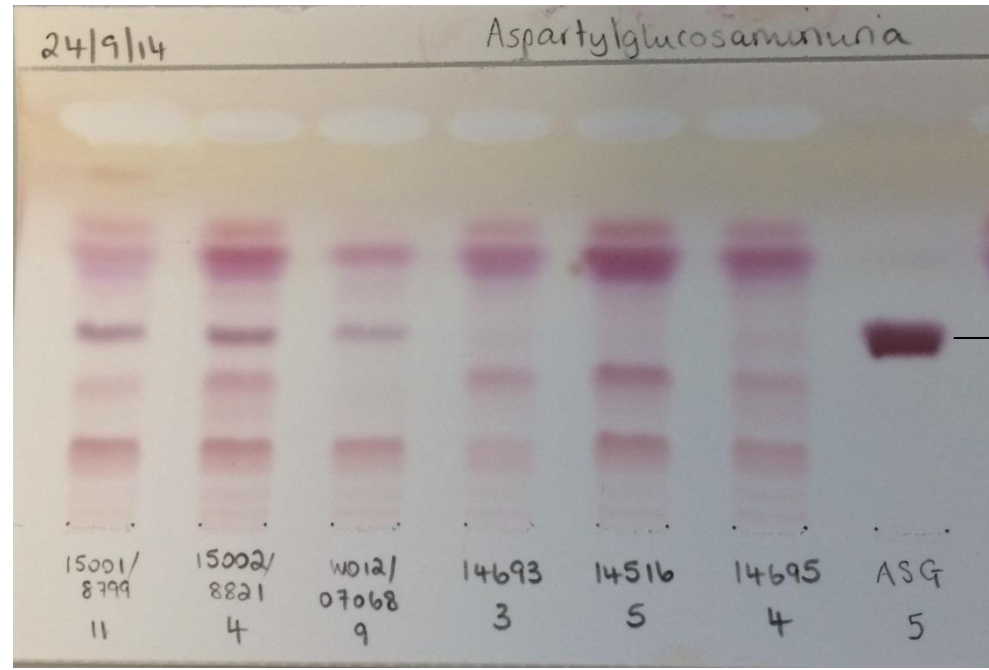
Examples of Thin Layer Chromatography TLC



Oligosaccharides showing pattern often seen in young / jaundiced babies

Examples of Thin Layer Chromatography

Aspartylglucosaminuria



Asg band

Test

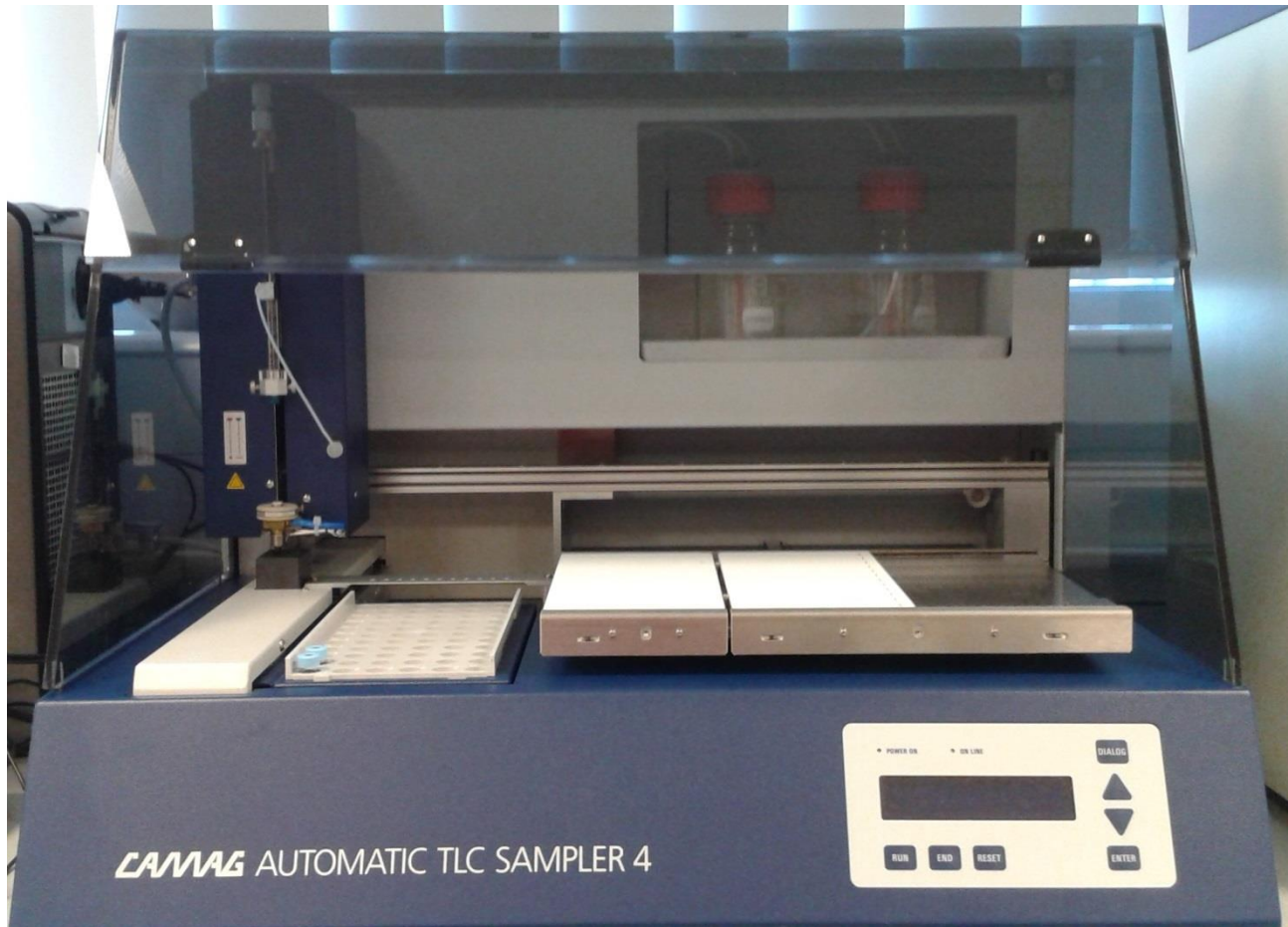
Positive control

Normal

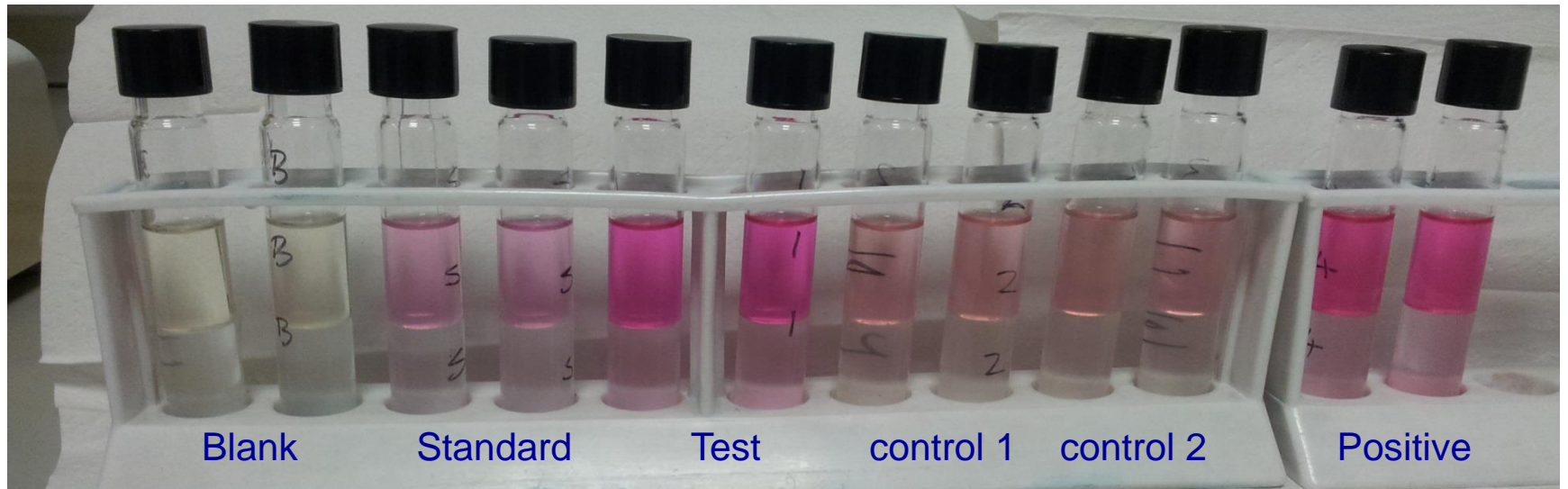
Plates fade quickly



CAMAG TLC Robot



Example of a test tube based assay

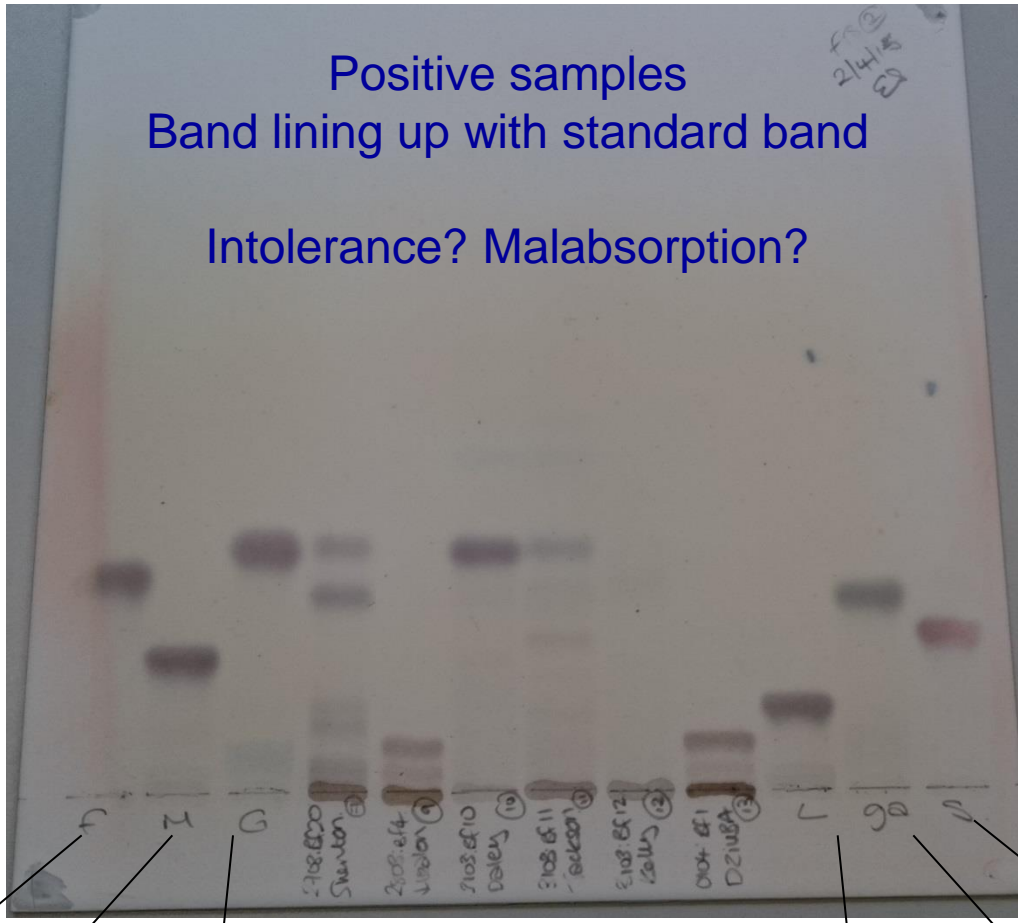


Free sialic acid estimation using a modified thiobarbituric acid assay

Very dark urine can cause assay failure ? Interference

Example of faecal sugar chromatography

Images from Deborah Riley Alder Hey Children's Hospital Liverpool



Fructose Maltose Glucose

Patient samples

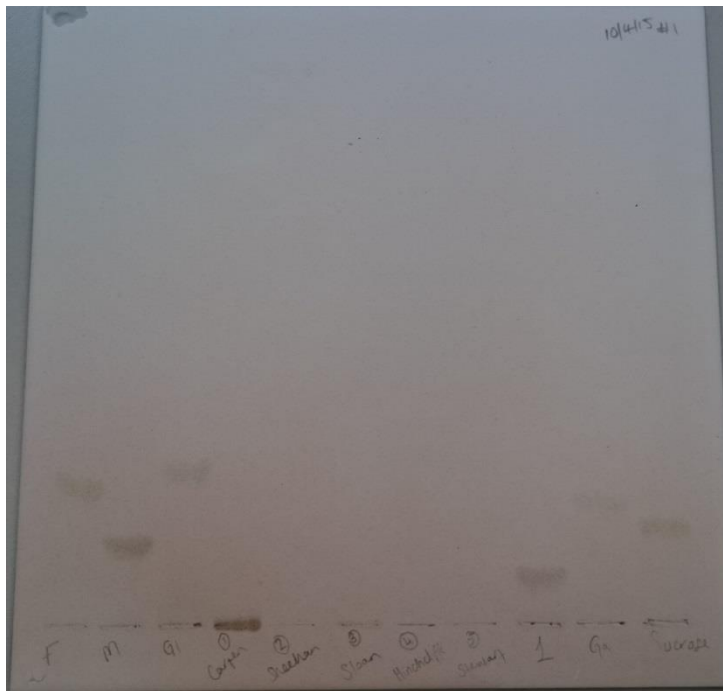
Lactose Galactose Sucrose



Example of faecal sugar chromatography

Problems

Patient samples too dilute



Standards distorted Plate scratched



Images from Deborah Riley Alder Hey Children's Hospital Liverpool

Thank You

