MPS & where we are now - Technical update on new membranes

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MPS screen

NHS
Birmingham Women's and Children's
NHS Foundation Trust

- Analysis of urinary GAGS (Glycosaminoglycans)

- Can also be used for monitoring treatment (ERT)

MPS screen

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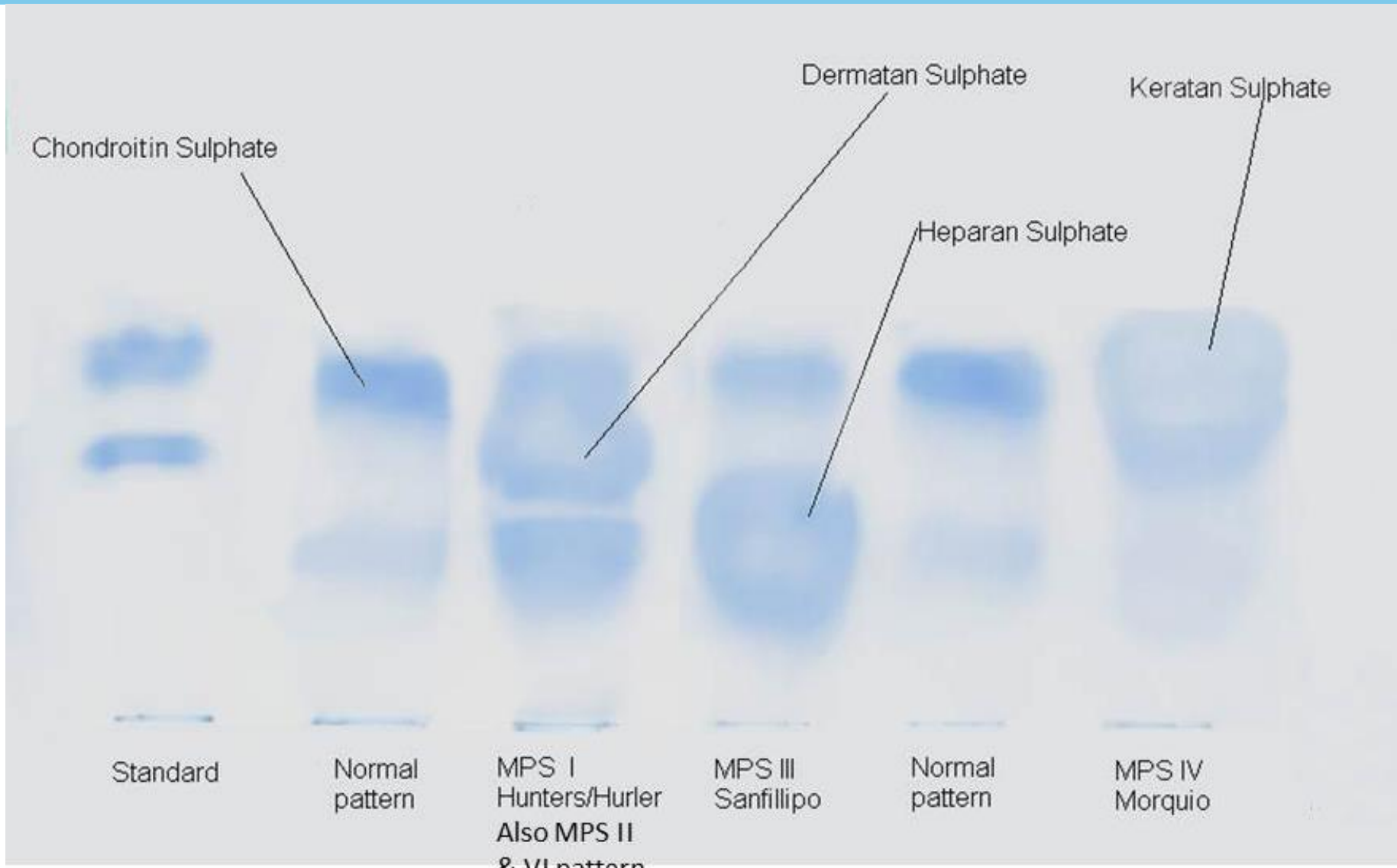

MPS screen

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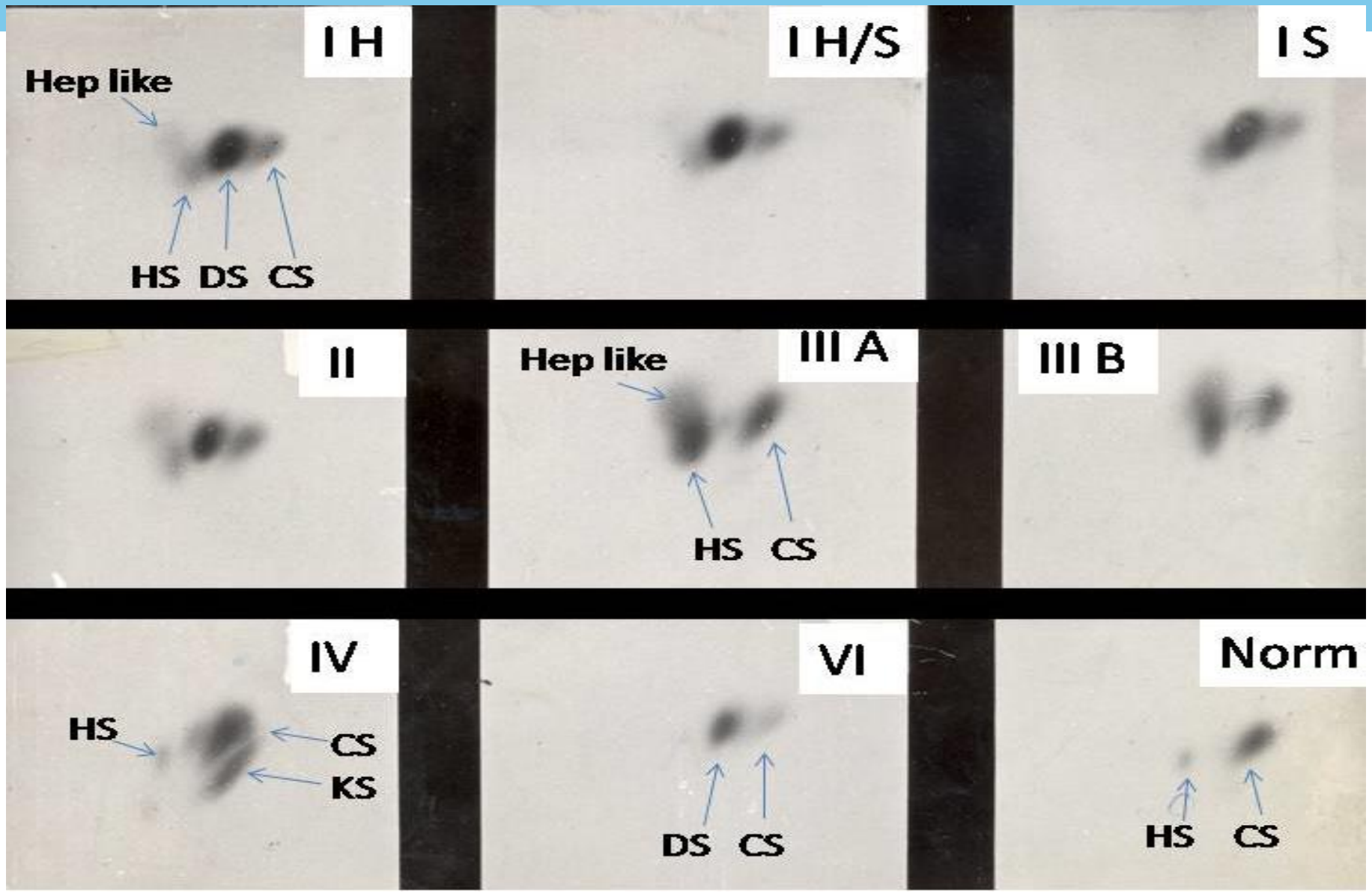

Basic method

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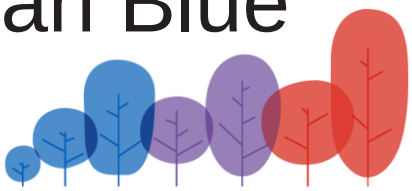
- Extract GAGS from 2ml urine using Alcian blue dye
- Quantification of GAGS using DMB dye
- Apply GAG extract to cellulose acetate membrane and perform electrophoresis
- 2D run routinely at Manchester, 1D at Birmingham
- Allow to dry and then stain with Acian Blue
- Destain with 5% acetic acid



No cellulose acetate

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- Biochrom no longer supply the CA-MEM-S cellulose acetate membranes
- Major issue as Manchester and Birmingham used this product
- Need to source new product

Manchester Feedback

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Supplier	Membrane	Findings
THERMO	Nitrocellulose product 88018	Faint GAG pattern very difficult to destain not suitable for use
BIOCHROM	CA-MEM-S (new manufacturer)	Faint pattern of GAGS difficult to destain
CELLOGEL	CELLASGEL (supplied wet)	Not able to be dried between 1st and 2nd dimensions (in 2D analysis)
CELLOGEL	Dry plates with myelar backing	GAG patterns not consistent
HELENA	Titan III SP membranes	Myelar backing blistered, pattern distortion, GAG ratios not in agreement with usual product
WHATMAN	OE60	Large fragile membranes needed to be cut. GAG spots smaller . Heparan and KS spots difficult to see
CELL START PROJECT	**PRODUCT AN556**	**Validated and in current use**

Manchester Feedback

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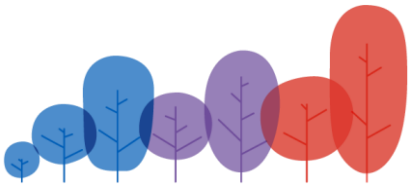
- Tested using previously analysed samples, normal, MPSI and MPS IVA.
- Membranes ran well, stained and de-stained but patterns were FAINT
- Abnormal patterns for MPS II,MPSIII (subtypes A,B and C) were tested again gave FAINT patterns.



Manchester Feedback

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- Increased loading volumes
- >6ul gave poor resolution
- To address this GAGS were concentrated by re-extraction and resuspension in half the usual calculated volume to optimise loading
- With adjusted conditions, comparisons between the CA-MEM-S and Cellstart project membranes showed similar patterns.

Manchester Feedback

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CELLSTART now in routine use at WILLINK

Summary points

1. Orientation may not matter but standardises procedure (cutting off corner)
2. GAG extracts need to be concentrated
3. Membranes suitable for urinary and amniotic fluid GAG analysis
4. Membranes are fragile and require care when handling
5. Each laboratory needs to validate the product for their own service provision

Birmingham Feedback

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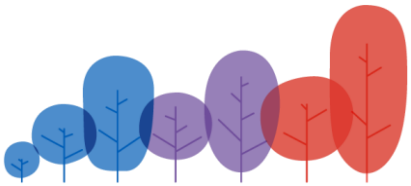
- Birmingham run the screen initially as 1D, follow up abnormals with 2D
- We tried nitrocellulose but poor staining- very blue background
- We have adjusted our protocol – now destain with 0.5% acetic acid (previously water)
- Loading the urines on the paper is more difficult. We have tried wet vs dry
- Staining is poorer/less intense on the new membranes
- Greater chance of missing a MPS IV
- We may have to double the loads on our 2D membranes, and they run and stain well but take longer to dry. We have done this with some EQA samples



Birmingham Feedback

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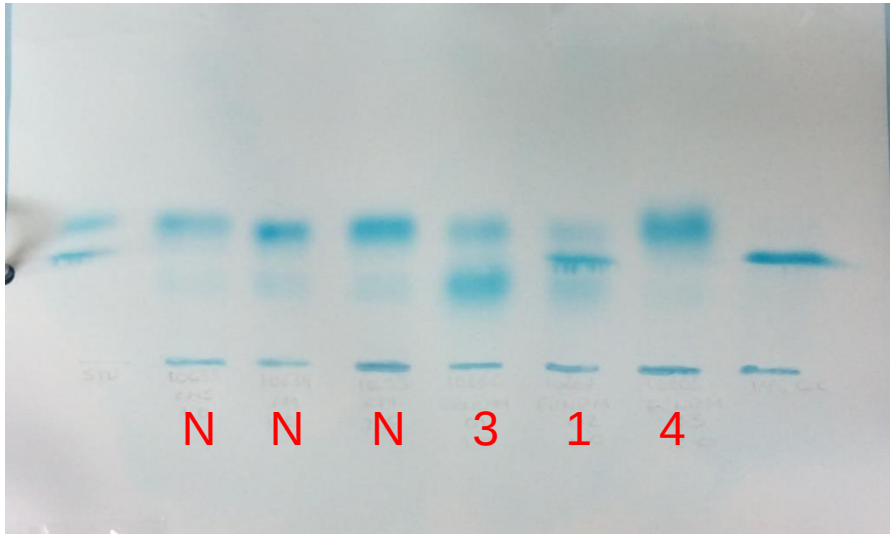
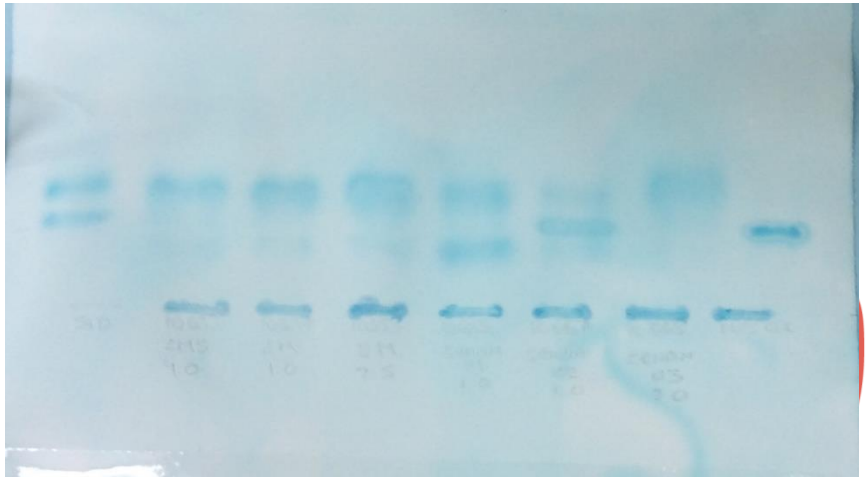


CA-MEM-S
Biochrom membranes
(OLD)

CELLSTART PROJECT
(NEW)



Birmingham Feedback

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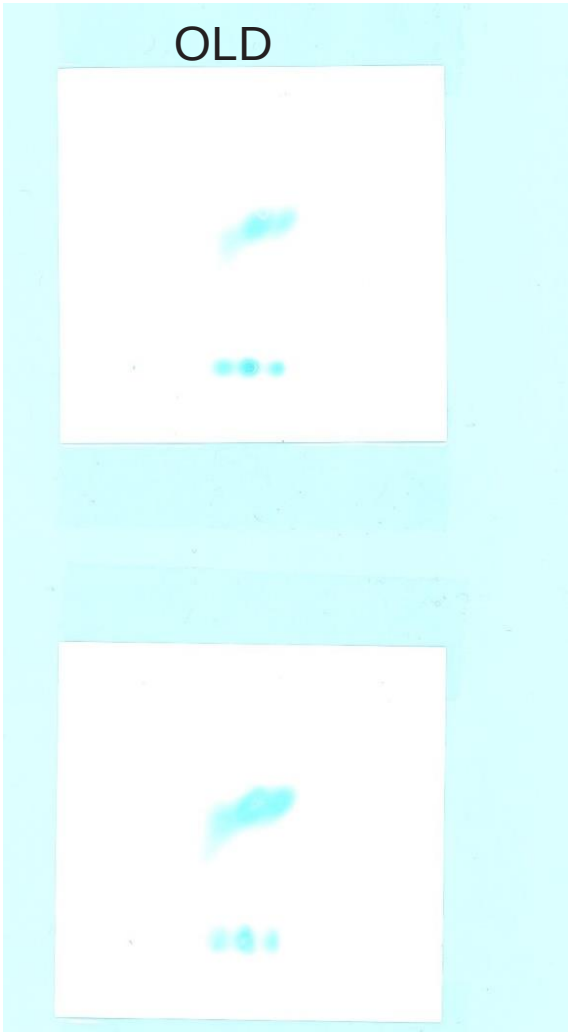

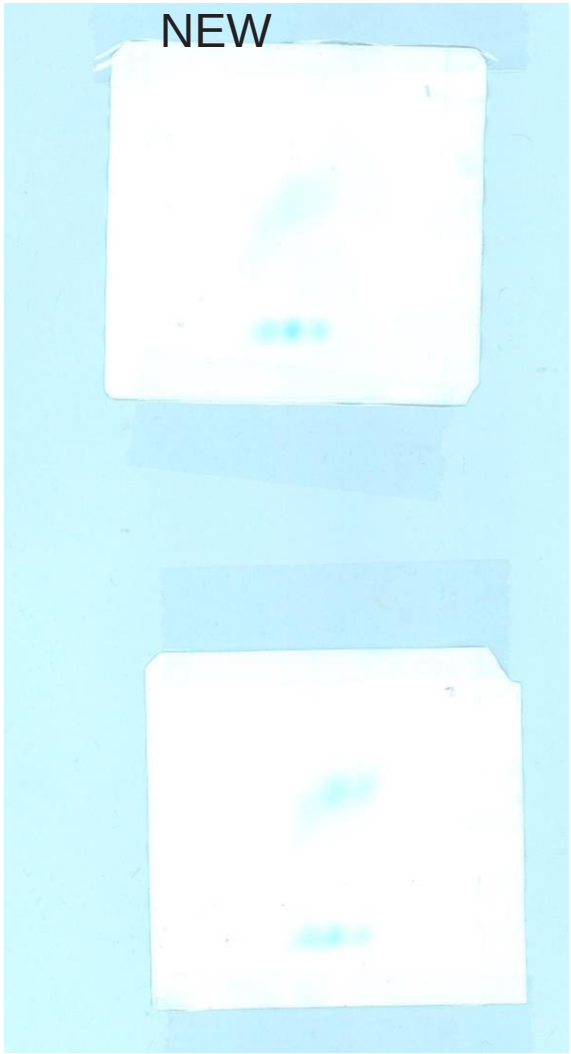

MPSI patient

2D direct comparison

Old paper is much clearer

New paper (fuzzy) And fainter

Birmingham Feedback

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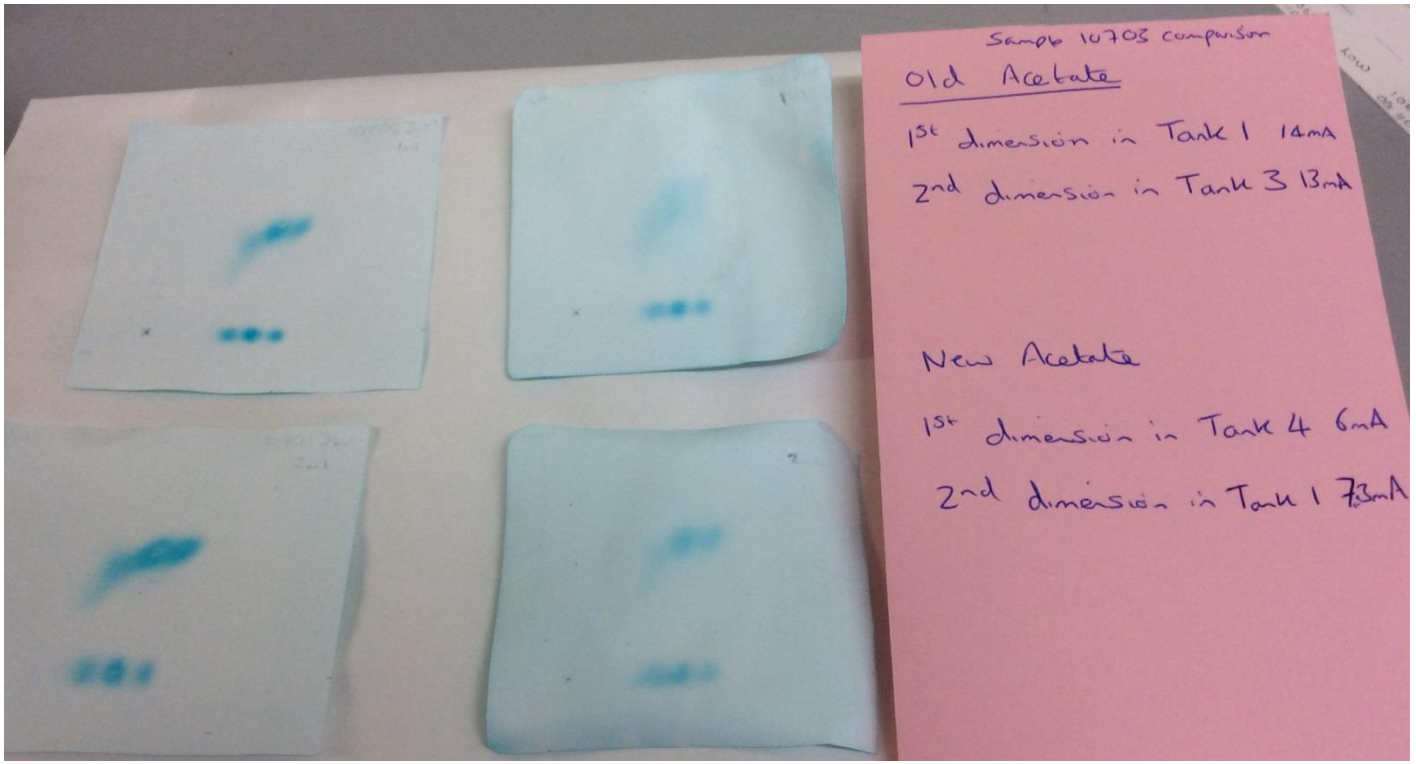

Birmingham Feedback

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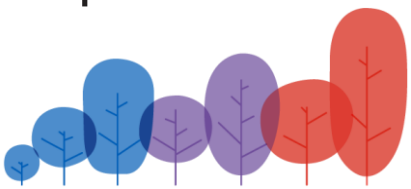
CELLSTART now in routine use at BCH for 1D (still have some old membranes for 2D)

Summary points

1. Not as good as the old membranes
2. New membranes are not consistent in quality
3. On the 1D the bands are not as tight/discreet, more fuzzy
4. Concerns about missing MPS IV (Keratan)
5. 2Ds work better on the new membranes than 1Ds, but not as good as before
6. Doubling the loads for 2Ds
7. Paper suffers from an uptake of dye and there is more background staining, but destain with acetic acid helps
8. Paper brittle when dried



Summary

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- New membranes now in use
- Not ideal, and require more adjustments/ see how it goes- difficult to get samples as patients are mostly on ERT/ HSCT so patterns not as abnormal
- Possibilities of new methodologies for GAGS (TMS)

Ordering Information:-

https://www.cell-startproject.net/en/products