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The protocol outlined here is based on the publication by Humbel & Collart (Clin Chim Acta 1975 60:143-145) and has been used for more than 20 years at Erasmus MC, Rotterdam.

1. REAGENTS

Reference standard mix oligo (0.5 mg/ml)
Dissolve the following compounds in 20 mL water

- 10 mg D(+)-lactose-monohydrate,
- 10 mg maltotetraose,
- 10 mg D(+)-galactose
- 10 mg D(+)-glucose-monohydrate

Eluent

75 ml butanol (Merck P.A.) + 37.5 ml acetic acid (Merck P.A.) + 37.5 ml water

Prepare fresh weekly

Orcinol dip reagent

Dissolve 80 mg Orcine-Monohydrate (Sigma) in 160 ml Acetone.

Add 8 ml concentrated sulphuric acid

Prepare fresh before use

2. MATERIALS

Chromatography tray (inside 20,5 x 8 cm)
Silica 60 TLC-plates, 200 x 200 mm (Merck 1.05721), or half TLC-plates 100 x 200 mm (Merck 1.05626)
CAMAG ATS-4 application robot with software

3. METHODS

3.1 Preparation

Centrifuge urine samples in an eppendorf centrifuge to remove debris
Pipet 0.5 ml urine in a vial and close with a crimpcap

3.2 Volume calculation

Calculate the quantity of urine that should be applied according to the table below. The minimum is 10 µl, the maximum 90 µl

µl urine = (age-dependent factor)/(creatinine [mmol/l])

Table age-dependent factor:

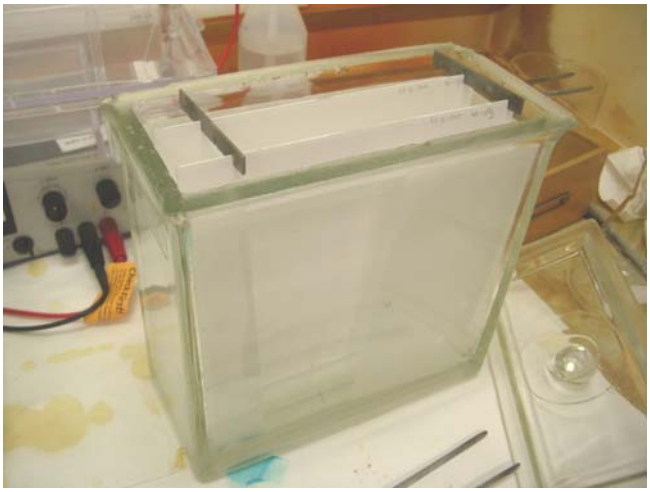
age (y)	factor
0 – 1	75
1 – 2	100
2 – 8	150
> 8	200

3.3 Aplication on TLC-plate

Apply urine samples using CAMAG ATS-4 robot about 1.5 cm from bottom side of the plate. Lanes should be approx. 1.5 cm wide with at least 1 cm open space between lanes. Lane 1 should be 2 cm from the left side and the last lane 2 cm from the right side. Use one lane for standard or apply the standard overlapping with last lane.

3.4 Chromatography

Fill chromatography tray with eluent. Take care that the liquid is below the application line (eluent level about 1 cm high).
Cover inside walls of chromatography tray with filter paper that is soaked with eluent.
Put up to 4 TLC-plates with samples in the tray. Use small notched rods to keep plates upright and evenly spaced.
Put lid on the tray using silicon fat to make an airtight chamber.
Take plates from the tray when the eluent is at or near the top of the plates (5 – 8 h) and dry in fumehood or stove. If required dried plates can be eluted again (to obtain better separation).



3.5 Staining of the TLC-plate

Preheat stove at 100°C.
Dip the dried plates in orcinol dip reagent (prepare fresh) using a flat tray.
Put dipped plates in stove for about 10 min (until colors have developed).
Dry backside of the plates and scan plates for electronic storage.

4. TROUBLESHOOTING

1. When applied volume should have been <10 uL or >90 uL, but instead 10 or 90 uL is applied: interpret with care.

2. When separation is suboptimal for the whole plate: prepare fresh eluent.

3. When separation is suboptimal for 1 sample: apply a smaller volume. Desalting the sample might also help.

4. Dextran hydrolysate may be used as an alternative standard

5. ADDITIONAL TESTING

Analysis of oligosaccharides in urine is a screening method. When an oligosaccharidosis is suspected on the basis of screening results, additional tests may be necessary. Finally, an enzyme test is required to established diagnosis.

1. The ß-mannosidosis storage compound GlcNAc-Man may co-elute with lactose using the system described above. Use the following eluent to separate GlcNAc-Man from lactose (GlcNAc-Man then elutes between lactose and galactose): 127.5 ml 1-propanol + 1.5 ml acetic acid + 22.5 ml water

2. Sialidosis pattern: stain TLC plate with Bial's reagent : dissolve 50 mg Orcine Monohydrate in 12.5 ml water, add 20 ml HCl (37%) and 0.5 ml FeCl3 . 6H2O (1%)
   Alternatively: determine conjugated sialic acid by HPLC or LC-MS/MS

3. Aspartylglucosaminuria pattern: stain TLC plate with ninhydrin
   Alternatively: determine aspartylglucosamine concentration by amino acid analysis