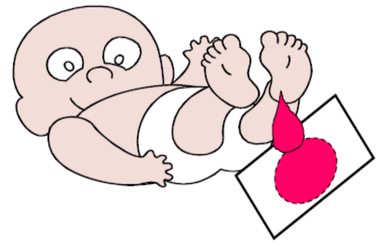
OPTIMISATION OF KERATAN SULFATE SEPARATION BY USING PELTIER SYSTEM FOLLOWING AN IMPROVED RAPID ISOLATION OF URINARY GLYCOSAMINOGLYCANS OF SMALL VOLUME OF URINE SAMPLES



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AIM :The procedure described here allows GAG isolation and high resolution GAG electrophoresis to be easily performed in routine clinical diagnostic laboratories.

INTRODUCTION :It is a modified method based on total GAG concentration in the urine rather than urine creatinine; for optimised isolation of total GAG for electrophoresis; instead of considering the urine creatinine concentration; 300 ug/mL GAG containing urine is considered to be the target concentration for the best precipitation with 1000 µl CPC/citrate buffer. Based on the effect of cold buffer using low voltage; GAGs high resolution electrophoresis (HRE) banding patterns described here enables a clear separation of keratan sulfate (KS) from chondroitin sulfate (CS) and GAG patterns are more clear, easily identified and provide a guide for the enzyme analysis deficient in the MPS disorders.

METHOD

Measurement of total GAG

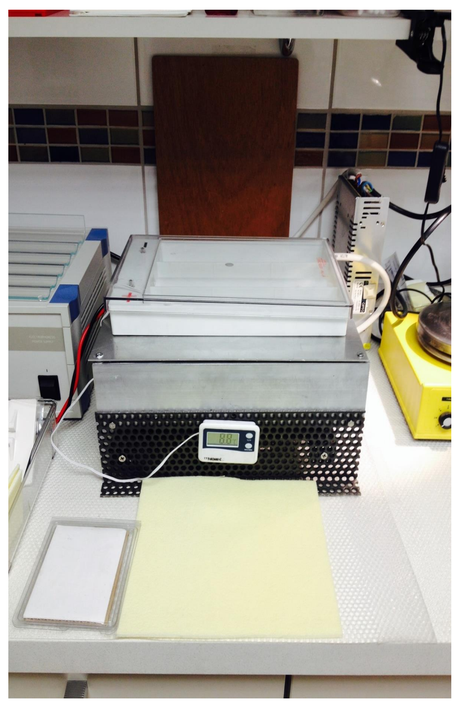
In this study, dimethylmethylene blue (DMB) dye method is used before GAG differentiation based on the method of (De Jong et al -1) Measurements were carried out on microtiter plates at double wavelength 580/690 nm using regression equation polynomial second order.

Isolation of GAGs

Briefly; 1000 µl CPC/citrate buffer was added to 300 µgr GAG containing urine samples. Following incubation at cold temperature (8-10 ºC degree) for overnight , the urine was centrifuged at 10.000 rpm for 5 minutes .The supernatant was decanted and the pellet was dissolved in 2 M lithium chloride and mixed with 800 uL absolute ethanol (1) . The mixture was centrifuged at 12.200 rpm for 5 minutes, supernatant is decanted and the pellet containing GAG s are dried under nitrogen (N2) gas. The final pellet was mixed with 20 µl phenol red 0.5 g/L.

High Resolution Electrophoresis of isolated urinary GAGs

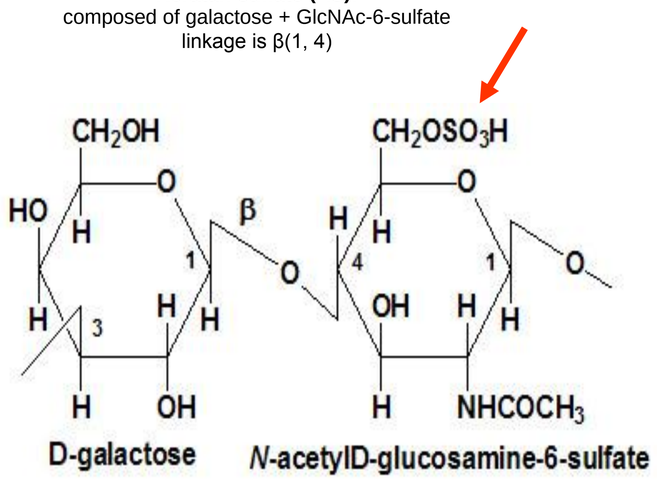
The cellulose acetate (CA) plate (TITAN III 60X76 mm, Helena laboratories) is pre-wett in buffer I (0,025 M Barium acetate (BaAc) ,pH 7.0) in the bufferizer for at least half an hour prior to electrophoresis. After blotting the plate; it is subjected to pre-run to condition the wicks prior to running sample plate for 10 minutes at 130 V. After blotting the plate; 8 µl of prepared specimens are loaded on the sample wells and Z – applicator (3 µL) is used to spot the samples onto the plate through cathode application. Electrophoresis is performed at constant 130 V , 4 mAmp 0-1 watt for 10 minutes , then the CA plate is removed and soaked in cold Buffer II (BaAc 0,025 M, pH 7.0 , 15 % ethanol) for 2 minutes, then it is blotted and re electrophoresed at constant 130 V , 4 mAmp 0-1 watt for 45 minutes. CA is once more removed and soaked in cold Buffer III II (BaAc 0,025 M, pH 7.0 , 50 % ethanol) for 2 minutes. After it is blotted , the CA plate is electrophoresed this time at 130 V , 4 mAmp 0-1 watt for 35 minutes. The main clue in the GAG electrophoresis is provide a cool medium. In order to maintain a cool medium, peltier is used . Peltier according to its structure is a kind of a substance that ; when the voltage is applied ;one side gets cooler while the other gets warmer. Cooling sides of the peltiers are in contact with aliminium plate 20X30 cm where the electrophoresis tank is placed on . The system is installed with 6 peltiers / 30 watt supported by the 380 watt power supply . The temperature of buffer in the electrophoresis tank can be cooled down to 8 - 10 degree centrigrade with this peltier system .
After the last run the plate is soaked in DMB dye.

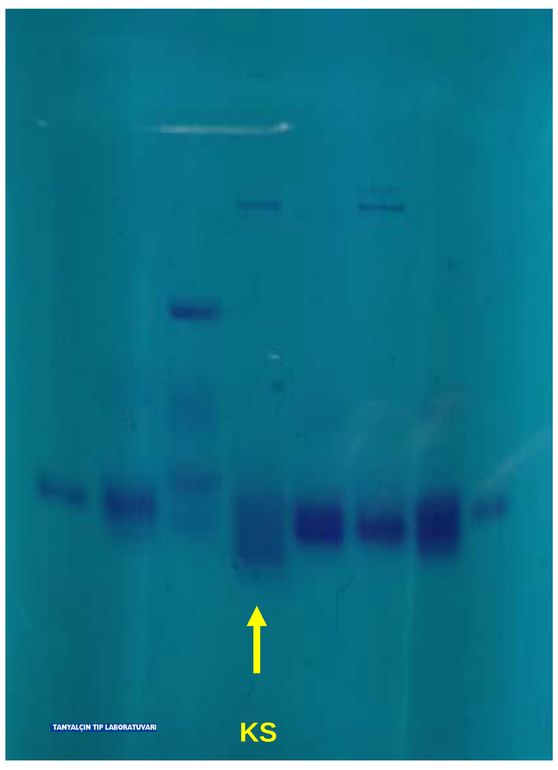


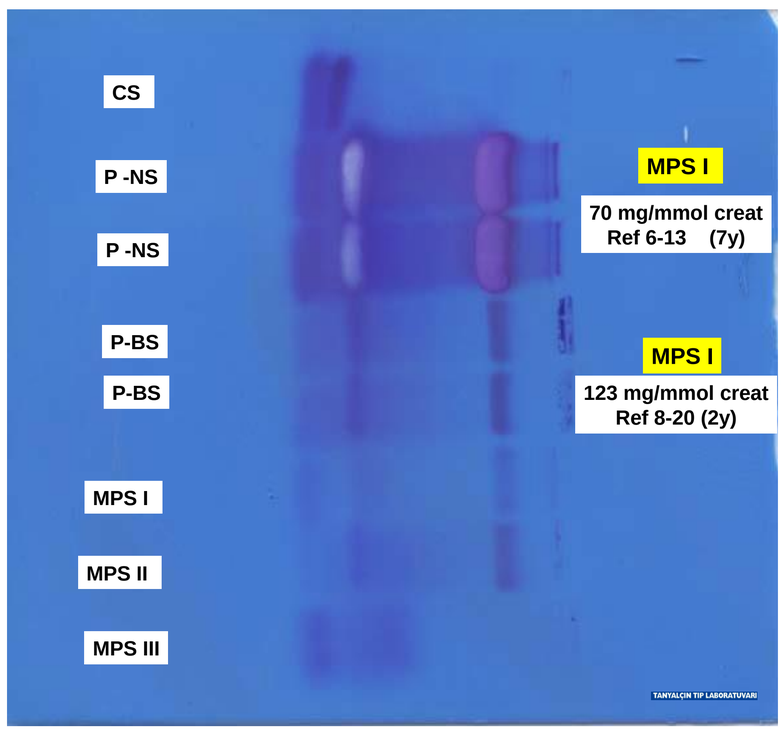
RESULTS

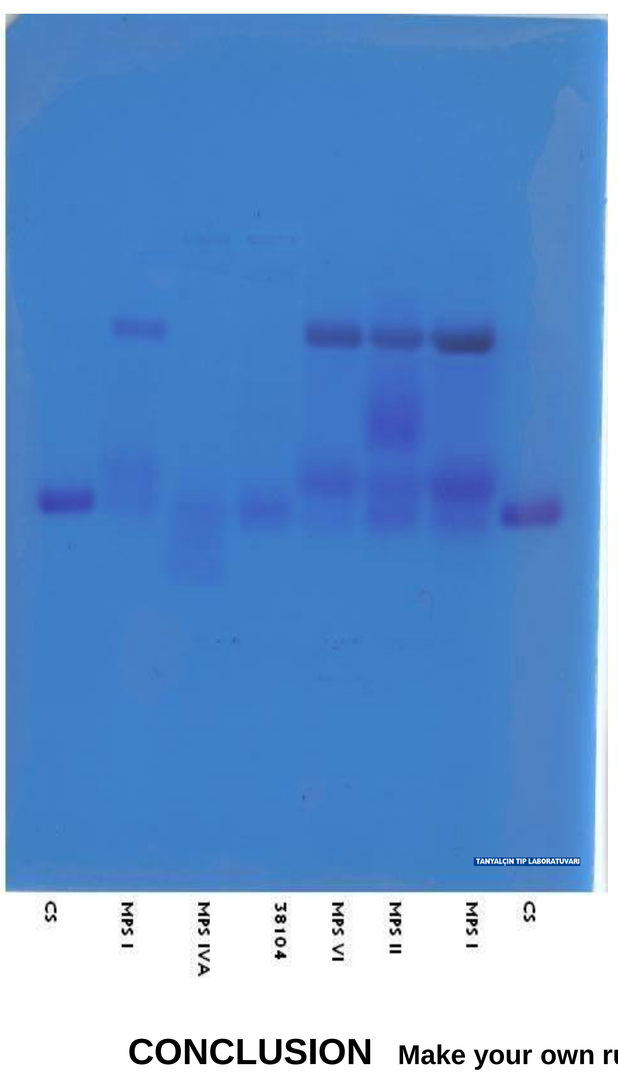
Below the pictures of cellulose acetate plates shows the clear separation of keratan sulfate in Morquio case (MPS IV A) that is from ERNDIM MPS QA Scheme sample (No:15), MPS II case is also from ERNDIM QA Scheme sample (No: 16). P means a patient sample, CS is the chondroitin sulfate standard included in that analytical run.

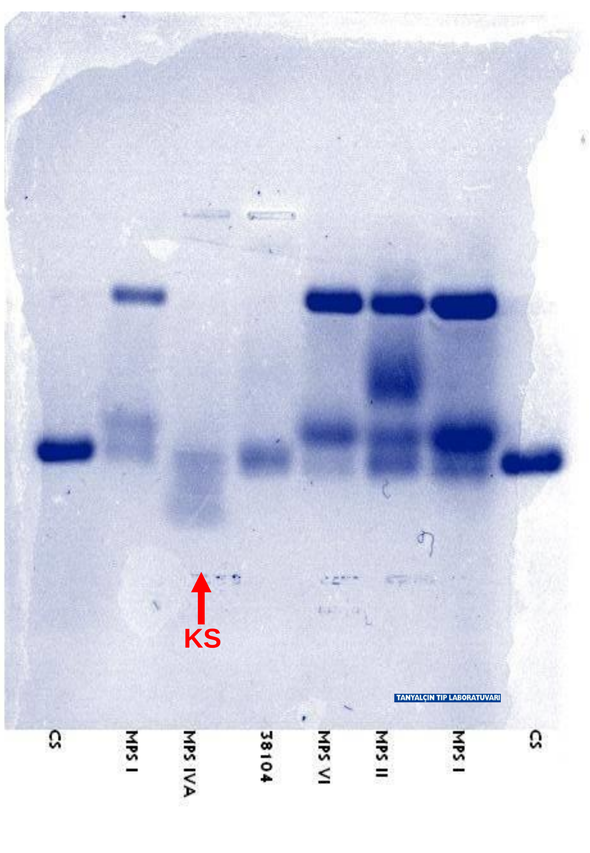
Keratan sulfate (KS):
composed of galactose + GlcNAc-6-sulfate
linkage is β(1, 4)

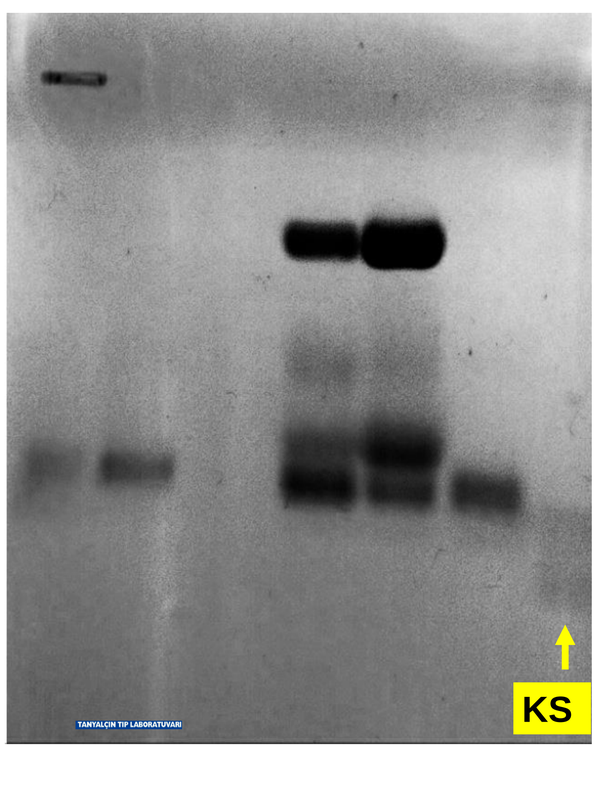




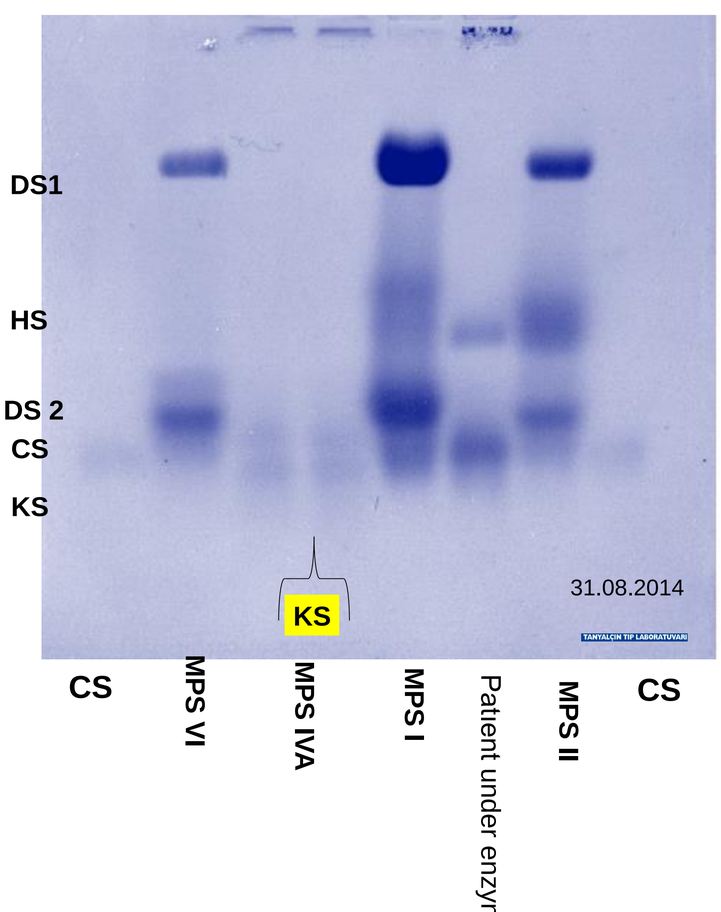








Quality Control Profile with Confirmed MPS case samples from REAL LIFE STUDY



CONCLUSION **Make your own rules for optimisation for the GAGE (GAG electrophoresis)**

1-Make accurate measurement of total GAG
2-Try to be realistic with the real life samples
Small volume of urine samples Standardisation CPC – GAG precipitation
300 ug/mL GAG – 1000 uL CPC
3- Buffer molarity , pH , Voltage , Temperature must be optimised according to the system used : COLD medium for Keratan Sulfate better separation
4-Make accurate interpretation of lab results (less false positives and NO!!! false negatives)

REFERENCES : Hopwood JJ & Harrison JR Analytical Biochemsitry (1982),119,12-128
Nor Azimah Azize et al.Malysian J Pathol (2010) 32:1; 35-42

Acknowledgment :
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