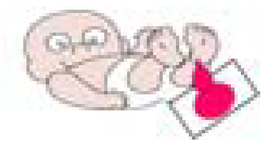
AN IMPROVED RAPID ISOLATION OF URINARY GLYCOSAMINOGLYCANS OF SMALL VOLUME OF URINE FOR HIGH-RESOLUTION ELECTROPHORESIS FOR ISOTYPING OF MUCOPLOYSACCHARIDOSIS: AN EXAMPLE WITH MPS VI - MAROTEAUX-LAMY



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AIM

This study presents a rapid isolation method for urinary glycosaminglycans (GAG) from a small volume of urine to perform GAG electrophoresis.

INTRODUCTION

The urine glycosaminoglycan (GAG) measurement for patients with mucopolysaccharidosis (MPS) is considered to be the first step for diagnosis of those heterogenous group of lysosomal storage disorders presenting clinical phenotype.

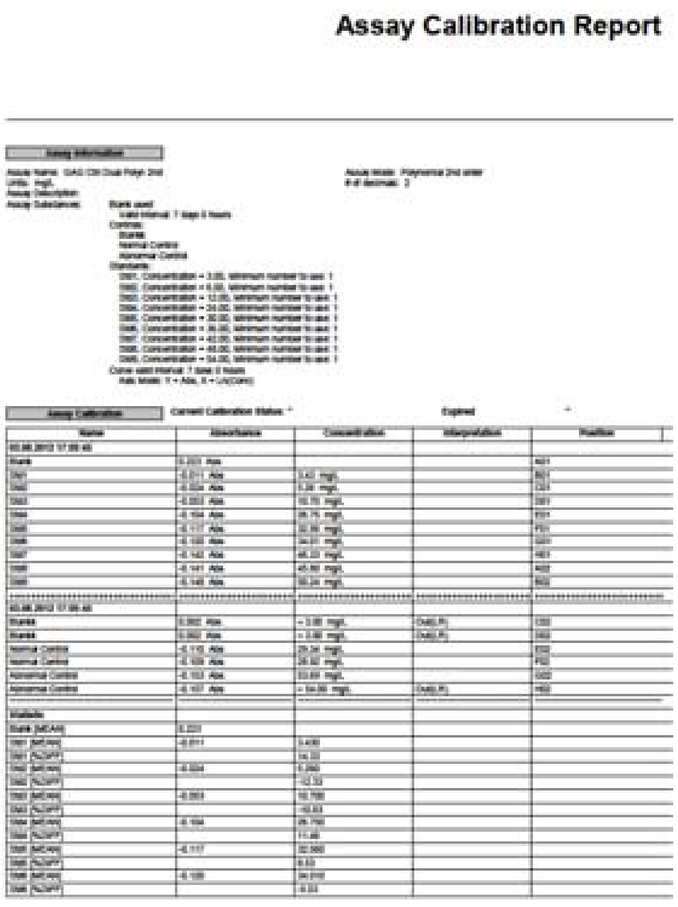
MATERIAL & METHOD

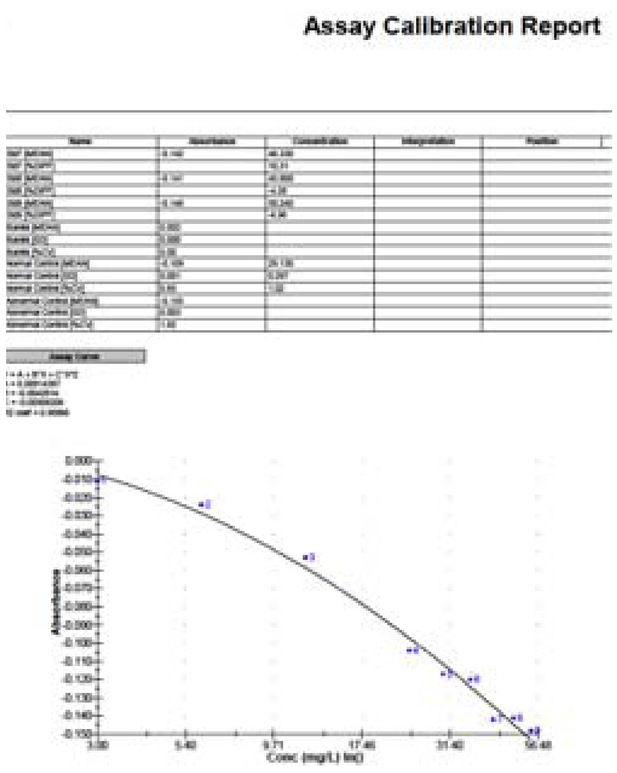
Patient information

Male , date of birth :16.12.2007, diagnosed as MPS VI at 22.02.2011. Clinical picture has coarsening of the facial features;gibbus and claw–hand deformity without organomeagly and goiter.There is no consanguinity marriage with 2 male and 1 female siblings alive and 1 died male sibling due to mucopolysaccaridosis.

Measurement of GAG

In this study, dimethylmethylene blue (DMB) dye method was used before GAG differentiation. Measurements were carried out on microtiter plates at double wavelength 580/690 nm using regression equation polynominal second order.





METHOD

Isolation of GAG

1000 µl CPC/citrate buffer was added to 300 µgr GAG containing urine. Following encubation at room temperature for 15 minutes , the urine was centrifuged at 12.200 rpm for 5 minutes .The supernatant was decanted and the pellet was dissolved in 2 M lithium chloride and mixed with absolute ethanol.The mixture was centrifuged at 12.200 rpm for 5 minutes, supernatant was decanted and the pellet containing GAG s was dried under nitrogen (N_2) gas. The final pellet was mixed with 50 µl phenol red 0.5 g/L. 8 µl of prepared specimen was loaded on the sample wells and Z –applicator (2 µL) was used to load onto the cellulose acetate plates (TITAN III 60X76 mm) and subjected to high resolution GAG electrophoresis.

RESULTS

1-The linear regression graph shows massive excretion of GAG for MPS VI case ; 1387 mg/g creatinine ; reference value 68-188 ; when compared to other patient samples. (Figure 1)
2-GAG electrophoresis shows large increase of dermatan sulfate compatible with a mucopolysaccaridosis type VI - Maroteaux-Lamy. (Figure 2)
3-Arylsulphatase B activity on dried blood spot was found to be as 0.13 µmol/L/h (reference value : 3.33 – 14.89 µmol/L/h). The arylsulphatase activity is below 10% of the mean of the normal population (<0.86 µmol/L/h).This result is compatible with MPS VI (Maroteaux-Lamy).
4- After treament with Naglazyme (galsulfase) for 9 months, there is a marked decrease in dermatan sulfate excretion at follow-up GAG electrophoresis.

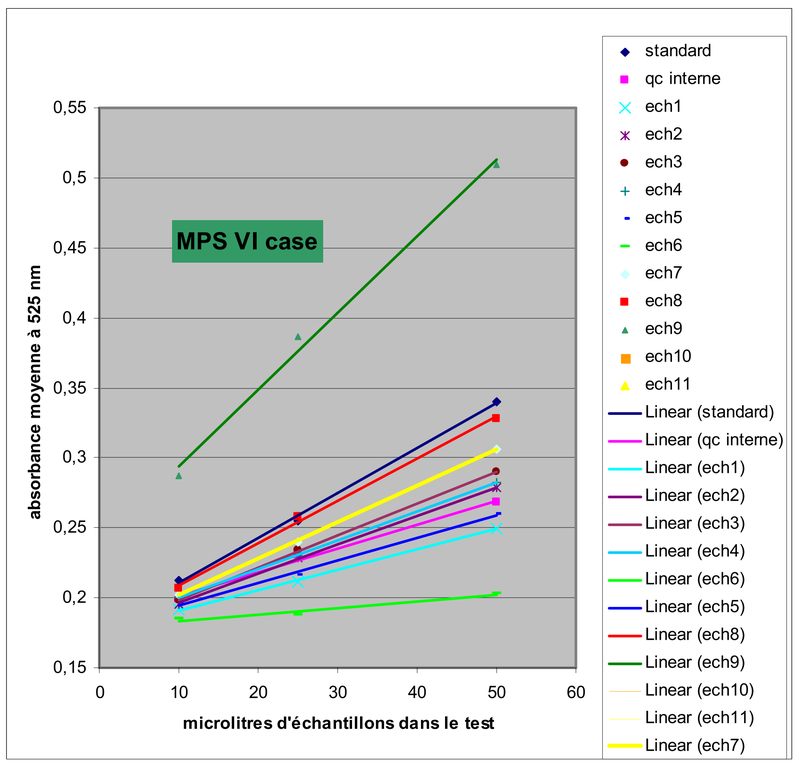


Figure 1

CONCLUSION

300 microgram of GAG is the most appropriate concentration that gives the best precipitation and clear pattern of high resolution GAG electrophoresis following high speed centrifugation at 12,200 rpm.
This procedure allows GAG isolation and high Resolution GAG electrophoresis to be easily performed in routine clinical diagnostic laboratories.

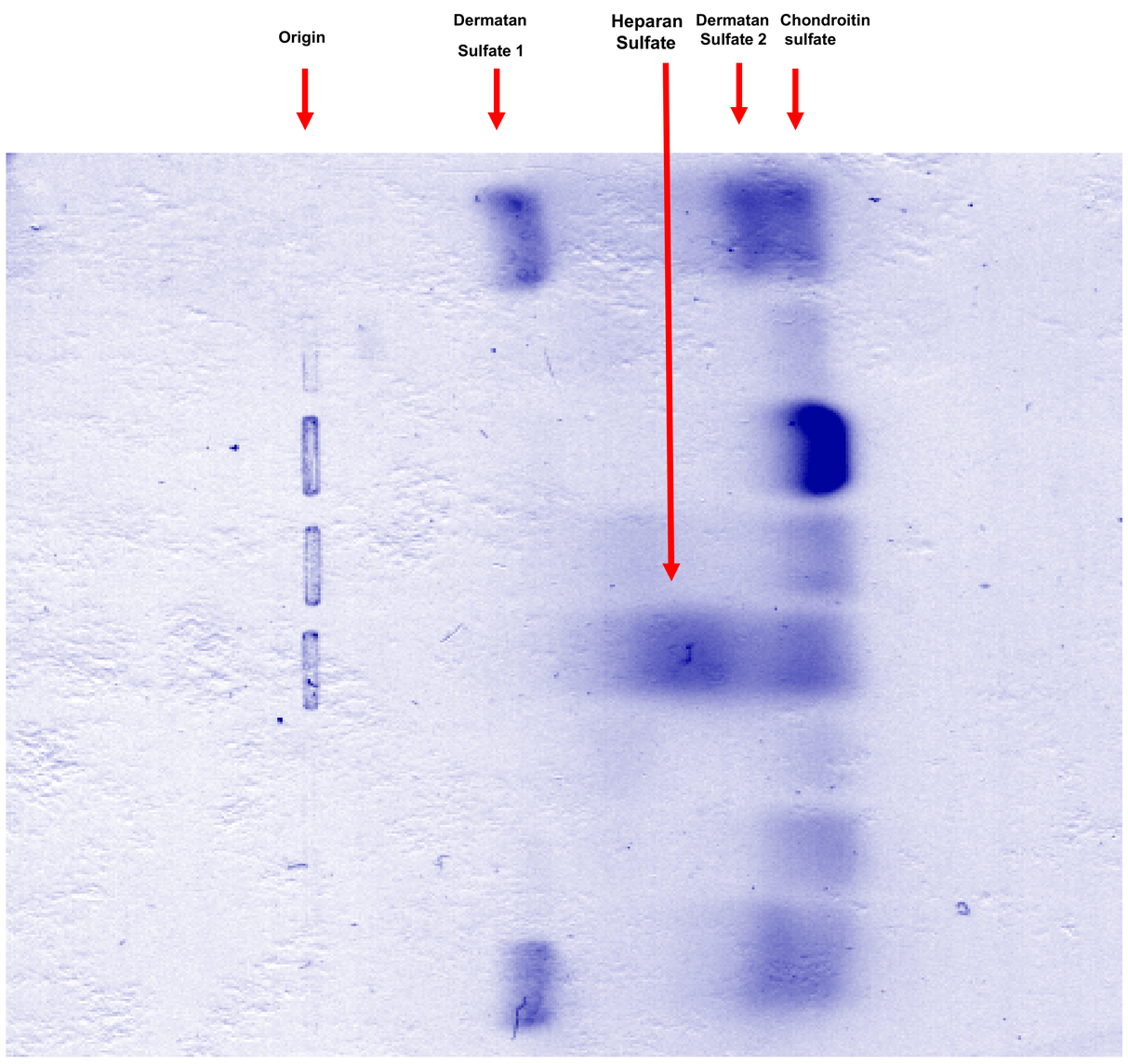


Figure 2