

Report of the Higher Specialist Trainee Workshop on Mucopolysaccharide and Oligosaccharide Analysis

Birmingham Childrens Hospital 17th November 2008

Introduction

This workshop was organised by Daniel Herrera and Donna Fullerton who are Higher Specialist Trainees in Paediatric and Inherited Metabolic Disease. It was specifically aimed at people in HST posts and in post-registration training in Inherited Metabolic Disease. The aim was to review the services within MetBioNet for mucopolysaccharide and oligosaccharide analysis and to provide informative lectures on the analytical aspects of these tests. Lectures were also given on the clinical aspects of their diagnosis and future developments in laboratory diagnosis. Prior to the meeting all MetBioNet laboratories involved in mucopolysaccharide and oligosaccharide analysis were sent a questionnaire asking for information on workload, methodologies used, reference data, policies, strategies for testing and problem areas of analysis.

Ten people in training posts attended the meeting including HSTs, post-registration trainees in Metabolic Disease and a Metabolic Registrar as well as the invited speakers.

Results of the Questionnaire

A total of 12 laboratories responded (75%) out of the 16 sent questionnaires.

Mucopolysaccharide Analysis

Daniel Herrera (Birmingham) summarized the results from the 12 centres in the UK providing a laboratory service for urine mucopolysaccharide analysis.

The workload can be split into three groups:

- 1- Two laboratories with a very high workload (about 1200 samples per year)
- 2- Seven laboratories offering a service with a workload between 280 and 650 samples per year
- 3- Three laboratories with a workload between 100 and 190 samples per year.

The majority of laboratories analyse all the samples requested by clinicians without a filtering system. One laboratory only analyses samples without prior discussion if the patient is less than 3 months of age. One laboratory filters the samples based on the clinical information provided by the clinician.

Ten laboratories use the DMB method for GAG quantitation, one is currently working on its development and one laboratory is solely using a separation technique. Seven centres use in house reference ranges and 3 centres use reference ranges from other centres or from the literature.

Six laboratories use the results of GAG quantitation to filter the samples to decide whether they will be subjected to a separation technique. Four laboratories use this information either to load specific amounts of GAG on electrophoresis, to aid in interpretation or to monitor ERT therapy.

The majority of the laboratories use 1D and/or 2D cellulose acetate electrophoresis as a separation technique. The centres using 1D electrophoresis (6) as a first analytical approach, use 2D electrophoresis or enzyme analysis as a second line test if there is an abnormal pattern on 1D electrophoresis or if there is a clinical suspicion of Morquio's disease. One laboratory uses TLC as its separation technique for GAG analysis.

Conclusions

- 1- Six laboratories rely on GAG quantitation as a first line test before using any separation technique. Different criteria are used as some laboratories filter as many as 90 % of the samples while others filter about 50%. The laboratories with more experience and a higher workload do not rely on quantitation to filter samples. They use a separation technique in conjunction with GAG quantitation or solely a separation technique for analysis of the samples.
- 2- All laboratories performing GAG quantitation use the DMB method but with some differences in methodology. The reference ranges provided from the different laboratories vary substantially which emphasises the need to use in-house reference ranges.
- 3- The majority of the laboratories acknowledge the limitations of 1D electrophoresis in the diagnosis of Morquio's Syndrome (MPS IV) and Sly's Syndrome (MPS VII). 2 D electrophoresis is regarded as a better separation technique but takes longer to perform and requires a lot of tanks to run large numbers of samples.

Oligosaccharide Analysis

Donna Fullerton (Birmingham) summarized the results from 10 centres in the UK providing a laboratory service for urine oligosaccharide analysis. The questionnaire was focused on the analytical aspects of oligosaccharide analysis.

Only eight laboratories perform oligosaccharide analysis with the remaining two referring the samples to another centre. That centre acts as a major referral centre for these other laboratories analysing approximately 1300 specimens per year accounting for 41 % of the total MetBioNet workload. There were three other main centres where large numbers of samples were analysed with workloads of 393, 500 and 956 requests per annum.

Most laboratories analysed the majority of samples received. However one laboratory analysed all samples from their own hospital but required prior arrangement for samples from other hospitals or clinics in their Region. Another refused requests on patients on whom oligosaccharide analysis had been previously performed. Only one laboratory filtered all the requests on the basis of clinical details.

The majority of laboratories spotted the urine directly onto chromatography plates. One laboratory however desalted and centrifuged their samples, using the supernatant for chromatography.

One laboratory rejected samples as too dilute for analysis on the basis of creatinine concentration (<1.0 and <0.6 mmol/L cited as suitable cut-off limits) and/or pH to identify infection (pH >8.0 or >8.5 cited as cut-off limit). Three laboratories had no rejection criteria but added suitable interpretative comments.

All laboratories used thin layer chromatography as the separation technique utilising orcinol/sulphuric acid and resorcinol/copper sulphate for staining unsialylated and sialylated oligosaccharides respectively.

All laboratories ran control material with patient samples, with the majority running both normal and positive controls. In all cases pooled urine from unaffected patients was used as a normal control although the positive control did differ between centres with laboratories using a GM1 Gangliosidosis urine, previous EQA samples or human milk. Four laboratories, in addition to running controls, also ran standards with the majority using lactose.

Interpreting the results of oligosaccharide chromatography can be difficult and the centres cited faint bands, dilute samples, drug interference, or dietary influences (glucose and/or other sugars) as the main causes of repeat requests. However only one centre reported a high rate of repeat requests (17%). All laboratories are aware that breast-fed infants can produce oligosaccharide patterns very similar to those observed in patients with alpha mannosidosis and so in these circumstances will request repeat samples. They were also aware that patients with less severe forms of the oligosaccharidoses may not show an abnormal pattern or that the excretion of abnormal oligosaccharides may be variable. In this case they would recommend enzyme analysis.

Due to the lack of an external quality assurance scheme for oligosaccharide analysis no information could be gathered on this subject, although the majority of laboratories participate in the ERNDIM Proficiency Scheme.

Conclusions

1. Eight metabolic laboratories perform oligosaccharide analysis but there only four main centres were identified as performing the test. However some of the laboratories send samples to the major laboratory performing this test.
2. The techniques used for oligosaccharide analysis are more or less standardised throughout the laboratories, with all laboratories using thin layer chromatography with orcinol/sulphuric acid and resorcinol/copper sulphate for staining unsialylated and sialylated oligosaccharides respectively.
3. All laboratories are aware that interpretation of oligosaccharide chromatography can be difficult because of dietary and drug interferences and that some patients may not show clear abnormalities.

After discussion it was agreed to circulate Daniel and Donnas presentations to the laboratories responding to the questionnaires for comments. In particular they were asked to say if they would result in any changes in practice. The results are expected in the New Year and will be reported separately along with their presentations.

GAG Quantitation and Electrophoresis –The Science, Interpretation Pitfalls and Quality Assurance

George Gray from Birmingham Childrens Hospital talked on the analytical aspects of mucopolysaccharide analysis. He discussed the basic science behind GAG quantitation and the separation techniques. He described the different types of abnormal patterns seen, problems with interpretation as well as both internal and external quality assurance.

Oligosaccharide Analysis-the science, interpretation and pitfalls

Margaret Thornley from the Willink Unit at Manchester Childrens Hospital talked about urine oligosaccharide analysis. She discussed the various methods available for oligosaccharide separation but concentrated on thin layer chromatography – the method of choice in all MetBioNet laboratories. She described the principles behind the method, the technical aspects and the sort of patterns one sees in patients with the various disorders of oligosaccharide metabolism. She also pointed out the pitfalls and limitations of this technique.

Clinical aspects of the mucopolysaccharidoses and oligosaccharidoses for biochemists – “Clues to early diagnosis”

Dr Anupam Chakrapani gave an overview of the clinical features of the mucopolysaccharidoses and oligosaccharidoses, particularly from the point of view of early symptoms. He stressed the need for early diagnosis now that more treatments are available.

Analysis of mucopolysaccharides and oligosaccharides – Future methods for analysis

Joan Keutzer of Genzyme Corporation gave a talk on future developments for diagnosing the mucopolysaccharidoses and oligosaccharidoses. The group all agreed that existing methods had their limitations as the technology was old and not suitable for rapid turnaround times or for mass work output. Joan reviewed alternative methods for diagnosing these disorders. These included bypassing the GAG or oligosaccharide quantitation by mass analysis of dried blood spots for a range of lysosomal enzymes. She also briefly discussed the quantitation of MS-MS of GAGs in plasma as well as urine both as a diagnostic test and for monitoring ERT.

Daniel Herrera, Donna Fullerton and George Gray