DIAGNOSTIC TESTS IN URINE FOR OLIGOSACCHARIDOSES

Glycoproteins and glycolipids contain covalently linked oligosaccharide side-chains. Oligosaccharidoses are caused by defects in lysosomal enzymes that are involved in the degradation of these glycan side-chains. Screening for these disorders occurs by detection of abnormal storage products (specific oligosaccharides) in urine. Currently, two approaches are generally used for screening these diseases: (i) conventional thin-layer chromatography and (ii) novel mass spectrometry-based approaches.
This document summarises a number of diagnostic tests commonly used to investigate oligosaccharides in urine aiming at identification of Oligosaccharidosis patients. Separately, a detailed protocol for oligosaccharide analysis by TLC is available from the ERNDIM website. Detailed protocols of mass spectrometry – based approaches are not included here; these can be found in the literature references provided.

1. ONE-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY (TLC)

Qualitative screening of oligosaccharidoses is performed by loading of native urine samples on silica gel plates, separation by thin-layer chromatography (TLC) and visualization by sulfuric orcinol staining. TLC plates are investigated for the presence of abnormal oligosaccharide patterns [1].
This traditional method is still used by the majority of diagnostic IEM laboratories. It is relatively easy to implement and does not require expensive chemicals or major capital investments, although application of urine sample onto the TLC plate can be cumbersome and, ideally, is performed by a robot applicator (see separate TLC protocol). Some modifications of the 1-dimensional TLC method have been proposed, but do not seem to be used widely [2, 3].
Interpretation of oligosaccharide excretion patterns by TLC is challenging and requires extensive experience, due to (1) the qualitative nature of the screening method, (2) its limited chromatographic resolution, and (3) low specificity due to drug- and nutrition-induced artefacts. Moreover, oligosaccharidosis are extremely rare disorders and laboratory specialists very infrequently encounter positive samples. Experience with positive samples is necessary to recognize pathological oligosaccharide patterns in screening samples.
A major pitfall of the TLC method is interference by milk oligosaccharides received from the mother via breast feeding or infant formula in newborn patient urines which may obscure pathological oligosaccharides and complicate interpretation. Another difficulty is that urinary oligosaccharide excretions decrease with age, which makes interpretation of screening results in older patients potentially more difficult than in children. Also, the sensitivity for some disorders is not optimal, e.g. GM2-gangliosidosis.
TLC can also be used for the detection of free sialic acid or sialyl-oligosaccharides. However, given the modest increase in sialic acid in some sialic acid storage disease patients, and the variability between samples, we recommend that sialic acid is measured by LC-MS/MS.

2. MASS SPECTROMETRY – BASED APPROACHES

To overcome the challenges and pitfalls of TLC, recently a number of reports have been published describing analysis of oligosaccharides by mass spectrometry:

- LC-MS/MS [4, 5, 6, 7]
- High-resolution MS [8, 9, 10]

The first LC-MS/MS method, published by Piraud et al [4], was based on original work using MALDI-TOF [9]. This method has high sensitivity and specificity and can also be used for prenatal testing in amniotic

fluid. Subsequently other labs have published LC-MS/MS methods. A method described by Semeraro et al includes the glycogen-derived tetraglucoside [5], while Mak & Cowan reported a method additionally including biomarkers of mucopolysaccharidoses, NGLY1 deficiency and MOGS-CDG [6]. Whereas the above mentioned approaches detect underivatised oligosaccharides, a method described by Huang et al [7] uses 4-aminobenzoate to derivatise oligosaccharides prior to LC-MS/MS analysis. Derivatisation increases sample processing time, but may lead to better ionization and detection.
High-resolution MS is a powerful technique to detect complex oligosaccharides, some of which have rather high molecular weights. Early reports used MALDI-TOF [8, 9], while more recently Hagemeijer et al reported a method using UHPLC-ESI-HRAM-MS (Orbitrap MS) [10]. In the latter method quantitative detection of sialic acid was included to facilitate diagnosis of sialic acid storage disease. A powerful feature of the method from Hagemeijer et al [10] is a bioinformatics pipeline producing z-score plots that are very easily interpreted. Such a pipeline can be incorporated in any MS platform producing (semi-)quantitative values.
A limitation of the current MS-based methods is that isotope-labeled internal standards of the oligosaccharides investigated are not commercially available and, hence, the values produced are at best semi-quantitative. This is not a problem to detect the usually grossly abnormal oligosaccharide excretions. Once therapy monitoring needs to be performed, e.g. during ERT of alfa-mannosidosis, better quantitation is probably required to detect changes in biomarker levels.

3. LITERATURE REFERENCES

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