DIAGNOSTIC TESTS IN URINE FOR MUCOPOLYSACCHARIDOSES

This document summarises a number of diagnostic tests commonly used to investigate glycosaminoglycans (GAG) in urine aiming at identification of Mucopolysaccharidosis (MPS) patients. More detailed information on methods can be found in a number of reviews [1, 2]
Detailed protocols are not included here; these can be found in the literature references provided.

1. QUANTITATIVE SCREENING TESTS

Quantitative screening tests for total GAG in urine include the following methods.

Spectrophotometric:
- Dimethylmethylene blue (DMB) [3]
- Alcian blue [4]

Hexuronic acid determination
- Carbazol [5]

Precipitation
- Cethylpyridiniumchloride (CPC) [6]

The DMB test [3] is the most commonly used assay. It is a relatively easy test to implement. Minuaturisation and automation are possible. False negative results have been reported for urine samples from mildly affected MPS I, III and IV patients. For this reason reference ranges (GAG normalized to creatinine), which are strongly age-dependent, should be established rigorously. New batches of the DMB dye must also be validated for this reason. A false-positive rate of around 5% is common. Urine samples with very low or very high creatinine values should be interpreted with caution.
The Alcian blue [4], Hexuronic acid [5] and CPC [6] tests are less commonly used. The Hexuronic acid test is specific for uronic acids and does not detect keratan sulfate.
Qualitative screening tests, such as the Berry spot test or the Ames test, should not be used to screen for MPS, since they have too high false-negative rates [7].

2. GAG SUBTYPE ANALYSIS (QUALITATIVE TESTS)

Qualitative methods for GAG subtype analysis include:
- 1-Dimensional electrophoresis [8]
- High-resolution (discontinuous) 1-dimensional electrophoresis [9]
- 2-Dimensional electrophoresis [10]
- Thin layer chromatography [11]

For these analyses GAG are isolated (see for example [4] or [10]) from a relatively large urine sample (2-5 mL) by precipitation, cleaned and subsequently applied to the cellulose acetate sheet or TLC plate. Please note that GAG may precipitate poorly from dilute urine samples. Following separation, the different GAG species are visualized by staining using e.g. Alcian blue. These methods are not quantitative, but rely on visual inspection of electrophoretic/chromatographic patterns and require experienced lab staff for interpretation. All electrophoretic methods use cellulose acetate sheets. The type of sheets is critical. In the past years several companies have discontinued manufacturing sheets and users may therefore be faced with supply issues.

3. QUANTITATIVE ANALYSES BASED ON LC-MS/MS

More recently developed tests use different approaches based on LC-MS/MS to diagnose mucopolysaccharidoses:

- Enzymatic hydrolysis of GAG followed by detection of disaccharides using LC-MS/MS [12]
- Methanolysis of GAG followed by detection of disaccharides using LC-MS/MS [13, 14]
- Detection of sulfated sugars and oligosaccharides by LC-MS/MS [15]

The first two methods can be used to quantitatively determine the different GAG species. They are very powerful to diagnose MPS and replace the screening- and GAG subtype tests described in sections 1 and 2. The method using enzymatic GAG hydrolysis [12] does not detect chondroitin sulfate, while the methanolysis method [13, 14] is less suitable for determination of keratan sulfate.
The third approach using LC-MS/MS quantitatively determines naturally occurring sulfated sugars/oligosaccharides as biomarkers for MPS [15]. Apparently, this method is able to specifically diagnose most MPS subtypes.
All three methods are capable to diagnose MPS, but in addition can be used for therapy monitoring.

4. LITERATURE REFERENCES

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