Detection of Homogentisic Acid in Plasma and Urine

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Laboratory proof of alkaptonuria in urine is based on the reducing properties of homogentisic acid. Even routine chemical examination of urine can lead to diagnosis of alkaptonuria on the basis of characteristic colouration with Fehling's solution. In patients with alkaptonuria who excrete 2–3 g of homogentisic acid/day, urine with Fehling's solution acquires a characteristic brown-black colouration even in cold condition. In milder forms of alkaptonuria, these colour reactions occur after warming up.

Older quantitative methods of detection of homogentisic acid in urine utilised the capability of homogentisic acid to reduce silver, phosphomolybdic acid or iodine. The drawback of these methods was the fact that they also detected other reducing compounds present in urine. These faults were manifested especially in case of lower concentration of homogentisic acid in urine of patients with alkaptonuria. Extraction of homogentisic acid with ether and subsequent iodometric detection represented certain improvement. Seegmiller et al. (1961) developed spectrophotometric enzymatic detection of homogentisic acid in plasma and urine using purified oxygenase of homogentisic acid. This enabled the authors to detect specifically

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even 1 μg of acid. Maleylacetoacetate acid formed by oxidation is detected by spectrophotometer at 330 nm. Stoner and Blivaiss (1965) developed relatively simple quantitative detection of homogentisic acid in urine. Homogentisic acid in alkaline environment with the presence of air oxygen forms 1,4-benzoquinone-2-acetic acid. This compound is subsequently conjugated with 2,4-dinitrohydrazine. Formed hydrazone in the presence of alcohol solution of sodium hydroxide creates characteristic lavender colour with absorption maximum at 570–580 nm. Later on, capillary electrophoresis (Presto Elgstoen and Jellum 1997) and gas chromatography (Oláh et al. 2003) were used for the detection of homogentisic acid. A very sensitive method for detection of homogentisic acid using liquid chromatography with amperometric detection was developed by Zoutendam et al. (1976). Recently a method for simultaneous quantification of urinary HGA and tyrosine using reverse phase LC-MS/MS has been developed (Hughes et al. 2014).

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