# 5-Hydroxyindoleacetic acid (urine, plasma)

## 1 Name and description of analyte

1.1 Name of analyte
5-Hydroxyindoleacetic acid

1.2 Alternative names
5-HIAA, 5-hydroxyindole-3-acetic acid, hydroxyindole acetic acid, 5-hydroxyindolamineacetic acid

1.3 NLMC code (to follow)

1.4 Description of analyte
5-Hydroxytryptamine (serotonin) is oxidised by hepatic monoamine oxidase to 5-hydroxyindoleacetaldehyde, most of which is further metabolised in the kidneys to 5-HIAA by aldehyde dehydrogenase. A small amount may be reduced to 5-hydroxytryptophol by aldehyde reductase. The major urinary excretion product of serotonin is 5-HIAA.

1.5 Function of analyte
5-hydroxyindoleacetic acid has no known function.

## 2 Sample requirements and precautions

2.1 Medium in which measured
Urine samples are predominantly used for measurement of 5-HIAA. A 24 h urine collection is the preferred sample. However, random urine samples are still accepted at some laboratories, with results often reported as a ratio to urinary creatinine. Random urine samples should be interpreted with caution as 5-HIAA production can be variable, even in patients with a carcinoid tumour; thus an overnight collection or early morning sample should be requested if a 24 h collection is not possible.

2.2 Precautions re sampling, handling etc.
Acid preservative is acceptable but not essential for 5HIAA analysis. The addition of acetic acid can stabilise indoles, but care should be taken not to reduce the pH to below 3 as 5HIAA is unstable in highly acidic solutions. Samples can be stored at 2–8 $^{o}$C if the sample will be analysed within 48 h; otherwise they should be frozen. Ideally the urine should be stored in a cool and dark place during the collection.

## 3 Summary of clinical uses and limitations of measurements

3.1 Uses
Measurement of 5-HIAA is often requested in the investigation of neuroendocrine tumours (NETs) that secrete serotonin (also known as carcinoid tumours). These are generally foregut or midgut tumours and can be associated with genetic syndromes

including multiple endocrine neoplasia type 1 (MEN-1), von Hippel-Lindau syndrome and neurofibromatosis type 1. Serotonin producing NETs can result in the carcinoid syndrome; this is a term used to describe clinical features including flushing, palpitation, diarrhoea and wheezing. Excess serotonin can also contribute to cardiac damage including endomyocardial fibrosis, tricuspid insufficiency and pulmonary valvular disease.

Quantification of urinary 5-HIAA is considered more useful than measurement of serotonin for the diagnosis of carcinoid tumours. Specificity is increased when dietary intake of 5-hydroxyindoles is excluded prior to sample collection. Therefore food containing high concentrations of indoles should be avoided before urine collection (see below).

3.2 Limitations

Secretion of serotonin may not be regular. Sensitivity is improved if a urine collection is conducted while the patient is symptomatic. A morning urine collection or overnight collection is recommended if a 24 h collection is not achievable. Hindgut tumours are not often associated with increased serotonin production or urinary 5-HIAA excretion.

Foods containing high hydroxyindole concentrations (e.g. aubergines, avocados, bananas, black- and red-currants, kiwi, gooseberries, melons, tomatoes, plums, pineapples and walnuts) as well as glyceryl guaiacolate (an ingredient in some cough medicines) can increase urinary 5-HIAA excretion. Pregnancy and sleep deprivation can also result in raised 5-HIAA excretion.

Medications and drugs such as levodopa, methyldopa, monoamine oxidase inhibitors, ethanol, imipramine, isoniazid, reserpine and *p*-chlorophenylalanine can reduce 5-HIAA production. Others, such as acetanilide, phenacetin, methocarbamol, cisplatin, fluorouracil, melphalan and rauwolfia can increase urinary 5-HIAA, as can 5-hydroxytryptophan, which is used to treat depression and is available over-the-counter.

## 4 Analytical considerations

4.1 Analytical methods

Urinary 5-HIAA is most commonly measured by high performance liquid chromatography (HPLC), coupled with either electrochemical or fluorescence detection. Urinary 5-HIAA can also be measured by liquid chromatography-tandem mass spectrometry (LC–MS/MS). Other methods include immunoassay (ELISA) or colorimetric methods; however, these have poorer analytical performance compared to HPLC. This article will focus on HPLC coupled with electrochemical detection.

4.1.1 HPLC-electrochemical detection (HPLC–ECD)

This is the most common method for measurement of urinary 5-HIAA, typically using reversed-phase chromatography. Detection of the compound is by voltammetric

oxidation of the hydroxyl group at carbon 5; thus the sample must be kept at a low pH to ensure that this hydroxyl group is not ionised.

4.1.2 LC–MS/MS

A more recent method for the detection and quantification of 5-HIAA acid is LC–MS/MS. After a protein precipitation step, acidified samples are injected into a reversed-phase column coupled to an ion-exchange column before ionisation and detection on the mass spectrometer. LC–MS/MS typically has a faster run time than HPLC–ECD.

4.2 Reference method

A reference method has not been defined.

4.3 Reference materials

Catecholamines Metabolites Mix, available from Cerilliant, Texas, USA.

4.4 Interfering substances

Analytical interferences to HPLC methods can arise from acetaminophen, mephenesin, methocarbamol and phenacetin, which can cause a falsely raised 5-HIAA. Conversely, acetic acid, dihydroxyphenylacetic acid, formaldehyde, gentisic acid, homogentisic acid, levodopa, methenamine, phenothiazine and salicylates (fluorescent methods only) can cause a falsely low result.

4.5 Sources of error

The patient must not consume food which contains large amounts of hydroxyindoles. The patient's medication should be reviewed for possible interference and, if possible, should not be taken during the collection period to reduce the risk of a false positive result. Food and medication interferences are described in section 3.2. Other sources of error may arise from incorrect collection of 24 h urine and during the storage of samples.

## 5 Reference intervals and variance

5.1.1 Reference interval (adults)

There are no harmonised reference ranges for urinary HIAA; however a reference range of <50 µmol/24 h is commonly used.

5.1.2 Reference intervals (others)

There are no other recommended reference ranges.

5.1.3 Extent of variation

5.1.3.1 Interindividual CV (HPLC): 33%

5.1.3.2 Intraindividual CV (HPLC): 20%

5.1.3.3 Index of individuality: 0.61

5.1.3.4 CV of method: HPLC– ED: 12.6%; LC–MS/MS: 13.5%

5.1.3.5 Critical difference: (assuming constant pre-analytical conditions) HPLC: 35%; LC-MS/

MS: 36%

5.1.4 Sources of variation

Diet, time of day, exercise, kidney and liver function may alter 5-HIAA excretion. Pregnancy and sleep deprivation can also cause increases.

## 6 Clinical uses of measurement and interpretation of results

6.1 Indications and interpretation

The measurement of 5-HIAA is most commonly used for the investigation of a suspected serotonin secreting neuroendocrine (carcinoid) tumour. A urine excretion greater than ten times the upper limit of normal indicates that a carcinoid tumour is extremely likely; however carcinoids can often be slow growing, resulting in more modest increases in urinary 5-HIAA. Carcinoids have also been reported as part of the MEN-1 syndrome, so that it may be useful additionally to investigate patients for suspected MEN-1.

Urinary excretion of 50–100 µmol/24 h can be caused by dietary sources of indoles. In this case a repeat sample should be requested omitting potentially interfering foodstuffs and drugs for at least 72 h.

Although the most common medium for 5-HIAA analysis is urine some laboratories are offering measurement in plasma. This reduces the errors associated with a 24 h urine collection and can reduce the amount of pre-analytical errors. As 5-HIAA is renally excreted, care should be taken interpreting plasma 5-HIAA results in patients with renal impairment; falsely high plasma 5-HIAA may be the result of a reduced GFR rather than a carcinoid.

The measurement of urinary 5-HIAA is mainly for diagnostic purposes; it can also be used as a prognostic marker although other tumour markers (for example, chromogranin A) are considered to have better prognostic accuracy.

6.2 Confounding factors

Ensure the patient has not consumed any hydroxyindole-containing food for at least three days prior to the urine collection. A decrease of 5-HIAA production in vivo can occur with raised corticotrophin, ethanol, imipramine, isoniazid, levodopa, MAO inhibitors and methyldopa concentrations. See section 3.2 for further dietary and medication requirements.

## 7 Causes of abnormal results

7.1 High values

7.1.1 Causes

Carcinoid syndrome is the most common reason for a greatly increased urinary excretion of 5-HIAA.

7.1.2 Investigation

The excretion of 5-HIAA may be mildly raised due to a carcinoid or due to a non-pathological cause (nutritional intake or medication). It is usually beneficial to perform a repeat 24 h urine collection to provide confirmation. As stated previously, carcinoids may be associated with MEN-1, therefore it may be beneficial to check serum calcium concentration and assess the pancreas (i.e. for a pancreatic mass).

Coeliac disease has also been reported to raise urinary 5-HIAA acid excretion. This is likely due to hyperplasia of enterochromaffin cells in the intestines resulting in increased serotonin release.

7.2 Low values

7.2.1 Causes

A decrease of urinary 5-HIAA excretion can occur with raised plasma corticotrophin, and with ingestion of aspirin, ethanol, imipramine, isoniazid, levodopa, MAO inhibitors and methyldopa. A low urinary 5-HIAA may also occur with depressive illness, mastocytosis, following small intestinal resection, and phenylketonuria or Hartnup disease.

CSF 5-HIAA concentrations are decreased in the following disorders: aromatic-L-amino acid decarboxylase deficiency, monoamine oxidase A deficiency, guanosine triphosphate cyclohydrolase deficiency, 6-pyruvoyl-tetrahydropterin synthase deficiency, dihydropteridine reductase deficiency, dopa-responsive dystonia and sepiapterin reductase deficiency (Rodan *et al*. 2015). Measurement of CSF 5-HIAA can assist with the diagnosis and monitoring of patients with inherited disorders of neurotransmitter metabolism, ataxia or neurological disorders which affect serotonin metabolism.

7.2.2 Investigation

Low urinary 5-HIAA concentrations would not be investigated further; however, low plasma or CSF concentrations could be the result of an enzyme deficiency, thus genetic testing should be requested.

7.3 Notes

None

## 8 Performance

8.1 Sensitivity, specificity etc. for individual conditions

The sensitivity and specificity of 5-HIAA in the presence of carcinoid syndrome are 70% and 90%, respectively. Midgut carcinoid tumours often produce more serotonin (and therefore more 5-HIAA) than foregut and hindgut carcinoid tumours. This is because the enzymes tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase are present in the midgut, whereas only tryptophan hydroxylase is present in the foregut and neither are present in the hindgut. These enzymes are involved in the metabolism of tryptophan to serotonin. As midgut carcinomas are most liable to cause the carcinoid syndrome higher sensitivities (>90%) have been reported.

## 9 Systematic reviews and guidelines

9.1 Systematic reviews

Deacon AC. The measurement of 5-hydroxyindoleacetic acid in urine. Ann Clin Biochem 1994;31:215–232.

Peaston RT, Weinkove C. Measurement of catecholamines and their metabolites. Ann Clin Biochem 2004;41:17–38.

9.2 Guidelines

O'Toole D, Grossman A, Gross D *et al*. ENETS consensus guidelines for the standards of care in neuroendocrine tumors: biochemical markers. Neuroendocrinol 2009; 90:194–202.

Ramage JK, Ahmed A, Ardill J *et al*. Guidelines for the management of gastroenteropancreatic neuroendocrine (including carcinoid) tumours (NETs). Gut 2012; 61:6–32.

9.3 Recommendations

Adaway JE, Dobson R, Walsh J *et al*. Serum and plasma 5-hydroxyindoleacetic acid as alternative to 24 h urine 5-hydroxyindoleacetic acid measurement. Ann Clin Biochem 2016;53:554–560.

Gedde-Dahl M, Thiis-Evensen E, Myklebust T *et al.* Comparison of 24-h and overnight samples of urinary 5-hydroxyindoleacetic acid in patients with intestinal neuroendocrine tumors. Endocrine Connections 2013;2:50–54.

Lips C, Lentjes E, Hoppener J. The spectrum of carcinoid tumours and carcinoid syndromes. Ann Clin Biochemistry 2003;40:612–627.

Rodan LH Gibson MK, Pearl PL. Clinical use of CSF neurotransmitters. Pediatr Neurol 2015;53:277–286.

## 10 Links

10.1 Related analytes: serotonin, 5-hydroxyindoleacetaldehyde

10.2 Related tests

Serum chromogranin A can be helpful in the diagnosis and prognosis of a carcinoid tumour. Urinary creatinine measurement will be required if a 24 h urine collection is not feasible. Serum calcium should be measured for the investigation of hyperparathyroidism in MEN-1. MEN-1 is also associated with pancreatic islet cell tumours, which can be screened for by measuring plasma insulin, glucagon and somatostatin.

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