Oxalate (urine, plasma)

1 Name and description of analyte

1.1 Name of analyte
Oxalate

1.2 Alternative names
None

1.3 NLMC code
To follow

1.4 Function of analyte
Oxalate is a metabolic end product primarily excreted by the kidneys. The poor solubility of the calcium salt makes it a risk factor for kidney stone formation. Oxalate, along with Cl\(^-\), SO\(_4^{2-}\), HCO\(_3^-\) and OH\(^-\), is a substrate of the SLC26A6 anion transporter found in the apical membrane of the gut and renal tubules and therefore has a minor role in Cl\(^-\) uptake. Faecal oxalate is usually of dietary origin except in end stage renal disease where some endogenous oxalate is possibly excreted into the gut.

2 Sample requirements and precautions

2.1 Medium in which measured
Urine
Plasma (EDTA)

2.2 Precautions re sampling, handling etc.

Urine: 24 h collections should be collected into acid preservative (HCl) not only to prevent non-enzymatic conversion of ascorbate to oxalate but also to increase oxalate solubility. Non-acidified samples should be acidified to pH < 2.0, mixed well and left overnight before further processing. Random collections should be acidified on receipt in the laboratory but [oxalate] may be influenced by dietary intake in the previous 2 h.

Plasma (EDTA): samples should be separated within 2 h of collection and the plasma frozen if not analysed immediately.

3 Summary of clinical uses and limitations of measurements

3.1 Uses
Oxalate is measured as part of the investigation of the underlying cause of renal stone disease, either secondary (e.g. due to excessive intake of oxalate or its precursors) or primary (endogenous oxalate overproduction).

3.2 Limitations
1. Random plasma samples can be influenced by dietary intake in the preceding 2 h.
2. Calculated oxalate:creatinine ratios may be misleading with dilute urine samples (creatinine < 0.8 mmol/L).
A raised urinary oxalate alone cannot distinguish between a diagnosis of primary or secondary hyperoxaluria. A clinical history and additional investigations are required.

4 Analytical considerations

4.1 Analytical methods

1. Enzymatic, using oxalate oxidase

Hydrogen peroxide is generated from oxalate through oxidation with oxalate oxidase (E.C 1.2.3.4). The reaction is:

\[ 2(\text{COO}^-)_2 + 2\text{H}_2\text{O} + \text{O}_2 \rightarrow (\text{oxalate oxidase}) \rightarrow 4\text{CO}_2 + 2\text{H}_2\text{O}_2 \]

The hydrogen peroxide formed reacts with 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-dimethylaminobenzoic acid (DMAB) in the presence of horseradish peroxidase (E.C. 1.11.1.7) to yield an indamine dye that is measured at 580 nm. Potential interference by ascorbic acid is suppressed by pre-treating the sample prior to analysis with buffered sodium nitrite at pH 3.5.

2. Enzymatic, using oxalate decarboxylase

Formate is produced from oxalate through decarboxylation with oxalate decarboxylase (E.C. 4.1.1.2); this reaction is coupled with formate dehydrogenation catalysed by formate dehydrogenase (E.C.1.2.1.2) in the presence of NAD\(^+\) and the reaction followed by measuring the increase in absorption at 340 nm. The reactions are:

\[ (\text{COO}^-)_2 + \text{H}_2\text{O} \rightarrow (\text{oxalate decarboxylase}) \rightarrow \text{H.CO}^- + \text{OH}^- + 2\text{CO}_2 \]
\[ \text{HCOO}^- + \text{NAD}^+ \rightarrow (\text{formate dehydrogenase}) \rightarrow \text{CO}_2 + \text{NADH} \]

This method has largely fallen out of favour although has the advantage that it is unaffected by ascorbate. It has a higher CV than the oxalate oxidase method.

3. Ion chromatography (Dionex, DX-500)

Anion exchange with conductivity detection. Ionpac® AS4A and AS11 columns are used with mobile phases of sodium bicarbonate and sodium carbonate or sodium hydroxide respectively.

4. HPLC

Ion-paired, reverse phase HPLC with electrochemical detection using a Spherisorb® ODS 2 column; the mobile phase is phosphate buffer, pH 7.0 and methanol (90:10 v/v).

5. Liquid chromatography-tandem mass spectrometry (LC-MS)

Initial separation is provided by a weak anion exchange online column, e.g. Oasis® WAX column (with the sample being loaded at low pH and eluted at high pH), followed by tandem MS in electrospray negative mode.

All methods are suitable for the analysis of urinary and plasma oxalate and have similar performance characteristics. Problems have been described in the reverse phase HPLC method as a result of a co-eluting compound.

4.2 Reference method

None
4.3 Reference materials
None

4.4 Interfering substances
Ascorbate: non-enzymatic conversion to oxalate at pH >5; can also cause colour suppression of the indamine dye (Section 4.1).

4.5 Sources of error
A co-eluting peak has been reported in HPLC methods. Samples acidified on receipt in the laboratory may give artefactually low results due to inadequate solubilisation of oxalate.

5 Reference intervals and variance

5.1.1 Reference interval (adults)
Urine: <460 μmol/24h; oxalate/creatinine ratio <38 mmol/mol
Plasma: <3 μmol/L

5.1.2 Reference intervals (others)
Children
Urine: <460 μmol/1.73m²/24h
Urinary oxalate:creatinine ratio
Infants; <1 year 4–98 mmol/mol
1–4 years 4–72 mmol/mol
5–11 years 3–71 mmol/mol
>12 years <38 mmol/mol
Plasma: <3 μmol/L

5.1.3 Extent of variation
5.1.3.1 Interindividual CV: 18.4%
5.1.3.2 Intraindividual CV: 24.0%
5.1.3.3 Index of individuality: 0.76
5.1.3.4 CV of method

<table>
<thead>
<tr>
<th></th>
<th>Urine</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Oxalate oxidase</td>
<td>4.3–5.6%</td>
<td>1.9–13%</td>
</tr>
<tr>
<td>2 Ion chromatography</td>
<td>3.4%</td>
<td>12%</td>
</tr>
<tr>
<td>3 Reverse phase HPLC</td>
<td>&lt;6%</td>
<td>2.8–8.4%</td>
</tr>
<tr>
<td>4 Tandem MS</td>
<td>6%</td>
<td>&lt;6%</td>
</tr>
<tr>
<td>5 Oxalate decarboxylase</td>
<td>4.3–8%</td>
<td>not used</td>
</tr>
</tbody>
</table>

5.1.3.5 Critical difference
67.9%

5.1.4 Sources of variation
Diet, variation in calcium intake

6 Clinical uses of measurement and interpretation of results

6.1 Uses and interpretation
Oxalate measurement is used in:
- the assessment of renal stone formers
- the assessment of patients with nephrocalcinosis noted on ultrasound
- the assessment of patients with oxalate crystals found in a renal biopsy in acute kidney injury.

6.2 Confounding factors
[Oxalate] in random plasma samples can be influenced by dietary intake before sampling.
Urine results may be misleading in renal failure.

7 Causes of abnormal results

7.1 High values
7.1.1 Causes
- Primary hyperoxaluria:
  - type 1 (PH1, alanine:glyoxylate aminotransferase deficiency)
  - type 2 (PH2, glyoxylate reductase/hydroxypyruvate reductase deficiency)
  - type 3 (PH3, 4-hydroxy-2-oxoglutarate aldolase deficiency)
These conditions tend to present at an early age, typically with haematuria or loin pain. In severe, early onset cases, an organic acidosis may be present as may renal failure. Nephrocalcinosis is a common finding in type 1.
- Secondary hyperoxaluria:
  - high dietary oxalate intake
  - gastrointestinal disease, e.g. Crohn's disease, short bowel syndrome, chronic pancreatitis, gastric bypass.
- Hyperoxalaemia
  - renal failure from any cause
    plasma [oxalate] >50 μmol/L is suggestive of primary hyperoxaluria but further, more specific testing, is required to establish this diagnosis.

7.1.2 Investigation
Secondary causes should be obvious from clinical history. X-ray and ultrasound examination are indicated to assess renal involvement. The presence of elevated [glycolate] and [glycerate] in urine support a diagnosis of PH1 and PH2, respectively but their absence cannot exclude either disease. Urinary oxalate is typically >700 μmol/1.73m²/24h in primary disease but can be lower and the history should be taken into account.
Further investigation of suspected primary disease requires either genetic or enzymological tests.

7.2 Low values
7.2.1 Causes
- Urine: of no clinical significance
- Plasma: of no clinical significance
7.2.2 Investigation
Not applicable

7.3 Notes
All patients with primary hyperoxaluria are at risk of recurrent calcium oxalate stone disease; the stones are typically >95% calcium oxalate. Failure to investigate renal stone formation adequately may lead to presentation in end stage renal failure. Outcomes are worse than ESRF from any other cause as a result of systemic oxalate deposition that has effects not only on other organs e.g. the heart, but also on future transplants.
8 Performance

8.1 Sensitivity, specificity etc. for individual conditions
Sensitivity and specificity data are not available as there are a large number of poorly investigated patients. Primary hyperoxaluria is likely if urinary oxalate is >700 μmol/24 h/1.73m² (or the oxalate/creatinine ratio is more than twice upper limit of normal), with no clinical evidence of enteric disease, and a history of recurrent stone disease.

9 Systematic reviews and guidelines

9.1 Systematic reviews
None identified

9.2 Guidelines

9.3 Recommendations
None identified

10 Links

10.1 Related analytes
Urinary glycolate
Urinary L-glycerate

10.2 Related tests
Alanine:glyoxylate aminotransferase catalytic activity
Glyoxylate reductase/hydroxypyruvate reductase catalytic activity
Genetic testing for mutations in the genes for the enzymes deficient in the primary hyperoxalurias (see 7.1.1). Sequence analysis is routinely available (UK Genetic Testing Network), provides a non-invasive means of diagnosis for these disorders and has largely superseded enzymology. Genetic testing is the only means of diagnosing PH3 and of prenatal diagnosis for these disorders.

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