Oxalate reagents are for the quantitative, enzymatic determination of oxalate in urine at 590 nm.

SUMMARY

Oxalate was confirmed as a normal constituent of urine in 1951, but only recently has the significance of calcium oxalate crystaluria and its relationship to urinary tract stone formation been fully recognized. Formation of the sparingly soluble calcium salt of oxalate in the urinary tract is considered the major factor in urolithiasis. Oxalate in urine may arise either as an end product of intermediary metabolism or from dietary sources. A decreased excretion of oxalate in the urine is associated with hyperoxaluria and hyperoxycitruria. An increased excretion of oxalate can be attributed to increased ingestion of oxalate precursors or oxalate rich foods, formation of oxalate due to metabolic defects such as in primary hyperoxaluria, and absorption of oxalate in a number of gastrointestinal disorders that produce severe fat malabsorption. This latter group includes patients with inflammatory bowel disease, ileal resection, biliary diversion, pancreatic insufficiency, sprue, small intestinal stasis with bacterial overgrowth, and following jeunonale bypass or resection for the treatment of obesity.1 2

Currently, urinary oxalate determination is performed by procedures based on isolation, dilution, gas and ion chromatography, as well as coupled enzyme reactions.1 2 4 These procedures are very time consuming and may require equipment not readily available in the clinical laboratory. The enzymatic method described below is based on the oxidation of oxalate by oxalate oxidase following measurement of hydrogen peroxide (H2O2) produced during the reaction by a peroxidase-catalyzed reaction.1 2 6 The procedure is specific for oxalate. It requires no special equipment and is easily adaptable for use on clinical automated analyzers.

PRINCIPLE

The enzymatic reactions involved in the assay procedure are as follows:

\[
\text{Oxalate Oxidase} \quad \text{Oxalate} + O_2 \rightarrow 2\text{CO}_2 + H_2O_2 \\
\text{H}_2O_2 + \text{MBTH} \rightarrow \text{Peroxidase} \rightarrow \text{Indamine Dye} + \text{H}_2O \\
\text{DMAB} \rightarrow \text{Oxalate} \rightarrow \text{carbon dioxide and hydrogen peroxide by oxalate oxidase. The hydrogen peroxide reacts with 3-methyl-2-benzothiazoline hydrazine (MBTH) and 3-(dimethylamino) benzoic acid (DMAB) in the presence of peroxidase to yield an indamine dye which has an absorbance maximum at 590 nm. The intensity of the color produced is directly proportional to the concentration of oxalate in the sample.}
\]

REAGENTS

**OXALATE REAGENT A**

- DMAB: 3.2 mmol/L
- MBTH: 0.22 mmol/L
- Buffer: pH 3.1 ± 0.1
- Nonreactive ingredients and stabilizers

**OXALATE REAGENT B**

- Oxalate Oxidase (Barley): 3000 U/L
- Peroxidase (Horseradish): 100,000 U/L

**SAMPLE DILUENT**

- EDTA: 10 mmol/L
- Buffer: pH 7.6 ± 0.1

**SAMPLE PURIFIER TUBES**

- Activated Charcoal

PRECAUTIONS:

The reagents are for "in vitro diagnostic use". Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state and federal laws.

Refer to Material Safety Data Sheets for any updated risk, hazard or safety information.

The following instruction should be adhered to when opening the red flip-seal cap as it has a sharp edge after opening:

- A tweezers, needle-nose pliers, forceps, de-cappers, spatula or similar type of object should be used to open and peel off the flip-seal from the vial. When doing this action, ensure it is done outwards, away from the body.
- Latex gloves should also be worn to provide further protection to the user.

PREPARATION

Reconstitute Oxalate Reagent A with volumes of deionized water indicated on vial label. If reagent is to be used in a discrete analyzer please refer to the respective application procedure for reagent preparation instructions. After the addition of water, stopper the vial and mix until it is completely dissolved.

Reconstitute Oxalate Reagent B with volume of deionized water indicated on vial label. Stopper the vial and immediately mix by gentle inversion. DO NOT SHAKE.

Sample Diluent is prepared as follows. Remove a sample diluent label from the kit and affix onto a clean dry container of appropriate size. Transfer the entire powder from a vial into the newly labeled container and add volume of deionized water indicated on the vial label. After addition of water, cap the container and immediately mix several times by inversion.

STORAGE AND STABILITY

Store the dry reagents refrigerated (2-8°C). Reagents are stable until the expiration date indicated on the vial. Store the sample purifier tubes at room temperature (15-26°C).

Reconstituted Oxalate Reagent A is stable for 1 day at ambient temperature (15 - 26°C) and 1 month when stored refrigerated (2-8°C).

Reconstituted Oxalate Reagent B is stable for 1 day at ambient temperature (18 - 26°C). Alternatively it can be stored for 30 days at 2-8°C or aliquoted and stored at -20°C for 30 days. Each aliquot should be used once and not re-frozen.

Reconstituted Sample Diluent is stable for 1 week at ambient temperature (18 - 26°C) and 3 months when stored refrigerated (2-8°C).

**NOTE:** Warm Oxalate Reagent B to approximately 37°C in order to dissolve any crystalline material which may form during storage in the refrigerator.

**DETERIORATION:**

Do not use dry Oxalate Reagent A, Oxalate Reagent B, or Sample Diluent if they indicate any moisture penetration.

**DISCRETE ANALYSER APPLICATIONS**

Application procedures using Trinity Biotech Oxalate reagents are available for various automated instruments. Please contact Trinity Biotech Technical Services Department for more information.

**SPECIMEN COLLECTION AND PREPARATION**

A 24-hour urine specimen is collected in a glass or plastic bottle containing 10 ml concentrated hydrochloric acid. Record the volume in litres. Oxalate in acidified urine is stable for 7 days when stored refrigerated or frozen. Ascorbic acid (vitamin C) at a very high concentration (exceeding 16 mmol/L) can interfere. It is recommended that patients refrain from taking excessive amounts of vitamin C or vitamin C rich food for at least 48 hours prior to urine collection. Prior to assay, dilute urine with equal volume of Sample Diluent. Please refer to sample preparation instructions given under "Manual Procedure" section for details.

**INTERFERING SUBSTANCES:**

Excessive amount of vitamin C in urine (exceeding 16 mmol/L) may affect the test results.

**MANUAL PROCEDURE**

**MATERIALS PROVIDED:**

- Oxalate Reagent A
- Oxalate Reagent B
- Sample Diluent
- Sample Purifier Tubes

**MATERIALS REQUIRED, BUT NOT PROVIDED:**

- Spectrophotometer, with temperature controlled cuvette compartment, capable of accurately measuring absorbance at 590 nm
- Pipette devices for the accurate delivery of volumes required for the assay
- Timer
- Plastic or glass container
- Oxalate Standard (0.5 mmol/L), Catalogue No. 591-3
- Centrifuge or Whatman filter paper

**PROCEDURE**

**Sample Preparation:**

1. Prepare Sample Diluent according to instructions.
2. Set up a series of labeled tubes for urine Sample and Controls.
3. Pipette 5 ml or any suitable volume of urine Samples and Controls into appropriately labeled tubes.
4. Add equal volume (as in Step 3) of Sample Diluent into each tube and mix.
5. Check the pH. It should be between 5.0 and 7.3. If not, adjust the pH using 1 N hydrochloric acid or 1 N sodium hydroxide.
6. Set up a series of sample purifier tubes for urine Samples and Controls.
7. Pipette 2 ml each of diluted urine Samples and Controls to appropriately labeled sample purifier tubes and mix for approximately 5 minutes by intermittent mixing. A rotator mixer is recommended for mixing.
8. Centrifuge the tubes for 5 minutes at 2000 rpm (1500g) or filter using Whatman filter paper.

Determine the oxalate concentration in the supernatants as described below.
Determination of Oxalate

1. Warm oxalate reagents to assay temperature (any temperature between ambient and 37°C).
2. Label tubes for Reagent Blank, urine Control and urine Sample.
3. Pipette 1 ml Oxalate Reagent A into each tube.
4. Pipette 50 µl of Supernatants or Filtrates ("Sample Preparation" section, Step 9), to respective tubes. Add 50 µl deionized water to Reagent Blank tube and 50 µl standard to tube labeled Standard.
5. Pipette 0.1 ml of Oxalate Reagent B into each tube and immediately mix by gentle inversion.
6. Incubate the tubes at desired temperature (18 - 37°C) for 5 minutes.
7. Read absorbances (A) of Blank, Standard, Control and urine Sample at 590 nm.
8. Determine the corrected absorbances (ΔA) of Standard, Control and Sample by subtracting Reagent Blank absorbance from the absorbance readings of Standard, Control and urine Sample.
9. To determine oxalate concentration in urine Sample, refer to "Calculations" section.

CALIBRATION:

The procedure is calibrated using aqueous Oxalate Standard, Catalogue No. 591-3. The concentration of oxalate in the sample is determined by comparing absorbance of the sample to that of the Oxalate Standard. Alternatively, the concentration of oxalate in unknown sample can also be extrapolated from a standard curve prepared using multi-level Oxalate Standard Set, Catalogue No. 591-11.

QUALITY CONTROL

The reliability of test results should be monitored by routine use of urine controls of known oxalate concentrations such as Trinity Biotech Oxalate Urine Control-E (elevated) and Control-N (normal), Catalogue Nos. O 6502 and O 6627, respectively. The oxalate concentration determined by this procedure should fall within the stated range of the controls.

Certified reference material (CRM) is traceable to NIST SRM 6140, Sodium Oxalate.

Calculations

Determine oxalate concentration in sample as follows:

Oxalate (mmol/L) = ΔA Sample x 0.5 x 2/ΔA Standard

Where:

0.5 = Concentration (mmol/L) of oxalate in standard
2 = Dilution factor

Quantity of Oxalate Excreted during 24-Hour Period = Oxalate (mmol/L) x Volume of Urine Voided during 24 hours (L)

EXAMPLE:

Volume of Urine Voided during 24 hours
ΔA Blank = 0.042
ΔA Standard = 0.751
ΔA Sample = 0.172
ΔA Sample - 0.042 = 0.130
ΔA Standard = 0.751 - 0.042 = 0.709

Urine Oxalate (mmol/24 h) = 0.130 x 0.5 x 2 x 1.43 = 0.262 mmol/24 hours
0.709

Multiply concentration in mmol/24 hr by 90 to obtain oxalate excretion in mg/24 hr.

LIMITATIONS

The reagents can measure urinary oxalate concentration up to 2 mmol, without further diluting the sample. If oxalate concentration in urine exceeds the upper limit of linear range, dilute 1 part sample with 1 part deionized water and reassay. Multiply the result by 2 to compensate for the dilution.

EXPECTED VALUES

Expected ranges were established using an oxalate reagent similar to the described product. Twenty-four hour urine specimens from 108 clinically healthy adult males and females and 12 children from 7 - 14 years old, on unrestricted diets were assayed for oxalate by a similar method. The mean value obtained for 67 adult males was 26 mg/24 hr and for 41 adult females was 17 mg/24 hr. The mean value obtained for children was 26 mg/24 hr. Normal ranges were calculated as the mean ± 2SD (SD = 9 for males, 7 for females, and 6 for children). Normal range for children under the age of 7 has not been determined. The expected ranges for oxalate concentration for adult males, adult females and children were determined to be as follows:

<table>
<thead>
<tr>
<th>mg/24 h.</th>
<th>mmol/24 h.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Males</td>
<td>7-44</td>
</tr>
<tr>
<td>Adult Females</td>
<td>4-31</td>
</tr>
<tr>
<td>Children</td>
<td>13-38</td>
</tr>
</tbody>
</table>

Similar normal ranges have been reported in the literature. Yribeni and Posen,11 using an oxalate decarboxylase-formate dehydrogenase, reported a range of 18 to 47 mg oxalate excreted per 24 hours for a mixed adult population. Biggs and Watts,14 using an isostioptide dilution method, reported a mean oxalate excretion rate of 33 mg per 24 hours (SD 6.5) for adult males and 35 mg per 24 hours (SD 6.7) for adult females. Similar values were reported by Hodgkinson and Williams11 for adults, but children under age 14 excreted 30 - 50% less per 24 hours. Pik and Kerckhoffs,18 however, reported oxalate excretion in children being very similar to that of adults with values between 10 and 45 mg per 24 hours. It is recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS

COMPARISON:

A total of 30 urine specimens with oxalate concentrations ranging from 0.1 - 1.2 mmol/L was assayed by the described method and by a similar procedure. Comparisons of oxalate values obtained by both the procedures yielded a correlation coefficient of 0.99 and the regression equation was y = 0.94x + 0.008.

SENSITIVITY:

An absorbance change of 0.150 measured in a 1 cm lightpath corresponds to oxalate concentration of 0.1 mmol/L when a spectrophotometer typically found in a clinical laboratory is used for the measurement under the stated conditions.

PRECISION:

Within-run and run-to-run precision studies yielded the following data:

<table>
<thead>
<tr>
<th>Urine 1</th>
<th>Within-Run</th>
<th>Urine 2</th>
<th>Urine 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mmol/L)</td>
<td>0.17</td>
<td>0.70</td>
<td>1.28</td>
</tr>
<tr>
<td>Standard Deviation (mmol/L)</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Coefficient of Variation (%)</td>
<td>1.45</td>
<td>0.94</td>
<td>0.96</td>
</tr>
<tr>
<td>Number of Assays</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urine 1</th>
<th>Run-to-Run</th>
<th>Urine 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mmol/L)</td>
<td>0.16</td>
<td>0.70</td>
</tr>
<tr>
<td>Standard Deviation (mmol/L)</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Coefficient of Variation (%)</td>
<td>2.18</td>
<td>1.69</td>
</tr>
<tr>
<td>Number of Assays</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

RECOVERY STUDIES:

Known amounts of oxalate were added to 59 urine specimens and the oxalate concentration in these samples was determined by this procedure to obtain percentage of oxalate recovery. The recovery of added oxalate ranged from 93-107%. The mean recovery was 100.5%.

Trinity Biotech warrants that its products confirm to the information contained in this and other Trinity Biotech publications. Purchaser must determine the suitability of the product for its particular use.

REFERENCES

ORDERING INFORMATION

KITS

<table>
<thead>
<tr>
<th>Catalogue No.</th>
<th>591-C</th>
<th>591-D</th>
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</thead>
<tbody>
<tr>
<td>Maximum Assays</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
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Contents – Catalogue Numbers

<table>
<thead>
<tr>
<th>Item</th>
<th>Catalogue No.</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalate Reagent A, 591-10</td>
<td>2 x 10 ml</td>
<td>10 x 10 ml</td>
</tr>
<tr>
<td>Oxalate Reagent B, 591-2</td>
<td>2 ml</td>
<td>5 x 2 ml</td>
</tr>
<tr>
<td>Sample Diluent, 591-4</td>
<td>100 ml</td>
<td>5 x 100 ml</td>
</tr>
<tr>
<td>Sample Purifier Tubes, 591-20</td>
<td>20 Tubes</td>
<td>-</td>
</tr>
<tr>
<td>Sample Purifier Tubes, 591-100</td>
<td>-</td>
<td>100 Tubes</td>
</tr>
</tbody>
</table>

INDIVIDUAL REAGENTS

<table>
<thead>
<tr>
<th>Catalogue No.</th>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>591-10</td>
<td>OXALATE REAGENT A</td>
<td>10 ml</td>
</tr>
<tr>
<td>591-2</td>
<td>OXALATE REAGENT B</td>
<td>2 ml</td>
</tr>
<tr>
<td>591-4</td>
<td>SAMPLE DILUENT</td>
<td>100 ml</td>
</tr>
<tr>
<td>591-3</td>
<td>OXALATE STANDARD, 0.50 mmol/L</td>
<td>25 ml</td>
</tr>
<tr>
<td>591-20</td>
<td>SAMPLE PURIFIER TUBES</td>
<td>20 tubes</td>
</tr>
<tr>
<td>591-100</td>
<td></td>
<td>100 tubes</td>
</tr>
</tbody>
</table>

REAGENT REQUIRED BUT NOT PROVIDED

<table>
<thead>
<tr>
<th>Catalogue No.</th>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>591-11</td>
<td>OXALATE STANDARD Set, 2x25 ml each of 0.25, 0.50 and 1.0 mmol/L</td>
<td>6 x 25 ml</td>
</tr>
</tbody>
</table>

OPTIONAL REAGENTS

<table>
<thead>
<tr>
<th>Catalogue No.</th>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>O 6502</td>
<td>Oxalate Urine Control-E</td>
<td>6 x 5 ml</td>
</tr>
<tr>
<td>O 6627</td>
<td>Oxalate Urine Control-N</td>
<td>6 x 5 ml</td>
</tr>
</tbody>
</table>

GUIDE TO SYMBOLS

- **REF** Catalogue number
- **IVD** For in vitro Diagnostic Use
- **LOT** Batch code
- **Store at 2-8°C**
- **Store at 18-26°C**
- **Reconstitute with** Manufacturer
- **H₂O** Use by
- **REAG A OXALATE** Oxalate Reagent A
- **REAG B OXALATE** Oxalate Reagent B
- **N** Normal
- **E** Elevated
- **DIL SPE** Sample Diluent