OXALATE KIT

REF 591C

REF 591D

For other languages | Para outras línguas
Pour d'autres langues | Για τις άλλες λώσσες
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INTENDED USE

Trinity Biotech 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 crystalluria and its relationship to urinary tract stone formation been fully recognized[1]. Formation of the sparingly soluble calcium salt of oxalate in the urinary tract is considered the major factor in urolithiasis.[2] 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 hyperglycinemia and hyperglycinuria.[3] An increased excretion of oxalate can be attributed to increases in 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 jejunoileal bypass or resection for the treatment of obesity.[4-12]

Currently, urinary oxalate determination is performed by procedures based on isotope dilution, gas and ion chromatography, as well as coupled enzyme reactions.[1,13] 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 followed by measurement of hydrogen peroxide (H_2O_2) produced during the reaction by a peroxidase-catalyzed reaction.[14] 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:

$$\text{Oxalate} + O_2 \xrightarrow{\text{Oxalate Oxidase}} 2CO_2 + H_2O_2$$

$$H_2O_2 + \text{MBTH} + \text{DMAB} \xrightarrow{\text{Peroxidase}} \text{Indamine Dye} + H_2O$$

Oxalate is oxidized to carbon dioxide and hydrogen peroxide by oxalate oxidase. The hydrogen peroxide reacts with 3-methyl-2-benzothiazolinone hydrazone (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)	100.00 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" and for professional use only. Normal precautions exercised in handling laboratory reagents should be followed. In case of damage, do not use. 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 volume 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
Standards
Store the Oxalate standards in the refrigerator (2-8°C). Do not use if there is evidence of microbial growth.

Store the dry reagents refrigerated (2- 8°C). Reagents are stable until the expiration date indicated on the labels. Store the sample purifier tubes at room temperature (18-26°C).

Reagent A
Reconstituted solution is stable for 1 day at 18-26°C.
Reconstituted solution is stable for 30 days at 2-8°C.

Reagent B
Reconstituted solution is stable for 1 day at 18-26°C.
Reconstituted solution is stable for 30 days at 2-8°C.
Reconstituted solution is stable for 30 days at -20°C when aliquoted. Each aliquot is to be used once and not refrozen.

Reconstituted Sample Diluent is stable for 1 week at 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.

Reconstituted Oxalate Reagent A is not suitable for use if the initial absorbance of the freshly reconstituted reagent measured in a 1 cm lightpath at 590 nm vs. water as reference is greater than 0.2.

The reconstituted reagents should be clear and free of particulate matter. If the reagents develop haziness due to bacterial contamination, they should be discarded.

DISCRETE ANALYSER APPLICATIONS

Please contact Trinity Biotech Technical Services Department for more information regarding applications procedures for Oxalate.

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 (2-8°C) or frozen (≤-20°C).

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
Pipetting 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 7.0. 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 (1500xg) 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, Standard, 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.

Oxalate Standard Set, Catalogue No. 591-11 & Oxalate Standard (0.5 mmol/L), Catalogue No. 591-3

EACH 591-11 SET CONTAINS

0.25 mmol/L– 2x25 ml
0.50 mmol/L– 2x25 ml
1.0 mmol/L– 2x25 ml

EACH 591-3 SET CONTAINS
0.50 mmol/L– 25 ml

Oxalate standards are a buffered preparation of Oxalic acid.

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.

Quality Controls should fall within their assigned ranges; otherwise the test run is invalid.

Certified reference material (CRM) is traceable to NIST SRM 8040, Sodium Oxalate.

CALCULATIONS

Determine oxalate concentration in sample as follows:

$$\text{Oxalate (mmol/L)} = \frac{\Delta \text{A Sample}}{\Delta \text{A Standard}} \times 0.5 \times 2$$

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	= 1.43 L
Δ BLANK	= 0.042
Δ STANDARD	= 0.751
Δ SAMPLE	= 0.172
Δ ASAMPLE	= 0.172 – 0.042 = 0.130
Δ ASTANDARD	= 0.751 – 0.042 = 0.709

$$\text{Urine Oxalate (mmol/24 h)} = \frac{0.130}{0.709} \times 0.5 \times 2 \times 1.43 = 0.262 \text{ mmol/24 hours}$$

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/L 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 re-assay. Multiply the result by 2 to compensate for the dilution.

EXPECTED VALUES

Normal and elevated urinary oxalate concentration ranges described in the literature have been established using various oxalate measurement methods. These include studies by Yriberri and Posen (1980) using an oxalate decarboxylase-formate dehydrogenase, who reported a range of 18 to 47 mg oxalate excreted per 24 hours for a mixed adult population. Gibbs and Watts (1969) using an isotope dilution method, reported a mean oxalate excretion rate of 33 mg per 24 hours for adult males and 35 mg per 24 hours for adult females. Similar values were reported by Hodgkinson and Williams (1972) for adults, but children under age 14 excreted 30 - 50% less per 24 hours. Pik and Kerckhoffs (1963) however, reported oxalate excretion in children being very similar to that of adults with values between 10 and 45 mg per 24 hours.

A recent publication (Chapter 43, Normal Laboratory Values and Drug Therapeutic and Toxic Ranges, The Medical Basis of Psychiatry, Fatemi and Clayton, Springer Science & Business Media, New York, 2016) describes expected values for adult males and females as follows

	mg/24hr
Adult Males	7 - 44
Adult Females	4 - 31

It is recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS

COMPARISON:
Platform System 1- A total of 30 urine specimens with oxalate concentrations ranging from 0.52 – 1.12 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 = 0.95x + 0.045."

Platform System 2 - A total of 30 urine specimens with oxalate concentrations ranging from 0.53 - 1.15 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 = 0.91x +0.059."

Platform System 3 - A total of 30 urine specimens with oxalate concentrations ranging from 0.52 - 1.16 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 = 0.97x +0.066."

SENSITIVITY:

An absorbance change of 0.15 measured in a 1 cm light path corresponds to oxalate concentration of 0.10 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 were performed on 3 different clinical chemistry analyser systems, yielding the following data.

	Platform Analyser 1			**Platform Analyser 2**			**Platform Analyser 3**		
	Within Run								
Sample	1	2	3	1	2	3	1	2	3
Mean Oxalate (mmol/L)	0.18	0.76	1.56	0.20	0.78	1.62	0.20	0.79	1.59
Standard Deviation	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01
%CV	0.0	1.0	0.7	4.7	1.9	0.6	0.0	1.2	0.8
No. Of Assays	20	20	20	20	20	20	20	20	20
	Run to Run								
Sample	1	2	3	1	2	3	1	2	3
Mean Oxalate (mmol/L)	0.22	0.94	1.75	0.23	0.95	1.79	0.24	0.97	1.77
Standard Deviation	0.01	0.03	0.09	0.01	0.04	0.08	0.01	0.03	0.07
%CV	6.1	2.6	4.9	5.4	4.2	4.2	4.5	3.2	4.2
No. Of Assays	40	40	40	40	40	40	40	40	40

RECOVERY STUDIES:
Known amounts of oxalate were added to 60 urine specimens and the oxalate concentration in these samples was determined by this procedure on three individual automated systems to obtain the mean oxalate recovery percentage. The mean percentage recovery was calculated as 100%, 107%, and 104% for the 60 samples tested on the system 1, 2 and 3 respectively. The mean % recovery ranged from 95-104% for system 1, 107% for the system 2 and 102-106% for the system 3. The mean recovery for the entire 180 sample preparations tested across the three systems was 104%. The mean recovery across all three systems ranged from 95% - 107%.

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

REFERENCES

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2. Robertson WG, Rutherford A: *Aspects of the analysis of oxalate in urine*. Scand J Urol Nephrol, Suppl 53, p 85, 1979.
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10. Stauffer JQ: *Hyperoxaluria and calcium oxalate nephrolithiasis after jejunoileal bypass*. Am J Clin Nutr 30:64, 1977.
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13. Zerwekh JE, Drake E, Gregory J, et al: *Assay of urinary oxalate: Six methodologies compared*. Clin Chem 29:1977, 1983.
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17. Hodgkinson A, Williams A: *An improved colorimetric procedure for urine oxalate*. Clin Chim Acta 36:127, 1972.
18. Pik C, Kerckhoffs HPM: *A simple method for the quantitative determination of oxalic acid in urine*. Clin Chim Acta 8:300, 1963.

ORDERING INFORMATION

KITS		
Catalogue No.	**591-C**	**591-D**
Maximum Assays	20	100
Contents – Catalogue Numbers		
Oxalate Reagent A, 591-10	2 x 10 ml	10 x 10 ml
Oxalate Reagent B, 591-2	2 ml	5 x 2 ml
Sample Diluent, 591-4	100 ml	5 x 100 ml
Sample Purifier Tubes, 591-20	20 Tubes	-
Sample Purifier Tubes, 591-100	-	100 Tubes

INDIVIDUAL REAGENTS		
Catalogue No.	**Item**	**Quantity**
591-10	OXALATE REAGENT A	10 ml
591-2	OXALATE REAGENT B	2 ml
591-4	SAMPLE DILUENT	100 ml
591-3	OXALATE STANDARD, 0.50 mmol/L	25 ml
591-20	SAMPLE PURIFIER TUBES	20 tubes
591-100	SAMPLE PURIFIER TUBES	100 tubes

REAGENT REQUIRED BUT NOT PROVIDED		
Catalogue No.	**Item**	**Quantity**
591-11	OXALATE STANDARD Set, 2x25 ml each of 0.25, 0.50 and 1.0 mmol/L	6 x 25 ml

OPTIONAL REAGENTS		
Catalogue No.	**Item**	**Quantity**
	OXALATE URINE CONTROLS	
O 6502	Oxalate Urine Control-E	6 x 5 ml
O 6627	Oxalate Urine Control-N	6 x 5 ml

GUIDE TO SYMBOLS


Consult instructions for use

Batch code

Catalogue number

In vitro diagnostic medical device

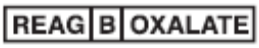
Recon.
Reconstitute with


Manufacturer

Water


Use-by date

Oxalate Reagent A



Oxalate Reagent B

N
Normal

E
Elevated

Sample Diluent

eIFU indicator


Temperature limit

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