



DCL MICROALBUMIN ASSAY

CATALOGUE NUMBER: 252-20

SIZE: R1: 4 x 20 mL; R2: 2 x 10 mL

INTENDED USE

For the quantitative determination of low levels of albumin in urine. For IN VITRO diagnostic use.

PRECAUTIONS

Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.

REAGENTS

Microalbumin Buffer Reagent (R1): a solution containing 100 mM tris (hydroxymethyl) aminomethane (pH 7.6 at 25°C) and a preservative.

Microalbumin Antibody Reagent (R2): a solution containing 20% anti-human albumin, goat anti serum, 100 mM tris (hydroxymethyl) aminomethane, and a preservative.

HISTORY

Low levels of protein are normally excreted in the urine of healthy individuals. The uriniferous tubules and glomeruli filter out most of these excreted mucoproteins. Albumin, a protein of molecular weight of 50,000, is not easily filtered out and small amounts are excreted into the urine. Increased excretion of albumin (microalbuminuria) is an early indicator of glomerular disease (1,2).

Microalbuminuria is characterized by increased urinary excretion of albumin in the absence of overt nephropathy (3,4). Microalbumin is recognized as a strong predictor of impending nephropathy in Type I Diabetics and its mortality risk in diabetic patients (5). Early detection of microalbuminuria may be beneficial for treatment programs for diabetics because renal damage may be reversible if diabetes is well controlled at this stage.

Many of the methods traditionally used for measuring albumin lack the sensitivity and precision required for measuring microalbumin. The DCL Microalbumin Assay uses an immunoturbidimetric format which provides the sensitivity required for accurate determination of urinary microalbumin.

PRINCIPLE

When a sample is mixed with anti-human albumin goat antiserum, agglutination is caused by the antigen-antibody reaction. The turbidity is measured at 340 nm and 700 nm and albumin in the sample is quantitatively determined.

REAGENT PREPARATION

The reagents are provided in a ready to use format.

REAGENT STABILITY AND STORAGE

The reagents included are stable until the expiry date stated on the labels when stored at 2-8°C.

Opened reagents can be used for one month if stored at 2-8°C. Recap and return reagents to 2-8°C promptly after use.

All stability claims are based on the real time studies in Diagnostic Chemicals Limited laboratories.

REAGENT DETERIORATION

The reagent solutions should be clear. Turbidity would indicate deterioration.

Elevating Chemistry to a Fine Art

INSTRUMENTS

Any instrument with two reagent and multiple point calibration capabilities and a temperature control of ± 0.5°C that is capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 340/700 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

SPECIMEN COLLECTION AND PREPARATION

The specimen should be a fresh or a 24 hour urine. Urine specimens should be refrigerated (2-8°C). The specimens may be stored refrigerated up to a week or frozen at -20°C. USE PLASTIC TUBES FOR STORING THE SAMPLES, DO NOT USE GLASS.

INTERFERING SUBSTANCES

Ascorbic acid, glucose, uric acid, creatinine, creatine, calcium, sodium chloride, magnesium, potassium chloride, and urea do not interfere with the assay.

Interferences from icterus and hemolysis were evaluated for this microalbumin method on a Hitachi analyzer using a significance criterion of > 10% variance from control.

Bilirubin ditaurate levels of 0-712 µmol/L (0-60 mg/dL) were studied with acceptable results to levels of 712 µmol/L (60 mg/dL).

Hemoglobin levels of 0-155 µmol/L (0-1000 mg/dL) were studied with acceptable results to levels of 77.5 µmol/L (500 mg/dL).

A summary of the influence of drugs on clinical laboratory tests may be found by consulting Young, D.S. (6).

PROCEDURE

Materials Provided

The reagents necessary for the determination of microalbumin are provided. Refer to the instrument manual and the guidelines provided for adaptation to specific automated analyzers or contact DCL Technical Services for instrument specific parameters.

Conditions

Automated Analyzer

Wavelength	340/700 nm
Temperature	37°C
Pathlength	1 cm
Mode	Endpoint
Reaction Time	5 minutes
Sample Volume	10 µL
Reagent Volume	300 µL (R1) + 100 µL (R2)
Total Volume	410 µL
Sample to Reagent Ratio	1:30:10

CALIBRATION

For albumin concentrations less than 100 mg/L a two point calibration curve can be made using a saline blank (0 mg/dL) and an albumin standard. When a sample with a high albumin concentration (> 100 mg/L) is assayed, it is recommended that a multipoint calibration curve be made. DCL recommends its catalogue number SE-252 Microalbumin Multi-Calibrator Set.

QUALITY CONTROL

A normal and abnormal level urine control should be analyzed with each run of samples and the results should fall within plus or minus two standard deviations of the established value.

CALCULATION AND RESULTS

Results

Albumin concentration is expressed as mg/L (mg/dL).

Limitations

A sample with an albumin level exceeding the linearity limit should be diluted 1 part sample with 4 parts isotonic saline including 0.05% Tween 20 and reassayed incorporating the dilution factor of 5 in the calculation of the value.

EXPECTED VALUES

< 21 mg/L (2.1 mg/dL)

The expected values of microalbumin were determined using 24-hour urine collected from 250 healthy donors.

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

PERFORMANCE CHARACTERISTICS

These performance characteristics were generated in DCL laboratories using automated procedures unless otherwise stated.

Reportable Range (NCCLS EP6-P)

Reportable range is dependent on the sample to reagent ratio used. The automated procedure described gives a reportable range from 5-300 mg/L (0.5-30.0 mg/dL).

Precision Studies (NCCLS EP5-T2)

Data was collected on two control urine in 40 runs conducted over 20 days.

Level		Total SD		Total CV %	Within Run SD		Within Run CV %
mg/L	mg/dL	mg/L	mg/dL		mg/L	mg/dL	
7.9	0.79	0.47	0.047	6.0	0.34	0.034	4.3
54.9	5.49	2.00	0.200	3.6	1.61	0.161	2.9

Accuracy (NCCLS EP9-P)

The performance of this method (y) was compared with the performance of a similar method (x) on a Hitachi analyzer. Sixty-four patient urine samples ranging from 0-197 mg/L (0.0-19.74 mg/dL) gave a correlation coefficient of 0.9981. Linear regression analysis gave the following equation:

$$\text{This method} = 0.949 (\text{reference method}) + 0.36 \text{ mg/L.}$$

REFERENCES

1. Harmoinen, A. et. al. Clinica Chimica Acta. 149: 269-274, 1985.
2. Morgensen, C.E., N. Engl. J. Med. 310: 356-360, 1984.
3. Morgensen, C.E., N. Christensen, C.K., N. Engl. J. Med. 311: 89-93, 1984.
4. V. berti, G. C., et al. Lancet. 1430-32, 1982.
5. Tietz, N.W. (Ed.), Fundamentals of Clinical Chemistry, W.B. Saunders Co., Toronto, 636-638, 937 (1970).
6. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, AACC Press, Third Edition, Washington, 1990.

IN25220-2
December 13, 2004