CE

TURBILYTE® MA

TURBIDIMETRIC IMMUNOASSAY FOR DETERMINATION OF MICROALBUMINURIA

SUMMARY

Urinary albumin excretion between 30-300 mg/day (Microalbuminuria), far below the levels found in clinical Proteinuria (> 300 mg/day) is a strong predictor of development of Diabetic nephropathy and vascular complications. Diabetic nephropathy leads to progressive loss of renal function or end stage renal disease (ESRD) and may necessitate need for dialysis or transplantation in most cases. The progression of Microalbuminuria is closely associated with progressive hypertension and loss of blood glucose control. The early presence of Microalbuminuria can be reversed by strict metabolic control and timely intervention of drugs early in the course of disease can arrest the progression of diabetic renal disease. Quantitative values of albumin are useful for differentiating Microalbuminuria from clinical proteinuria and the effective monitoring of intervention strategies.
Annual screening of Microalbuminuria is recommended by 'WHO' and 'International Diabetes Foundation' in all patients with IDDM over the age of 12 years and who have had diabetes for five years or more. Microalbuminuria is also a significant risk marker of cardiovascular diseases. Its presence can be regarded as an index of increased cardiovascular vulnerability and a signal for correction of known risk factors.
Information regarding the concentration of albumin in urine for the detection of Microalbuminuria can be obtained by using **TURBILYTE®-MA** reagents.

REAGENT

1. **TURBILYTE®-MA** Activation Buffer (R1): Ready to use.
2. **TURBILYTE®-MA** Latex Reagent (R2):Ready to use uniform suspension of polystyrene latex particles coated with anti-human albumin antibody.
3. **TURBILYTE®-MA** Calibrator (S): Ready to use albumin solution and is equivalent to the stated amount of albumin on mg/L basis. The **TURBILYTE®-MA** calibrator is traceable to the IFCC reference material CRM 470.
   Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity, and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagents at 2-8°C. DO NOT FREEZE.
2. The shelf life of the reagents is as per the expiry date mentioned on the respective vial labels.

PRINCIPLE

TURBILYTE®-MA is a turbidimetric immunoassay for the detection of albumin in urine and is based on the principle of agglutination reaction. The test specimen is mixed with the **TURBILYTE®-MA** activation buffer (R1) and latex reagent (R2) and allowed to react. Presence of albumin in the test specimen forms an insoluble complex producing a turbidity, which is measured at wavelength 546 nm. The resulting turbidity corresponds to the concentration of albumin in the test specimen.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagents that are derived from human source have been tested for HBsAg and Anti-HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
3. Reagents contain 0.095% Sodium Azide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagents be verified using known controls periodically.
5. As the reagents within lots have been matched, reagents from different lots must not be interchanged.
6. Calibrators of different manufacturers must not be used with **TURBILYTE®-MA** reagents.
7. **TURBILYTE®-MA** can be used on any semi automated analyzer with appropriate programming facility. Fully automated analyzers may be used, provided the reagent has been standardized on the system.
8. The procedures mentioned in this pack insert are based on a minimum reading volume of 500 µl (0.5 ml). In case of instruments where minimum volume required for reading absorbance is 1.0 ml, use double the quantity of reagents and samples mentioned in the test procedure.
9. Do not use damaged or leaking reagents.

SPECIMEN COLLECTION AND PREPARATION

Though random urine specimen can be used, preferably first morning urine specimen should be collected in clean dry glass or plastic containers free from detergents and even traces of proteins. Specimen should be tested immediately preferably within 12 hours of collection. Specimen can be, stored upto 2 days at 2- 8°C provided they are not contaminated. Specimen should be free from particulate matter. Turbid or particulate urine specimen must be clarified by centrifugation at 2000 rpm for 10 minutes. Use the clear supernatant for testing.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.
This product requires the handling of human specimens. It is recommended that all human sourced material are considered potentially hazardous and are handled in accordance with the OSHA standard on blood borne pathogens.
Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

ADDITIONAL MATERIAL REQUIRED

Spectrophotometer with 546 nm wavelength filters, stopwatch, well calibrated micropipettes, disposable tips, isotonic saline, particulate free distilled water, test-tubes, test-tube rack, incubator/ waterbath set at 37°C, optically clean disposable/glass **semi micro cuvettes**.

TEST PROCEDURE

Bring reagent and sample to room temperature before use.
Assay conditions;

Wavelength	546 nm
Reaction Temperature	37°C
Path length	1 cm

The **TURBILYTE®-MA** calibrator is ready to use for MA calibration. The Concentration (S) of MA calibrator is as mentioned on the calibrator vial label.

Pipette into the cuvette:

	For calibration	For sample
R1	450 µl	450 µl
R2	50 µl	50 µl
Mix well and incubate for 5 minutes		
Calibrator	10 µl	-
Sample	-	10 µl
Mix well and read absorbance A1 at 10 seconds and A2 at 2 minutes.		

CALCULATIONS

1. Calculate ΔA:
   ΔA = (A2-A1).
2. Concentration of MA in sample = $\frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}}$ x Concentration (S) of calibrator

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity

The Linearity of **TURBILYTE®-MA** is upto 150 mg/L. The linearity limit depends on the sample to reagent ratio as well as the analyzer used. It will be higher by decreasing the sample volume, though the detection limit of the assay will be proportionately decreased.

Detection limit / Analytical Sensitivity

Detection limit: 10 mg/L

The detection limit represents the lowest measurable albumin concentrations that can be distinguished from zero.

Prozone limit

No prozone effect was observed with albumin concentration upto 1000 mg/L.

Precision

Within Run	n	Mean mg/L	SD	CV(%)
Sample 1	10	14.5	1.27	8.79
Sample 2	10	20.5	1.07	5.21
Sample 3	10	121.3	1.25	1.03

Between run	n	Mean mg/L	SD	CV(%)
Sample 1	10	14.3	1.27	8.88
Sample 2	10	20.7	1.07	5.16
Sample 3	10	121.3	1.26	1.04

Interference

No interference was observed by Glucose upto 400 mg/dl, Bilirubin upto 50 mg/dl and Haemoglobin upto 500 mg/dl.

REFERENCE VALUES

The reference values of urinary albumin in normal population are ≤ 20 mg/L. The reference values vary with regards to the time of collection of the urine sample.

REMARKS

(1) Microalbuminuria is classified as: Albumin excretion rate : 20-200 µg/min, Albumin/Creatinine ratio .2.5 - 25 mg/mmol, Albumin/Creatinine ratio 30-300 mg/g, Albumin concentration (early morning urine) : 30-300 mg/L. (2) For determining albumin excretion rate, ideally a 24-hr urine must be used as a sample. (3) Usage of well-calibrated equipment and accessories and procedures is critical for achieving correct results. (4) Samples with values beyond the linearity limit have to be diluted with isotonic saline and retested. The values obtained must be multiplied with the dilution factor for calculating the result. (5) Microalbuminuria also occurs in response to acute inflammatory conditions such as Ischaemia, trauma and thermal injury, surgery, pancreatitis and inflammatory bowel diseases. In many of these conditions the albumin excretion increases within minutes or hours of the initiating stimulus and only lasts for 24 - 72 hours. (6) Albumin excretion has also been associated with urinary tract infections. (7) Use only urine as test specimen. Do not use serum. (8) Contaminated and turbid urine samples could produce erroneous albumin values. (9) Albumin excretion is increased after physical activity. It is therefore recommended to use urine sample that has been produced at rest whenever random urine specimen is used. (10) As albumin excretion is subject to physiological fluctuations it is necessary to take two measurements in consecutive days, in case of contradictory results three measurements on different days must be done preferably within a week. (11) Liquid intake of the patient must be in the normal range i.e. 1.5 - 2 litres/day. (12) To diagnose incipient nephropathy, microalbuminuria must be present in at least 2 out of 3 specimens over a 3-6 month period. (13) It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis. (14) Do not read results beyond two minutes.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

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Manufactured by:

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