



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 **Quantia[®]-MA** reagents.

REAGENT:

Quantia[®]-MA assay contains:

1. **Quantia[®]-MA** Activation Buffer (R1): Ready to use.
2. **Quantia[®]-MA** anti-human albumin Reagent (R2): A ready to use solution of anti-human albumin antibody.
3. **Quantia[®]-MA** calibrator: Ready to use albumin solution and is equivalent to the stated amount of albumin on mg/L basis. The **Quantia[®]-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 reagent vial labels.
3. The working reagent for **Quantia[®]-MA** be prepared by mixing R2 and R1 in the ratio 1:6.
4. The mixed stability of the working reagent (R1+ R2) is 7 days when stored at 2-8°C.

PRINCIPLE

Quantia[®]-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 activation buffer (R1) and **Quantia[®]-MA** anti-human antibody solution (R2) and allowed to react. Presence of albumin in the test specimen forms an insoluble complex producing a turbidity, which is measured at wavelength 340 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. All the materials derived from human source have been tested for HBsAg and Anti-HIV antibodies and found to be non-reactive.
3. The reagents contain 0.095% sodium azide as preservative. Avoid contact with skin or 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 reagents be verified by testing with known controls periodically.
5. Shake the **Quantia[®]-MA** anti-human albumin reagent well before use to improve test performance.
6. Do not use vortex mixers for mixing. Gently mix the reagents and samples during test procedures.
7. The **Quantia[®]-MA** assay is recommended only for analyzers with cuvette mode. Though any semi automated analyzer

with appropriate programming facility can be used, for best results it is recommended to use **Quantiamate** analyzer. Fully automated analyzers may be used, provided the reagent has been standardized on the system.

8. Calibrators of different manufacturers must not be used with **Quantia[®]-MA** reagents.
9. The calibration curve must be validated periodically with known controls.
10. As the reagents within lots have been matched, reagents from different lots must not be interchanged.
11. 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.

SAMPLE 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.

ADDITIONAL MATERIAL REQUIRED

Spectrophotometer with 340 nm wavelength and cuvette mode, stopwatch, well calibrated micropipettes, disposable tips, isotonic saline, particulate free distilled water, test-tube rack, optically clean disposable cuvettes such as **Quantimate semi micro cuvettes** / glass cuvettes.

TEST PROCEDURE

Bring reagents and specimen to room temperature before use.

Assay conditions:

Wavelength	340 nm
Reaction Temperature	37°C
Cuvette	1 cm path length

Method for preparation of MA Calibration Curve

The concentration (S) of **Quantia[®]-MA** calibrator is as mentioned at the end of this package insert.

Dilute the **Quantia[®]-MA** calibrator serially as mentioned below for preparation of calibration curve.

Test Tube No.	1	2	3	4	5
Calibrator dilution No.	D1	D2	D3	D4	D5
Vol. of Calibrator	100 µl	50 µl D1	50 µl D2	50 µl D3	50 µl D4
Vol. of Isotonic Saline	-	50 µl	50 µl	50 µl	50 µl
Conc. of albumin in mg/L	S	S/2	S/4	S/8	S/16

The above five dilutions of the calibrator including the highest 400 mg/L (D1) and lowest 25 mg/L (D5) concentrations of measuring range must be used for the preparation of the calibration curve.

Note: During calibration on instrument with programming facilities, increasing concentration of the standards must be used for preparing calibration curve.

Test procedure for preparation of calibration curve

1. Zero the instrument with distilled water.
2. Pipette 500 µl of **Quantia[®]-MA** buffer (R1) and 100 µl of **Quantia[®]-MA anti-human abumin** reagent (R2) in the measuring cuvette. Mix well and incubate for 5 minutes at 37°C.

OR

- Pipette 600 µl of the **Quantia[®]-MA** reagent in the measuring cuvette. Mix well and incubate for 5 minutes at 37°C.
3. Add 10 µl of calibrator D1, mix gently and start the stopwatch simultaneously.
4. Read absorbance (A1), **exactly** at ten seconds, and absorbance (A2) again at the end of **exactly** two minutes.
5. Repeat steps No. 2 - 4 for each diluted calibrator (D2 to D5) for preparing the calibration curve.

- Calculate $\Delta(A_2-A_1)$ for each calibrator (D1 to D5). Plot a graph of ΔA versus concentration of albumin on the graph paper provided with the kit.

“The calibration curve”so obtained is valid only for the same lot of Quantia[®]-MA reagents.

Test procedure for specimen

For determination of albumin concentration in the test specimen:

- Follow steps 2 - 4 as mentioned in the above procedure for calibration curve using the test specimen in place of the calibrator.
- Calculate $\Delta(A_2-A_1)$ for the test specimen.

VALIDATION CRITERIA

If the ΔA of the test specimen is less than ΔA obtained for the calibrator of highest concentration (D1) then the concentration of albumin in the test specimen can be determined directly by interpolating ΔA of the test specimen on the obtained calibration curve.

If the ΔA of the test specimen is higher than ΔA of standard with highest concentration (D1) then the test has to be rerun by carrying out serial dilutions of test specimen such as 1:2, 1:4 etc. till the ΔA of the diluted test specimen is less than ΔA of D1.

The albumin concentration for such samples can be determined as mentioned in **calculations**.

CALCULATIONS

- Interpolate ΔA of the diluted test specimen on the calibration curve and obtain the albumin concentration 'C' of the diluted test specimen.
- Multiply the albumin concentration 'C' with the dilution factor (F) of the test specimen for obtaining the concentration of albumin in the neat test specimen.

$$\text{Concentration of albumin in the neat test specimen in mg/L} = C \times F$$

(Where 'F' is the dilution factor of the test specimen, for e.g. 2 for 1:2 dilution of test specimen and so on)

SPECIFIC PERFORMANCE CHARACTERISTICS

Measuring range

The Quantia[®]-MA reagent has been designed to measure albumin concentrations in the range of about 25 - 400 mg/L and is linear within the measuring range. The exact range is dependent on the Calibrator value, which is lot specific.

Detection limit / Analytical Sensitivity

Detection limit: 20 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

Intra-assay precision	n	Mean mg/L	SD	CV(%)
Sample 1	15	30	1.53	5.16
Sample 2	15	150	3.6	2.41
Sample 3	15	325	7.18	2.22

Inter-assay precision	n	Mean mg/L	SD	CV(%)
Sample 1	15	30.07	2.31	7.68
Sample 2	15	150.53	5.71	3.79
Sample 3	15	325.47	12.99	3.99

Interference

No interference was observed with:

Interference Factor	No Interference upto
Glucose	400 mg/dl
Bilirubin	50 mg/dl
Haemoglobin	500 mg/dl
Creatinine	6 g/L
Urea	2g/L

REFERENCE VALUES

The reference values of urinary albumin in normal population are ≤ 25 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 μ g/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. When ΔA obtained for the test specimen is greater than the ΔA of the standard with highest concentration then, it indicates that the concentration of albumin in the test specimen is beyond the measuring range of the **Quantia[®]-MA** assay. Such specimens should be rerun with further dilutions.
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.

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