FUJIFILM

Code No. 999-06001

Autokit Micro Albumin

Intended use

The Autokit Micro Albumin test is an in vitro assay for the quantitative determination of albumin in human urine.

Summary and explanation of the test

Diabetic nephropathy, which is accompanied by irreversible kidney damage and persistent proteinuria, is a major cause of death in persons with insulin-dependent diabetes mellitus and a main reason to initiate hemodialysis.
Therefore, detection of kidney (glomerular) damage that is minimal and reversible is important. Microalbuminuria is a condition characterized by increased urinary excretion of albumin in the absence of overt nephropathy. It has been reported in several studies to predict development of diabetic nephropathy and its mortality risk in both diabetes mellitus of insulin-dependent and non-insulin-dependent.
Because microalbuminuria may be reversible if diabetes is well controlled, its early detection may be very beneficial in treatment programs for diabetes.
Microalbuminuria is not generally measurable by the test ordinary used to detect proteinuria because it lacks sensitivity. For the detection of microalbuminuria, RIA, ELISA and nephelometry assay are used. However they have complicated procedures and are time demanding.
Autokit Micro Albumin is a turbidimetric immunoassay using polyclonal antibodies against human albumin and allows accurate and reproducible measurement of microalbuminuria with a simple procedure.

Principle of the method

When a sample is mixed with Buffer and Antibody, albumin in the sample combines specifically with anti-human albumin antibody (goat) in the Antibody to yield an insoluble aggregate that causes increased turbidity in the solution. The degree of turbidity of solution can be measured optically and is proportional to the concentration of albumin in the patient's sample.

Reagents

(1) Buffer

50 mmol/L Good's buffer (pH 7.4) containing 0.09% sodium azide.
Store at 2-10℃.

(2) Antibody

100 mmol/L Tris-HCL buffer (pH 7.4) containing 1.8 mg/mL anti-human albumin, Goat and 0.09% sodium azide.
Store at 2-10℃.

Warnings and precautions

- For in vitro diagnostic use.
- Not to be used internally in humans and animals.
- Do not use reagents past the expiration date stated on each reagent container label.
- Do not use the reagents described above for any purpose other than described herein.
- Do not use the reagent which was frozen by mistake.
- After opening the reagent, it is not recommended to store it for more than 1 month at 2-10℃. When the opened reagent is stored, cap the bottle and keep it under the specified conditions.
- In some instances, falsely high or low results occur due to non-specific turbidity. If a result is questionable, inspect the reaction course or dilute the sample and repeat analysis.
- If the reagents come in contact with mouth, eye or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
- Buffer and Antibody contain 0.09% sodium azide as a preservative. Sodium azide may react with copper or lead plumbing to form explosive compounds. Even though the reagents contain minute quantities of sodium azide, drains should be well flushed with a large amount of water when discarding the reagents.

Physical or chemical indications of instability

The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent instability.

Instruments

The reagent is designed to be used on commercially available automated analyzers such as TBA120FR/c8000® analyzer. Refer to the operating manual for a description of instrument operation, specifications and calibration.

Specimen collection and preparation

Use urine as a specimen. Collect urine either overnight, during 24 hours or as a random, midstream sample in a glass or plastic collection container. Samples can be stored for 4 weeks at 4-8℃ for 6 months frozen at −20℃ without significant effect on the measured values. Repeated freezing and thawing should be avoided.
Preservatives such as toluene, xylene, hydrochloric acid, formalin, chloroform and acetic acid have no influence on the assay when employed in their usual amounts.
Ascorbic acid, glucose, urea, calcium, inorganic phosphorus, hippuric acid, hemoglobin, bilirubin and urobilinogen do not interfere with measurement.

Warning/Biohazard

It is recommended that specimen collection be carried out in accordance with NCCLS Document M29-A.
Since all specimen are potentially infectious, they should be handled at the Biosafety Level 2 as recommended for any potentially infectious body fluid in the USA Center for Disease Control / USA National Institutes of Health manual ''Biosafety in micro biological and Biomedical Laboratories'', and in accordance with any other local or national regulations relating to the safe handling of such materials.

Procedure for TBA120FR/c8000® analyzer

Materials supplied

Refer to the section entitled ''Reagents.''

Materials required but not supplied

TBA120FR/c8000® analyzer
Quality control material
Calibrator
All analyzer applications should be validated in accordance with CLIA recommendations. For further assistance call Wako Diagnostics Technical Service Department at 1-800-992-9256.

Reagent preparation

Reagent 1: Use Buffer as supplied.
Unopened Reagent 1 is stable until expiration date.
After opening the bottle, it is satable for 1 month at 2-10℃.

Reagent 2: Use Antibody as supplied.
Unopened Reagent 2 is stable until expiration date.
After opening the bottle, it is stable for 1 month at 2-10℃.

Test procedure

Parameter setting (TBA120FR/c8000®)

Temperature: 37℃

ANALYZE	Autokit Micro Albumin
CH TEST	Micro Albumin
REACTION MODE	END
UP/DOWN	UP
TEST/BLANK WAVELENGTH	340 / 700
TEST READ TIMING	31 - 33
END STABILITY	0.05
SAMPLE BLANK TEST	Micro Albumin
BLANK READ TIMING	14 - 16
SAMPLE VOLUME/DIL VOLUME	6.0 / 0.0
R1 VOLUME	180
R2 VOLUME	30
FACTOR / BASE	1.0 / 0.0
DECIMAL POINT LOC.	1
UNIT	μg/mL
BLANK/STD.REP	3 / 3
CALIB.METHOD	SPLINE
BLANK	0.0 - *2
STD.(1) CONC.-POS.	*1 - *2
STD.(2) CONC.-POS.	*1 - *2
STD.(3) CONC.-POS.	*1 - *2
STD.(4) CONC.-POS.	*1 - *2
STD.(5) CONC.-POS.	*1 - *2

*1 Input the assigned value of the calibrator.
*2 Input the position of the calibrator.

Results

The final results are automatically calculated and printed in activity. The results are given in μg/mL.

Calibration

The Autokit Micro Albumin assay produces a standard curve by plotting absorbance vs. concentration. It is recommended to perform calibration as required by changes in quality control results. Refer to the TBA120FR/c8000® Operator's Manual for details on performing calibration.

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. The values obtained for controls should fall within the manufacturer's acceptable ranges. If values are to be established for unassayed control material, the laboratory should assay each level of control material a sufficient number of times to generate a valid mean and acceptable range.

Limitations of the procedure

In the case of multi-point calibration, the linearity of Autokit Micro Albumin is up to 500 μg/mL. If the albumin value exceeds the upper limit of measurable range, dilute the sample with saline, repeat the assay and multiply the result by the dilution factor.
Prozone phenomenon is not observed up to 10000 μg/mL.

Expected values[1]

1.0-14.0 mg/dL

Urinary Albumin Excretion

	μg/min	mg/24 hr	Albumin:Creatinine
Normal	<10	<15	<0.01
IDDM	20-200	30-300	0.02-0.2
Diabetic nephropathy	>200	>300	>0.2

Since expected values are affected by age, sex, diet, geographical location and other factors, each laboratory should establish its own expected values for this procedure.

Performance characteristics

Accuracy

The accuracy of this method was demonstrated by a recovery study.

No.	Expected value (μg/mL)	Obtained value (μg/mL)	Recovery (%)
1	33.1	32.6	98.4
2	58.1	56.9	97.8
3	118.1	117.6	99.6

Precision

Within-run precision

Sample #	Replicates	Mean (μg/mL)	SD	CV (%)
1	21	4.97	0.09	1.81
2	21	105.81	0.79	0.75
3	21	510.84	3.91	0.77

Total precision

The data was collected according to NCCLS EP5-T2 Guideline.

Number of assay days	Mean (μg/mL)	SD	CV (%)	Swr	ST
21	4.90	0.14	2.86	0.23	0.23
21	511.21	7.68	1.50	7.65	9.78

Sensitivity

The minimum detectable level of this method is estimated to be 0.33 μg/mL. A known sample with zero activity was run 21 times. The mean and 2sd was calculated. The lower limit of sensitivity/detection was calculated as the mean plus 2sd.

Correlation

60 samples were correlated between the Autokit Micro Albumin assay and a commercially available product. This correlation study resulted in a correlation coefficient of 0.9984 and a regression equation of y = 1.0179x − 0.9619.

Specificity

Additive study

Ascorbic acid (mg/dL)	None	50	100	200	400
Albumin (μg/mL)	27.3	26.9	27.1	26.9	27.1

Glucose (mg/dL)	None	500	1000	2000	4000
Albumin (μg/mL)	27.0	27.2	26.9	26.9	26.6

Uric acid (mg/dL)	None	12.5	25.0	50.0	100.0
Albumin (μg/mL)	26.6	26.8	26.9	26.4	26.2

Bilirubin (mg/dL)	None	3.1	6.2	12.5	25
Albumin (μg/mL)	26.1	26.2	26.6	26.3	26.3

Urea (mg/dL)	None	50	100	200	400
Albumin (μg/mL)	26.9	27.0	26.8	26.7	27.0

Urobilinogen (mg/dL)	None	2.5	5.0	10	20
Albumin (μg/mL)	25.0	25.2	25.2	25.6	26.3

Creatinine (mg/dL)	None	50	100	200	400
Albumin (μg/mL)	26.9	26.9	26.7	26.7	26.9

pH	3	4	5	6	7	8	9	10
Albumin (μg/mL)	25.4	26.3	26.6	26.6	26.8	26.5	26.0	26.5

Sodium chloride (mg/dL)	None	250	500	1000	2000
Albumin (μg/mL)	27.0	26.8	26.9	26.6	26.7

6N Hydrochloric acid (mL/dL)	None	0.5	1.0	2.0	4.0
Albumin (μg/mL)	26.5	26.2	26.4	25.5	24.4

Potassium chloride (mg/dL)	None	125	250	500	1000
Albumin (μg/mL)	27.0	26.8	26.9	26.8	26.8

Toluene (μL/dL)	None	125	250	500	1000
Albumin (μg/mL)	26.5	26.7	26.7	26.2	26.1

Calcium (mg/dL)	None	50	100	200	400
Albumin (μg/mL)	26.9	27.4	27.0	27.1	27.3

Chloroform (μL/dL)	None	125	250	500	1000
Albumin (μg/mL)	26.5	27.1	26.3	26.6	25.9

Inorganic phosphorus (mg/dL)	None	50	100	200	400
Albumin (μg/mL)	26.9	26.9	27.0	26.7	26.9

Acetic acid (μL/dL)	None	125	250	500	1000
Albumin (μg/mL)	26.4	26.0	25.8	25.6	25.9

Hippuric acid (mg/dL)	None	50	100	200	400
Albumin (μg/mL)	26.9	26.8	26.6	26.7	26.3

Thymol (mg/dL)	None	6.3	12.5	25	50
Albumin (μg/mL)	26.9	27.4	27.0	27.1	27.3

Hemoglobin (mg/dL)	None	62.5	125	250	500
Albumin (μg/mL)	26.4	26.6	26.7	26.5	26.5

Formalin (μL/dL)	None	125	250	500	1000
Albumin (μg/mL)	26.5	26.7	26.6	26.6	26.6

Xylene (μL/dL)	None	125	250	500	1000
Albumin (μg/mL)	26.3	26.3	26.4	26.3	26.6

References

1. Burtis, C. A. and Ashwood, E. R., Ed. : Tietz Textbook of Clinical Chemistry, 2nd Ed., Saunders, Philadelphia, 1994.
2. Lothar Thomas, M. D., Ed. : Clinical Laboratory Diagnostics.
3. DG Klinische Chemie Mitteilungen 26 (1995) page5.
4. Mount.J.N. : Journal of Clinical Pathology, **22**, 12 (1986).
5. Rosenstock, j. and Raskin,P. : Diabetes Care, **9**, 529 (1986).
6. Mogensen, C. E., Christensen, C. K. and Vittinghus, E. : Diabetes, 32 (Suppl.2), 64 (1983).
7. Viberti, G. C. et al : Kidney International, **21**, 714 (1982).
8. Parving, H-H, et al : Acta Endocrinologica, **100**, 550 (1982).
9. Viberti, G. C. et al : Lancet, **1**, 1430 (1982).
10. Schmitz, A. and Vaeth, M. : Diabetic Medicine, **5**, 126 (1988).
11. Mogensen, C. E. et al : N. Engl. J. Med., **311**, 89 (1984).
12. Mogensen, C. E. : N. Engl. J. Med., **310**, 356 (1984).

Ordering information

Code No.	Product	Package
999-06001	Autokit Micro Albumin	
	Buffer	2 × 45 mL
	Antibody	1 × 15 mL
995-06101	Autokit Micro Albumin Calibrator Set	5 conc. × 2 mL
991-06201	Autokit Micro Albumin Control Set	2 × 2 conc. × 2 mL

Revision date ; Apr. 1, 2018

Manufactured by
FUJIFILM Wako Pure Chemical Corporation

1-2, Doshomachi 3-Chome, Chuo-Ku Osaka 540-8605, Japan
Tel : +81-6-6203-3749
Fax : +81-6-6203-1917
www.wako-chem.co.jp

Distributed by
FUJIFILM Wako Diagnostics U.S.A. Corporation

1025 Terra Bella Ave., Mountain View, CA 94043 U.S.A.
Tel : 877-714-1924
Fax : 804-271-0449
www.wakodiagnostics.com

18.03.19K04