Alinity c Microalbumin (Microalb)-19

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BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity c Microalbumin assay is used for the quantitative measurement of albumin in human urine on the Alinity c analyzer.

SUMMARY AND EXPLANATION OF THE TEST

Microalbuminuria is a condition characterized by increased urinary excretion of albumin in the absence of overt nephropathy, and can be used to predict diabetic nephropathy.[1, 2] Diabetic nephropathy is a major cause of death in individuals with insulin-dependent diabetes; and because it is accompanied by irreversible kidney damage and persistent proteinuria, it is a major factor in the decision to initiate hemodialysis.[3, 4]

Early detection of glomerular damage, when it is minimal and reversible, is extremely important. Monitoring urinary microalbumin is an important component of treatment for both Type I and Type II diabetes mellitus.[3] Methods for monitoring microalbuminuria include measurement of protein excretion in 24 hour, timed, or overnight collections, and determination of the albumin:creatinine ratio in an untimed "spot" urine specimen. Twenty-four hour and timed urine collections may be associated with collection errors including improper timing, missed samples, and incomplete bladder emptying. The concentration of protein in a spot urine sample provides an estimate of the protein excretion rate, but is affected by patient hydration. The ratio of protein or albumin to creatinine in a spot urine sample corrects for variations in hydration and avoids the sources of error associated with 24 hour and timed urine collection.[5]

PRINCIPLES OF THE PROCEDURE

The Microalbumin assay is a turbidimetric immunoassay that uses polyclonal antibodies against human albumin. When a specimen is mixed with the reagents, albumin in the specimen combines with the anti-human albumin antibody (goat) in the reagent to yield an insoluble aggregate that causes increased turbidity in the solution. The degree of turbidity is proportional to the concentration of albumin in the specimen, and can be measured optically.

Methodology: **Turbidimetric/Immunoturbidimetric**

For additional information on system and assay technology, **refer to the Alinity ci-series Operations Manual, Section 3.**

REAGENTS

Kit Contents

Alinity c Microalbumin Reagent Kit 08P04

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	**08P0420**
Tests per cartridge	320
Number of cartridges per kit	2
Tests per kit	640
R1	67.8 mL
R2	15.6 mL
R1 Active ingredients: Good's buffer (1.03%), Sodium chloride (1.48%), Sodium hydroxide (< 0.14%). Preservative: sodium azide (0.09%).	
R2 Active ingredients: TRIS buffer (1.17%), Anti-human albumin antibody (goat) (0.17%), Sodium chloride (1.14%), Hydrochloric acid (< 0.9%). Preservative: sodium azide (0.09%).	

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY
- R2 contains non-sterile goat polyclonal antibodies.

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[6], [7], [8], [9]

The following warnings and precautions apply to: R1	
Contains 4-morpholinopropanesulphonic acid* and sodium azide.	
H316*	Causes mild skin irritation.
EUH032	Contact with acids liberates very toxic gas.
Response	
P332+P313*	If skin irritation occurs: Get medical advice / attention.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

* Not applicable where regulation EU 1272/2008 (CLP) or OSHA Hazard Communication 29CFR 1910.1200 (HCS) 2012 have been implemented.

The following warnings and precautions apply to: R2	
Contains tris hydroxymethyl aminomethane* and sodium azide.	
H316*	Causes mild skin irritation.
EUH032	Contact with acids liberates very toxic gas.
Response	

P332+P313*	If skin irritation occurs: Get medical advice / attention.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

* Not applicable where regulation EU 1272/2008 (CLP) or OSHA Hazard Communication 29CFR 1910.1200 (HCS) 2012 have been implemented.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 8.

Reagent Handling

- Reagents are shipped refrigerated or on cold packs.
- Upon receipt, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 8 hours before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 7.**

Reagent Storage

- Do not freeze.

	Storage Temperature	**Maximum Storage Time**	**Additional Storage Instructions**
Unopened	2 to 8°C	Until expiration date	Store in upright position.
Onboard	System Temperature	28 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
			performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, refer to **the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, refer to **the Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Alinity c Microalbumin assay file must be installed on the Alinity c analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the Alinity ci-series Operations Manual, Section 2.

For information on printing assay parameters, refer to the Alinity ci-series Operations Manual, Section 5.

For a detailed description of system procedures, refer to the Alinity ci-series Operations Manual.

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit
µg/mL	1.0	mg/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen type used for this assay is urine only.

The specimen types listed below were verified for use with this assay.

Specimen Type	Collection Vessel	Special Conditions
Urine (random midstream specimens (spot collection))	Clean, unused plastic or glass container with preservatives	A urine creatinine result is required to calculate a spot collection microalbumin-to-creatinine ratio.
Urine (timed specimens)	Clean, unused plastic or glass container with preservatives	
Urine (24 hour)	Clean, unused plastic or glass container with preservatives	

The following are acceptable preservatives: **6N hydrochloric acid, acetic acid, chloroform, formalin, toluene, and xylene.**

- The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Urine (random midstream specimens (spot collection))	4°C	6 days*10*	For spot samples, which require the analysis of both albumin and creatinine, analyze fresh specimens if possible.
Urine (timed or 24 hour specimens)	4°C	2 weeks*3*	Analyze specimens as soon as possible following collection.
	-70°C	5 months*3*	

Avoid multiple freeze/thaw cycles.

Stored specimens must be inspected for particulates. If present, mix with a low speed vortex or by inversion and centrifuge the specimen to remove particulates prior to testing.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

RESULTS

Calculation

The Alinity c Microalbumin assay utilizes the Spline data reduction method to generate a calibration and results.

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

Interpretation of Results

As with all analyte determinations, the microalbumin value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.

Measuring Interval

Measuring interval is defined as the range of values in µg/mL (mg/L) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity c Microalbumin assay **is 5.0 to 500.0** µg/mL (5.0 to 500.0 mg/L).

PROCEDURE

Materials Provided

08P04Alinity c Microalbumin Reagent Kit

Materials Required but not Provided

- Alinity c Microalbumin assay file
- 08P0401 Alinity c Microalbumin Calibrators
- 08P0410 Alinity c Microalbumin Controls or other commercially available controls
- Saline (0.85% to 0.90% NaCl) for specimen dilution

For information on materials required for operation of the instrument, refer to the Alinity ci-

series Operations Manual, Section 1.

For information on materials required for maintenance procedures, refer to the Alinity ci-series Operations Manual, Section 9.

Assay Procedure

For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
- Minimum sample volume requirements:
  - Sample volume for single test: 6.0 µL.

    NOTE: This amount does not include the dead volume plus the additional over-aspiration volume. For total sample volume requirements, refer to the Alinity ci-series Operations Manual, Section 4.
- Refer to the Alinity c Microalbumin Calibrators package insert and Alinity c Microalbumin Controls package insert or commercially available control material package insert for preparation and usage.
- For general operating procedures, refer to the Alinity ci-series Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

Samples with an albumin value exceeding 500.0 µg/mL (500.0 mg/L) are flagged with the code "> 500.0 µg/mL" (> 500.0 mg/L) and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a **1:4 d**ilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor.

Manual Dilution Procedure

Dilute the sample with saline (0.85% to 0.90% NaCl).

The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result.

If the operator does not enter the dilution factor, the result must be manually multiplied by

the appropriate dilution factor before reporting the result. If a diluted sample result is less than the lower value of the measuring interval of 5.0 µg/mL (5.0 mg/L), do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to the Alinity ci-series Operations Manual, Section 5.

Calibration

CAUTION: The Microalbumin assay uses polyclonal antibodies against human albumin that can react with closely related proteins such as bovine albumin. **Therefore, calibrators containing non-human protein should not be used with this assay.** The Alinity c Microalbumin Calibrators contain only human albumin.

For instructions on performing a calibration, refer to the Alinity ci-series Operations Manual, Section 5.

Calibration is stable for approximately **28 days (672 hours),** but is required with each change in reagent lot. Verify calibration with at least 2 levels of controls according to the established quality control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

CAUTION: The Microalbumin assay uses polyclonal antibodies against human albumin that can react with closely related proteins such as bovine albumin. This cross-reactivity may vary among reagent lots and cause shifts in control values. **Therefore, control material containing non-human protein should not be used with this assay.** The Alinity c Microalbumin Controls contain only human albumin and are not affected by cross-reactivity.

As appropriate, refer to your laboratory standard operating procedure(s) and/or quality assurance plan for additional quality control requirements and potential corrective actions.

- At least two levels of controls one normal and one abnormal every day testing performed.
- If quality control results do not meet the acceptance criteria defined laboratory procedure, sample results may be suspect. Follow the established quality control procedures to troubleshoot. Recalibration may be necessary. For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10.**
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Quality Control Guidance

Refer to “Basic QC Practices” by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[11]

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

LIMITATIONS OF THE PROCEDURE

- In some instances, falsely high or low results occur due to non-specific turbidity. Prozone may be observed at albumin concentrations greater than 10 000 µg/mL. If a result is questionable, dilute the sample and repeat the analysis.

Refer to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS, CALIBRATION, QUALITY CONTROL PROCEDURES and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert.

EXPECTED VALUES

It is recommended that each laboratory determine its own reference range based upon its particular locale and population characteristics.

According to the American Diabetes Association (ADA) microalbumin levels can be measured from 24 hour, timed, or random urine specimens. Normal albumin levels, microalbuminuria, and macro (clinical) albuminuria levels for each specimen type are listed below.[12, 13]

	Spot collection[a]		**24 hour collection[b]**	**Timed collection[c]**
	Microalbumin:Creatinine ratio			
	(µg/mg) or (mg/g)	**(mg/mmol)**	**(mg/24 hr)**	**(µg/min)**
Normal	< 30	< 2.5 (male) < 3.5 (female)	< 30	< 20
Microalbuminuria	30 to 299	2.5 to 29 (male) 3.5 to 29 (female)	30 to 299	20 to 199
Macro (clinical) albuminuria	≥ 300	> 30 (male) > 30 (female)	≥ 300	≥ 200

a. Spot collection = (microalbumin (µg/mL) ÷ creatinine urine (mg/dL)) × $\frac{100\ \text{mL}}{\text{dL}}$

or microalbumin (mg/L) ÷ creatinine urine (mmol/L)

b. 24 hour collection = (microalbumin (µg/mL) × Volume mL) ÷ $\frac{1000\ \mu\text{g}}{}$

mg

c. Timed collection = (microalbumin (µg/mL) × Volume mL) ÷ Time (min)

Due to variability in urinary albumin excretion, at least two of three test results measured within a 6-month period should show elevated levels before a patient is designated as having microalbuminuria.[14] Exercise within 24 hours, infection, fever, congestive heart failure, marked hyperglycemia, and marked hypertension may elevate urinary albumin excretion over baseline values.[12]

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity c analyzer, and the ARCHITECT c System and AEROSET System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity c analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2.[15] Testing was conducted using 1 lot of the Alinity c Microalbumin Reagent Kit, 1 lot of the Alinity c Microalbumin Calibrators, 1 lot of control material containing albumin and 1 instrument. Two controls were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

Sample	n	Mean µg/mL (mg/L)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Control Level 1	119	32.0	0.31	1.0	1.47	4.6
Control Level 2	120	91.4	0.91	1.0	2.05	2.2

[a]Includes within-run, between-run, and between-day variability.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.[17] Testing was conducted using 3 lots of the Alinity c Microalbumin Reagent Kit on each of 2 instruments over a minimum of 3 days. The maximum observed Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below.

	µg/mL (mg/L)
LoB[a]	0.2
LoD[b]	0.4
LoQ[c]	**1.3**

[a]The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b]The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c]The LoQ was determined from n ≥ 60 replicates of low-analyte level samples and is defined as the lowest concentration at which a maximum allowable precision of 20 %CV was met.

Linearity

A study was performed based on guidance from CLSI EP06-A.*16*

This assay is linear across the measuring interval of 5.0 to 500.0 µg/mL (5.0 to 500.0 mg/L).

Interference

This study was performed on the AEROSET System.

Potentially Interfering Substances

A study based on CLSI protocol NCCLS EP7-P*18* using urine specimens containing approximately 27 µg/mL microalbumin supplemented with the individual compounds listed below.

Potential interference in the microalbumin assay is ≤ 10% for the urine preservatives and interferents listed below when used in the amounts shown.

Potentially Interfering Substance	Interferent Level	
	Default Units	**Alternate Units**
6N Hydrochloric acid	4 mL/dL	40 mL/L
Acetic acid	1000 µL/dL	10 mL/L
Chloroform	1000 µL/dL	10 mL/L
Formalin	1000 µL/dL	10 mL/L
Thymol	50 mg/dL	500 mg/L
Toluene	1000 µL/dL	10 mL/L
Xylene	1000 µL/dL	10 mL/L

Potentially Interfering Substance	Interferent Level	
	Default Units	Alternate Units
Ascorbic acid	400 mg/dL	22 712 µmol/L
Bilirubin	25 mg/dL	427.5 µmol/L
Calcium	400 mg/dL	100 mmol/L
Creatinine	400 mg/dL	35 360 µmol/L
Glucose	4000 mg/dL	222 mmol/L
Hemoglobin	500 mg/dL	5 g/L
Hippuric acid	400 mg/dL	4 g/L
Inorganic phosphorus	400 mg/dL	130 mmol/L
Potassium chloride	1000 mg/dL	10 g/L
Sodium chloride	2000 mg/dL	20 g/L
Urea nitrogen	400 mg/dL	142.8 mmol/L
Uric acid	100 mg/dL	5.9 mmol/L
Urobilinogen	20 mg/dL	33.8 µmol/L

Urine samples ranging in pH levels from 3 to 10 demonstrated ≤ 10% interference.

Method Comparison

A study was performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method.[19]

		Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity c Microalbumin vs ARCHITECT Microalbumin	Urine	µg/mL (mg/L)	123	1.00	2.01	0.99	7.0 - 487.0

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