SIEMENS

DCA™ Systems

Microalbumin/Creatinine Reagent Kit

For Use With DCA™ Analyzers

A Quantitative Assay for Microalbumin/Creatinine in Urine

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INTENDED USE

The DCA™ Microalbumin/Creatinine assay is a convenient, quantitative method for measuring low concentrations of albumin, creatinine, and the albumin/creatinine ratio in urine. The method is designed for decentralized testing using random, overnight, or timed specimens with results available to the patient within seven minutes. Testing for microalbuminuria (low concentrations of albumin in the urine) is recommended in patients with insulin-dependent diabetes mellitus (IDDM) as well as patients with non-insulin-dependent diabetes mellitus (NIDDM).[1,2,3] This assay is intended for use in both screening for, and monitoring treatment of, microalbuminuria.[4,5,6,7]

The DCA Microalbumin/Creatinine assay is for use in laboratories such as physician office laboratories, clinics, and hospitals.

SUMMARY AND EXPLANATION

Subjects with both IDDM and NIDDM are at increased risk of kidney damage. Diabetic nephropathy is the number one (30 – 40%) cause of need for kidney dialysis and/or kidney transplant, and is the cause of serious morbidity and mortality in diabetes mellitus. Microalbuminuria is the earliest stage of diabetic nephropathy.

The generally accepted definitions of microalbuminuria[13] are:

Albumin Excretion—30 – 299 mg/24 h

Albumin/Creatinine Ratio—2.5* – 25 mg/mmol (30 – 300 mg/g)

*3.5 as lower limit has been proposed in females because of lower creatinine excretion.

Early recognition of kidney disease associated with diabetes may lead to early medical intervention with the possibility of delaying or halting the future need for kidney dialysis. Reduction of protein intake in the diet, control of hypertension by drug therapy, and near normalization of blood glucose, have been shown to be successful in delaying the progression of the disease.[9,10,11]

Historically microalbuminuria was detected by measuring the albumin excretion rate (AER) using 24-hour or other timed urine specimens. Recently, calculation of the albumin to creatinine ratio was shown to reduce the need for timed collections as the albumin concentration is normalized using the creatinine concentration.[4,5,6,7] The DCA Microalbumin/Creatinine assay allows both the measurement of the AER, and the albumin to creatinine ratio.

CHEMICAL PRINCIPLES OF PROCEDURE

All of the reagents for measuring albumin and creatinine are contained in the DCA Microalbumin/Creatinine REAGENT CARTRIDGE Reagent Cartridge (Figure 1). Both the concentration of albumin and creatinine are measured in the reagent cartridge. The albumin and creatinine concentrations, and the ratio are reported on the same display screen of the DCA Analyzer.

DCA Microalbumin/Creatinine Reagent Cartridge

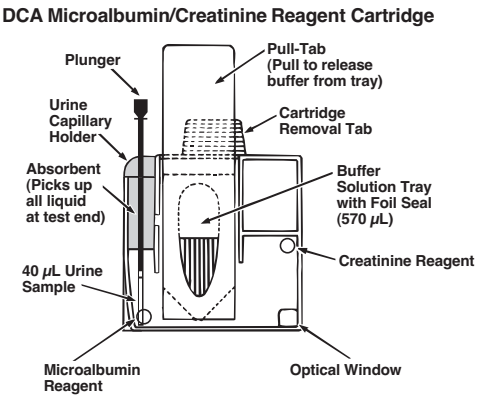


Figure 1

For the measurement of albumin, a specific antibody binds with albumin in the presence of polyethylene glycol. The albumin-antibody complexes that are formed cause increased turbidity, which is measured as absorbance at 531 nm. The albumin is then quantified using a calibration curve of absorbance versus albumin concentration.

The creatinine assay is based on the Benedict/Behre chemistry[12] in which creatinine complexes with 3,5-dinitrobenzoic acid at high pH to form a colored complex which is measured at 531 nm. The creatinine is then quantified using a calibration curve of absorbance versus creatinine concentration.

The albumin to creatinine ratio, A/C, is then calculated.

All measurements and calculations are performed automatically by the DCA Analyzer. The instrument's screen displays albumin concentration (mg/L), creatinine concentration (selectable as mg/dL or mmol/L), and albumin to creatinine ratio (no units displayed) at the end of the assay.

Note: If creatinine units are selected as mg/dL, then the albumin to creatinine ratio is reported as mg/g. If the creatinine units are selected as mmol/L, the albumin to creatinine ratio is reported as mg/mmol.

KIT CONTENTS:

10—Reagent Cartridges
10—Urine Capillary Holders with Plungers
1—Calibration Card
2—Package Inserts

REAGENTS

Albumin Reagent: 3.3 – 10% purified polyclonal goat anti-human albumin antiserum in 50 mM TRIS; 8.6% w/v non-reactive ingredients (15 µL dried in each reagent cartridge).

Creatinine Alkaline Reagent: 28% potassium hydroxide, 5% non-reactive ingredients (30 µL dried in each cartridge).

Buffer Solution: 0.22% w/v 3,5-dinitrobenzoic acid, 4% polyethylene glycol in 25 mM HEPPS buffer, with 2% non-reactive ingredients (0.57 mL in each cartridge).

CAUTION

- DCA Microalbumin/Creatinine Reagent Cartridges are for IVD *in vitro* diagnostic use.
- Safety glasses, gloves, and lab coat are recommended when using the DCA System.

HUOMAUTUS:

- DCA-mikroalbumiini- ja -kreatiniinireagenssikasetit on tarkoitettu diagnostiseen *in vitro* IVD -käyttöön.
- On suositeltavaa käyttää suojalaseja, käsineitä ja laboratoriotakkia, kun käytät DCA-laitetta.

WARNING:

- To prevent injury, do not force removal of a cartridge from the instrument. Consult the operator's guide to verify the proper removal technique. Contact your technical service provider if the problem cannot be solved.

VAROITUS:

- Jotta ei syntyisi vammoja, älä poista kasettia laitteesta väkisin. Katso oikea poistotapa käyttöoppaasta. Jos ongelma ei poistu, ota yhteys huoltohenkilökuntaan.

STORAGE

2°C 8°C — Store reagent cartridges refrigerated at 2° – 8°C (36° – 46°F).

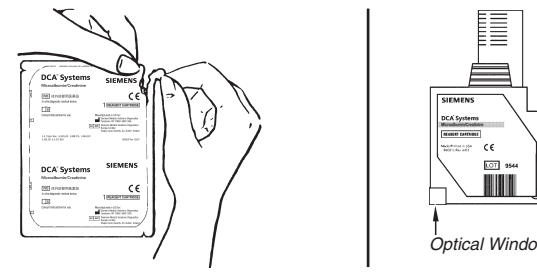
15°C 30°C — Capillary holders may be stored refrigerated or at room temperature (15° – 30°C/59° – 86°F).

USE LIFE

Reagent cartridges can be kept for up to three months at room temperature anytime before the ⌛ (**EXP**) expiration date. Record on the carton, the date the carton was placed at room temperature.

RECOMMENDED PROCEDURES FOR HANDLING REAGENT CARTRIDGES

To open the foil pouch, tear down from the corner notch (until the entire long side of the pouch is open).

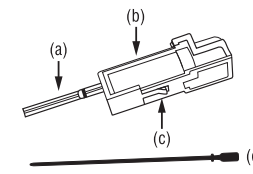


Discard the reagent cartridge if the cartridge is damaged, the flexible pull-tab is loose or missing, the desiccant is missing, or if loose desiccant particles are found inside the foil pouch.

Upon removal from refrigerated storage, allow the reagent cartridge to warm up at room temperature for 15 minutes (in the unopened foil pouch). **After opening the foil pouch, the reagent cartridge must be used within 10 minutes.**

RECOMMENDED PROCEDURES FOR HANDLING URINE CAPILLARY HOLDERS

Unused urine capillary holders and plungers may be saved and used with any lot of reagent cartridges. Discard the urine capillary holder if any of the following are missing from the holder: (a) glass capillary, (b) absorbent pad, (c) latching mechanism, (d) plunger. Also discard if the starch plug is missing or at the bottom of the capillary tube.



STABILITY OF REAGENT CARTRIDGES

Reagent cartridges are stable through the last day of the expiration month.

SPECIMEN COLLECTION AND PREPARATION

The urine sample may be random, overnight, or 24-hour. Use no preservative.

Urine specimens may be stored at 2°–8°C (36°–46°F) for up to two weeks. **Do not freeze.** Allow urine specimens to reach room temperature naturally. Mix urine specimen thoroughly before use.

TESTING PROCEDURE

See the Quick Reference Guide and Operator's Guide for detailed illustrated directions.

CALIBRATION

Instrument: The DCA Analyzer is calibrated by the manufacturer. Thereafter, the instrument automatically self-adjusts during first-time power-up and during each assay. In the event the system is unable to make appropriate internal adjustments, an error message displays.

Reagent: Each lot of reagent cartridges undergoes a thorough analysis and characterization before release by the manufacturer. Values of calibration parameters are determined that provide for optimal reagent performance. The values for the calibration parameters are encoded onto the calibration card provided with each lot of reagent cartridges. Prior to use of each new lot of reagent cartridges, scan the calibration card into the analyzer.

Before the sample can be analyzed, the reagent cartridge barcode (containing the lot number and test name) is scanned. This accesses the appropriate calibration parameter values (calibration curve) for the particular lot number of reagent cartridges in use. If no calibration curve is in the instrument for the particular lot number of cartridges in use, the instrument prompts the user to scan the calibration card.

The instrument can store two calibrations for the DCA Microalbumin/Creatinine Assay. Each of the two calibrations is for a different lot number.

When reagent cartridges are stored and used properly, acceptable performance up to the expiration date is ensured. To verify proper functioning of the DCA System, analyze DCA Microalbumin/Creatinine Controls (refer to Quality Control section).

The calibrators used to determine the calibration parameter values are made using a liquid preparation of albumin known to consist of 99% monomer, which is the form of albumin found in urine. The calibrators have been assayed against the RPPHS** and NIST creatinine, and have been shown to produce identical results.

**RPPHS is a certified reference material developed conjointly through the College of American Pathologists (CAP), the Bureau Communita de Reference (BCR) and the International Federation of Clinical Chemists (IFCC).

QUALITY CONTROL

To assure quality of both testing procedures and patient results for Microalbumin/Creatinine, the DCA System performs 48 optical, electronic, mechanical, and reagent systems checks during the course of each specimen assay. These checks include calibration verification during every test. If an assay or system error occurs during any individual measurement, the system automatically reports an error message, preventing the reporting of erroneous patient results.

The staff at each laboratory site can benefit by establishing a quality assurance plan, based on their institution's policies. Run quality control specimens under the following conditions:

- At regular intervals determined by the laboratory procedures
- With each new lot of reagents
- Each time a calibration card is scanned
- To train and confirm performance acceptability for new analysts
- When results do not match the patient's clinical condition or symptoms.

Good laboratory practices include a well-designed and implemented quality control process. These practices, for example, may involve:

- Proper storage and handling of reagent kits
- Careful sample collection and handling procedures
- Training of testing personnel
- Routine review of sample and control results
- Periodic quality system reviews
- Retention of quality control testing records.

If the problem cannot be corrected, or the reason for an out-of-limits result cannot be determined, contact the Authorized Representative nearest you.

RESULTS

Albumin: The displayed test result requires no further calculation. Albumin concentrations in the following range are reported: 5 to 300 mg/L. The test is linear throughout this range.

Creatinine: The displayed test result requires no further calculation. Creatinine concentrations in the following range are reported: 15 to 500 mg/dL (1.3 to 44.2 mmol/L). The test is linear throughout this range.

Albumin/Creatinine Ratio: The displayed test result requires no further calculation. Albumin/Creatinine ratios can be reported in the following range: 1 to 2000 mg/g (0.11 to 226 mg/mmol).

Albumin or Creatinine result preceded by a less than sign (<): A less than sign in the display indicates a concentration below the lower limit of the test (under range). This method does not provide for re-assay using a larger sample.

Albumin or Creatinine result preceded by a greater than sign (>): A greater than sign in the display indicates a concentration above the upper limit of the test (over range). This method does not provide for re-assay using a diluted sample. To obtain a more quantitative test value, use another test method.

Ratio result preceded by a less than (<) sign or greater than (>) sign or (---): If the albumin or creatinine result is under or over range, the ratio will also be reported as under or over range. In certain cases, no ratio is reported (---).

Example 1: If the albumin result is >300 mg/L and the creatinine result is 100 mg/dL (8.84 mmol/L), then the ratio is reported as >33.9 mg/mmol (>300 mg/g).

Example 2: If the albumin result is 75 mg/L and the creatinine result is <15 mg/dL (<1.33 mmol/L), then the ratio is reported as >500 mg/g (>56.4 mg/mmol).

Example 3: If the albumin result is >300 mg/L and the creatinine result is >500 mg/dL (>44.2 mmol/L), then no ratio is reported (---).

Example 4: If the albumin result is <5 mg/L and the creatinine result is <15 mg/dL (<1.33 mmol/L), then no ratio is reported (---).

All laboratory tests are subject to random error. If the test result is questionable, or if clinical signs and symptoms appear inconsistent with test results, re-assay the sample or confirm the result using another method.

LIMITATIONS OF PROCEDURE

Avoid using urine specimens that are visually hemolyzed or highly pigmented. Urine specimens containing in excess of 2000 mg/L of albumin may give assay results within the range of the DCA System because of the antigen excess effect for direct agglutination assays. If such gross proteinuria is suspected, assay the sample using Multistix® 10 SG Reagent Strips or Albustix® Reagent Strips.

The amount of albumin excreted in the urine can vary according to changes in posture, amount of hydration, physical activity, blood pressure in the individual, and during pregnancy. Urine samples should not be obtained following strenuous activity. The test should not be performed if the sample exhibits significant bacterial growth or if the patient shows signs of a urinary tract infection.

Interferences

Albumin Assay: No cross-reactivity exceeding 5% has been found with IgG, hemoglobin, transferrin, or microglobulin. Elevated glucose (2500 mg/dL or 138.8 mmol/L) and urea (2400 mg/dL or 400 mmol/L) have been shown not to affect the assay results. No interference has been shown by drugs commonly found in the urine or prescribed to persons with diabetes.

Creatinine Assay: Elevated glucose (2500 mg/dL or 138.8 mmol/L) and urea (2400 mg/dL or 400 mmol/L), ascorbate (80 mg/dL or 4.5 mmol/L), bilirubin (3.6 mg/dL or 0.06 mmol/L), acetoacetate (80 mg/dL or 7.8 mmol/L), and hemoglobin (4.7 µmol/L or 30 mg/dL) have been shown not to affect the assay results. No interference has been shown by drugs commonly found in the urine or prescribed to persons with diabetes.

EXPECTED VALUES:

The reference ranges suggested by the study shown below should be regarded as guidelines only. Because of differences which may exist between laboratories and locales with respect to population, diet, laboratory technique and selection of reference groups, it is important for each laboratory to establish by similar means the appropriateness of adopting the reference ranges suggested by this study.

Albumin Timed specimens: A total of 95 timed, 24 hour and overnight urine samples were collected from normal volunteers at two widely separated laboratories and assayed with the DCA Microalbumin/Creatinine Reagent Kit. The albumin results ranged from <5 to 45.6 mg/L. The average of the 90th and 91st (95th percentile) sample results was *16 mg/L albumin, which was established as the upper limit of the normal range.*

Albumin Random specimens: A total of 95 random urine samples were collected from normal laboratory volunteers at two widely separated laboratories and assayed with the DCA Microalbumin/Creatinine Kit. The results ranged from <5 mg/L to 92.1 mg/L albumin. The average of the 90th and 91st (95th percentile) sample results was *37 mg/L, which was established as the upper limit of the normal range.*

Creatinine specimens: Normal daily creatinine excretion varies widely depending on muscle mass, fluid intake, and physical activity. The ranges of creatinine concentration obtained in 109 normal individuals were *<15 mg/dL to 500 mg/dL (<1.3 mmol/L to 44.2 mmol/L) for random specimens, and from 36 mg/dL to >500 mg/dL (3.2 mmol/L to >44.2 mmol/L) for timed specimens.*

Albumin/Creatinine Ratio: A total of 95 overnight timed specimens and a total of 95 random specimens, were collected from normal volunteers at two widely separated laboratories and assayed with the DCA Microalbumin/Creatinine Reagent Kit. For the overnight timed specimens, the albumin/creatinine ratio ranged from <1 to 13 mg/g (<0.1 to 1.5 mg/mmol). The average of the 90th and 91st (95th percentile) sample results *was 12 mg/g (1.4 mg/mmol), which was established as the upper limit of the normal range.* For the random specimens, the albumin/creatinine ratio ranged from <1 to 55 mg/g (<0.1 to 6.2 mg/mmol). The average of the 90th and 91st (95th percentile) sample results was *16 mg/g (1.8 mg/mmol), which was established as the upper limit of the normal range.*

SPECIFIC PERFORMANCE CHARACTERISTICS

The precision and correlation data are results obtained by the staff at separate physician offices and a large diabetes treatment/research center. The statistical calculations were performed following CLSI procedures.

Precision: Multiple DCA 2000 Microalbumin/Creatinine assays of two different commercially prepared artificial urine base controls were performed by three independent investigators. The assigned values listed were determined from studies conducted by the manufacturer. Within-run precision was evaluated by including low and high controls, in duplicate, in each run of clinical specimens.

Albumin

Control	Site No.	Assigned Value (mg/L)	Mean Value (mg/L)	No. Runs	No. Assays	Within-Run S.D.	Within-Run % C.V.	Overall S.D.	Overall % C.V.
Low	1	30.8	31.8	27	54	1.37	4.3	1.85	5.8
Low	2	30.8	33.0	31	62	2.00	6.1	2.02	6.1
Low	3	30.8	32.9	33	66	1.54	4.7	2.18	6.6
High	1	200	202	27	54	3.42	1.7	5.41	2.7
High	2	200	216	31	62	6.31	2.9	7.46	3.4
High	3	200	217	33	66	6.17	2.8	7.37	3.4

Creatinine

Control	Site No.	Assigned Value (mg/dL)	Mean Value (mg/dL)	No. Runs	No. Assays	Within-Run S.D.	Within-Run % C.V.	Overall S.D.	Overall % C.V.
Low	1	99.9	104	28	56	2.03	2.0	2.95	2.8
Low	2	99.9	106	31	62	2.30	2.2	2.81	2.6
Low	3	99.9	106	33	66	2.19	2.1	3.80	3.6
High	1	396	412	28	56	5.51	1.3	8.92	2.2
High	2	396	421	31	62	7.41	1.8	10.88	2.6
High	3	396	424	33	66	9.09	2.1	11.42	2.7

Correlation: The concentration of albumin and creatinine in clinical specimens ranging from 5 mg/L – 350 mg/L albumin and 15 mg/dL – 370 mg/dL (1.3 mmol/L – 32.7 mmol/L) creatinine was determined using the DCA 2000 Microalbumin/Creatinine System (y) and RIA (Diagnostic Products Co., Double-Antibody Kit), turbidimetry (Behring Turbitimer), and nephelometry (Beckman Array 360) (x) for albumin; and automated Jaffe (x) for creatinine. At Site 1, random, timed overnight, and 24-hr. timed specimens were collected. At Sites 2 and 3, random and timed overnight specimens were collected. Results are as follows:

Albumin

Site No.	Sample Type	Comparative Method	No. of Assays	Regression Line (mg/L)	Standard Error of Estimate	Correlation Coefficient
1	Random	RIA	281	$y = (-)1.0 + 0.869x$	8.0	0.99
1	Timed Overnight	RIA	221	$y = (-)0.4 + 0.878x$	6.8	0.99
1	24-hr. Timed	RIA	67	$y = (-)0.5 + 0.855x$	10.4	0.99
2	Combined Random and Timed Overnight	Turbidimetry	137	$y = 0.1 + 0.857x$	6.2	0.99
3	Combined Random and Timed Overnight	Nephelometry	129	$y = 0.6 + 1.06x$	7.0	0.99

Creatinine

Site No.	Sample Type	Comparative Method	No. of Assays	Regression Line (mg/dL)	Standard Error of Estimate	Correlation Coefficient
1	Random	Automated Jaffe	276	$y = (-)0.2 + 1.06x$	5.1	0.99
1	Timed Overnight	Automated Jaffe	266	$y = (-)2.1 + 1.08x$	4.2	1.00
1	24-hr. Timed	Automated Jaffe	70	$y = (-)3.2 + 1.08x$	3.8	1.00
2	Combined Random and Timed Overnight	Automated Jaffe	224	$y = (-)2.3 + 1.02x$	5.6	1.00
3	Combined Random and Timed Overnight	Automated Jaffe	196	$y = (-)3.0 + 1.12x$	4.9	0.99

AVAILABILITY

DCA Microalbumin/Creatinine Reagent Kit is available as 6011A.
DCA Microalbumin/Creatinine Low and High Control Kit is available as 6012A.

GLOSSARY OF ACRONYMS

AER: Albumin excretion rate
CLSI: Clinical and Laboratory Standards Institute
IDDM: Insulin dependent diabetes mellitus
IFCC: International Federation of Clinical Chemistry
NIDDM: Non-insulin dependent diabetes mellitus
NIST: National institute of standards and technology
RPPHS: Reference Preparation for Proteins in Human Serum

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