



## HemoCue® Urine Albumin Microcuvettes and the HemoCue® Urine Albumin Analyzer

### Intended use

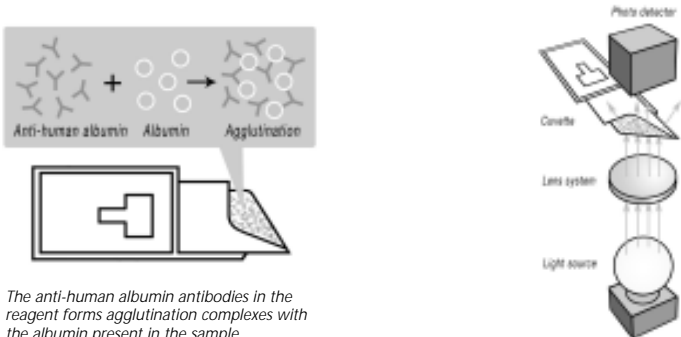
The quantitative, rapid, turbidimetric immunoassay of albumin in human urine using a specially designed analyzer, the HemoCue Urine Albumin Analyzer. The system can be used for the quantitative determination of low concentrations of urine albumin for the purpose of screening for, monitoring and to supplement the clinical evidence in the diagnosis and treatment of microalbuminuria. The system is designed for testing using spot samples or timed collections. A quantitative result is obtained within 90 seconds. HemoCue Urine Albumin Microcuvettes are for in vitro diagnostic use only. The HemoCue Urine Albumin Analyzer is only to be used with HemoCue Urine Albumin Microcuvettes. **For professional use only.**

### Summary and explanation of the test

Microalbuminuria is defined as a urinary albumin excretion rate of between 20–200 mg/L in the first morning sample, 20–200 µg/min in a timed overnight or 24 hour sample on at least 2 of 3 occasions within a period of 6 months<sup>1</sup>. An albumin excretion rate of 20–200 µg/min is approximately equivalent to 30–300 mg/24 hours. The test can be performed with spot urine, preferably first morning urine, as well as urine collected over night or during 24 hours.

Nephropathy is a leading cause of morbidity and mortality in diabetes. Microalbuminuria is a powerful predictor for the future development of diabetic nephropathy, retinopathy and cardiovascular complications to type 1 diabetes<sup>2,3</sup>. For type 2 diabetes and patients with hypertension<sup>4</sup>, there is an increased risk of cardiovascular disease if microalbuminuria is present<sup>5,6,7</sup>. According to the American Diabetes Association guidelines, annual screening in individuals with type 1 diabetes should begin after 5 years' disease duration or at the age of 12, and from the time of diagnosis of type 2 diabetes<sup>8</sup>. Microalbuminuria is also suggested as a risk marker for cardiovascular complications and increased cardiovascular risk in hypertension<sup>9,10</sup>. Microalbuminuria is also an independent risk factor for cardiovascular disease among patients without diabetes or hypertension<sup>11</sup>. Several studies have shown that early detection of microalbuminuria will delay or prevent further deterioration through early treatment<sup>12</sup>. Microalbuminuria before pregnancy is the strongest predictor of pre-eclampsia in Type I diabetes<sup>13</sup>. The turbidimetric method utilized by the HemoCue Urine Albumin Microcuvette is an immunochemical antigen-antibody reaction using antihuman antibodies specific for human albumin. The reaction, which is enhanced by polymers, form agglutination complexes that create turbidity. When used with the HemoCue Urine Albumin Analyzer, the end-point reaction within the cuvette yields turbidity proportional to the concentration of the albumin in the sample.<sup>1</sup>

The HemoCue Urine Albumin Microcuvette is disposable once the test is completed and the result is noted.



The anti-human albumin antibodies in the reagent forms agglutination complexes with the albumin present in the sample.

Within 90 seconds, the immunochemical reaction is completed and the turbidity is measured photometrically at 610 nm.

### Principles of the procedure

#### The technique

The HemoCue Urine Albumin technique is based on an optical measuring cuvette with a small and exact volume and a short light path. The cuvette cavity contains reagents deposited on its inner walls. The urine sample is drawn into the cavity by capillary action. The filled cuvette is inserted into the HemoCue Urine Albumin Analyzer where the contents of the cuvette are mixed through vibration. Within 90 seconds, the immunochemical reaction is completed and the turbidity is measured photometrically at 610 nm. The albumin concentration is proportional to the turbidity. When the end point is reached, the result is displayed in mg/L. Thus the technique makes it possible to obtain a measured amount of specimen, mix with reagents, follow the chemical reaction to endpoint and determine the result all within the microcuvette.

#### The Microcuvette

The cuvette is made of polystyrene and contains a cavity that holds approximately 15 µL of specimen. The distance between the walls of the optical window is about 0.5 mm, permitting photometric determination of albumin in urine.

#### The Chemistry Principles

A specific rabbit anti-human albumin antibody (polyclonal) forms an agglutinate with human albumin in the sample. The agglutination is enhanced by polymers. The turbidity of the agglutinates created due to aggregate formation is measured photometrically at 610 nm.

### Reagents

3 % w/w rabbit anti-human albumin antibody (polyclonal), 39 % w/w PEG, 17 % w/w Tris/HCl-buffer, 5 % w/w polymer and 36 % w/w non-reactive ingredients

### Warnings and precautions

HemoCue Urine Albumin Microcuvettes are for In Vitro Diagnostic use only. The chemicals deposited in the cavity of the microcuvette are harmful if swallowed. Although the reagents are present in the cuvette in extremely low quantities, consult local environmental authorities for appropriate disposal. Always handle body fluids with care, including urine samples, as they might be infectious.

### Storage and handling of HemoCue Urine Albumin Microcuvettes

Store HemoCue Urine Albumin Microcuvettes in their package in a refrigerator, at 35–46 °F (2–8 °C). The cuvettes may NOT be stored in a freezer. Use HemoCue Urine Albumin Microcuvettes prior to their expiration date. The expiration date is printed on each container as well as on each individual package. The reagents within HemoCue Urine Albumin Microcuvettes are moisture and temperature sensitive. Open a package and remove the cuvette which is for immediate use. As this test method relies on photometric measurement, care should be taken not to hold the microcuvette by the filling end. Take care to wipe away all contaminating substances from the outer surface of the filled cuvette. Unused HemoCue Urine Albumin Microcuvettes should be kept in the original package.

### Instrument

HemoCue Urine Albumin Microcuvettes are specifically designed for use with the HemoCue Urine Albumin Analyzer. The system is calibrated against a turbidimetric method using CRM 470 (Certified Reference Material).

### Specimen collection and preparation

The test can be performed with spot urine, preferably first morning urine, as well as urine collected over night or during 24 hours without additives. Preanalytical factors are important. Factors that influence the excretion of albumin in the urine are for example, physical activity, urinary tract infections, fever and high water intake. There is also a large between-day as well as intraindividual variation of albumin excretion which is the reason why microalbuminuria is defined as a urinary albumin excretion rate of between 20–200 mg/L in a spot sample or 20–200 µg/min in an overnight or 24 hour sample on at least 2 of 3 occasions within a period of 3–6 months<sup>14</sup>.

#### Spot samples

The first morning urine specimen, after rest is recommended since muscle activity influences the excretion of albumin in urine. Spot samples during the day may be used, but higher results can be expected.

### Timed urine collection

To determine the urine albumin excretion rate, a timed collection should be performed. The urine collected before sleep will be discarded and the time noted. All the urine from this point until morning is collected. Note the time for the last collected portion. Measure the total volume<sup>5</sup>.

### Procedure

Read the Operating Manual for the HemoCue Urine Albumin Analyzer to ensure correct use of the instrument.

### Materials Provided

HemoCue Urine Albumin Microcuvettes

### Materials required but not provided

HemoCue Urine Albumin Analyzer. Gauze or lint free wipe.

### Proper temperatures

The HemoCue Urine Albumin system is designed for use at room temperature 64–86 °F (18–30 °C).

### Directions for use

- Make sure that the analyzer lid is closed. Press the button on the back of the analyzer to the "ON" position. The display shows the version number of the program, after which it will say "U-ALBUMIN CHECKING...". During "U-ALBUMIN CHECKING..." the analyzer will automatically verify the performance of the optronic unit. After this, the display will show "U-ALBUMIN OPEN THE LID" indicating that the analyzer is ready for use. Also see the Operating Manual for the HemoCue Urine Albumin Analyzer.
- Use of HemoCue Urine Albumin Microcuvettes
  - Take out only as many cuvettes from the package as you need for the immediate use. Follow the instructions, given under "Handling of HemoCue Urine Albumin Microcuvettes".
  - The shape of the cuvette and the names of the different parts of the cuvette can be seen in figure 1.
  - Pipette a drop of urine onto a hydrophobic surface (for example a plastic film). Note: Visibly turbid urine must be centrifuged at a minimum of 1200 g for 10 minutes. The Turbidity scale in the Operating Manual can be used as guidance.
  - Hold the cuvette opposite the filling end. Bring the filling end of the cuvette into contact with the urine sample, see figure 2. Always avoid contamination of the optical eye. It is also possible to fill the cuvette directly from the sample, if it is not to be used for any other analysis. Allow the cavity of the cuvette to fill completely in one step. **Do not refill the cuvette!**
  - When completely filled, carefully wipe off the excess urine from the outside of the cuvette with a clean lint free wipe, see figure 3. Make sure that no sample is drawn out of the cuvette during this procedure. The filled cuvette should be visually inspected to check that the cuvette is properly filled, i.e. completely filled up to the edge and without air bubbles in the optical eye. **If air bubbles are seen in the optical eye of a filled cuvette, the cuvette should be discarded and another cuvette filled for analysis.**
  - Open the lid and push the filled cuvette into the cuvette holder, see figure 4a. It is important that the cuvette "snaps" properly into the cuvette holder, see figure 4b. **The filled cuvette must be placed into the analyzer within 30 seconds of filling.**
  - Gently close the lid. The display should read "U-ALBUMIN MEASURING...".
  - Within 90 seconds, the result is displayed in mg/L. The result will remain on the display until the lid is opened. Measurements above 150 mg/L are displayed as "HHH". Measurements below 10 mg/L are displayed as "LLL". If the display shows a number equal to or greater than 900, together with "ERROR", an error has occurred. See the Troubleshooting Guide and the Maintenance section in the Operating Manual for additional information<sup>14</sup>.
  - Do not remeasure the cuvette!**
  - Open the lid and discard the used cuvette.
  - Close the lid. When the display shows "U-ALBUMIN OPEN THE LID", the analyzer is ready for a new measurement. If the display shows ERROR 902, the optronic unit must be cleaned and the sample must be analyzed with a new cuvette.



Fig. 1

Fig. 2

Fig. 3

Fig. 4a

Fig. 4b

### Stability of the final reaction

Measurement of a HemoCue Urine Albumin Microcuvette in a HemoCue Urine Albumin Analyzer must be performed immediately or at the latest within 30 seconds after the microcuvette is filled with specimen. Do not remeasure the cuvette!

### Quality control

The HemoCue Urine Albumin Analyzer has an internal electronic "SELFTEST". Every time the analyzer is turned on it will automatically verify the performance of the optronic unit of the analyzer. The system can be verified by measuring a commercially available urine albumin control. Considerable variability exists in different types and production methods for liquid urine controls. Contact HemoCue Inc, Technical Support, for advice.

### Results

The result is read directly from the HemoCue Urine Albumin analyzer in mg/L.

The system is linear up to 150 mg/L. Measurements above 150 mg/L are displayed as "HHH".

Measurements below 10 mg/L are displayed as "LLL".

### Limitations of the procedure

- Visually turbid samples should only be analyzed after centrifugation, see instructions under Directions for use.
- The following substances have been tested without interfering with the system. The highest concentration tested is referred to in brackets. Acetaminophen (50 mg/dL), acetoacetate (102 mg/dL), acetone (697 mg/dL), acetyl salicylic acid (50 mg/dL), ascorbic acid (50 mg/dL), betanin (0.8 mg/dL), β<sub>2</sub> microglobulin (0.1 mg/dL), bilirubin (15 mg/dL), calcium (64 mg/dL), creatinine (271 mg/dL), hemoglobin (0.2 g/dL), IgG (1.5 mg/dL), potassium (180 mmol/L), uric acid (6 mg/dL). For glucose > 1982 mg/dL, urea > 1541 mg/dL, sodium > 300 mmol/L and pH < 4.5 or > 9.0 the result should be interpreted with caution.
- Results obtained from patients undergoing renal dialysis should be interpreted with the utmost caution.

### Expected values

According to the literature the following values are indicators for normal and pathological urine albumin results respectively.

Category	First morning spot sample (mg/L)	24-h collection (mg/24 h)	Timed collection (µg/min)
Normal	< 20	< 30	< 20
Microalbuminuria	20-200	30-299	20-199
Clinic albuminuria	> 200	≥ 300	≥ 200

For screening purposes, a spot sample can be used, preferably the first morning sample. The cut off used should be 20 mg/L. Microalbuminuria is defined as 20-200 mg/L and macroalbuminuria > 200 mg/L. Measurements from spot urines during the day may give slightly higher results due to physical activity. In these circumstances, normal values are < 30 mg/L<sup>1</sup>.

### Specific performance characteristics

The results presented below for within batch and total precision come from one instrument and one batch of cuvettes. No recalibration was performed during the study period. The precision study was performed according to NCCLS EP5-A 1999 Vol 19 No 2<sup>16</sup>.

The results in the correlation study come from a HemoCue instrument that was not recalibrated during the period.

The evaluation has been performed according to NCCLS EP9A 1995 Vol 15 No 17.

#### Precision

The precision was determined using commercially available controls at two different levels. Each control was measured in duplicate twice a day during twenty consecutive days.

Level mg/L	No of days	No of determinations (n)	Mean mg/L	Within run		Total precision	
				Standard-deviation mg/L	Coefficient of variation %	Standard-deviation mg/L	Coefficient of variation %
20	20	80	22.1	2.8	12.7	2.9	13.1
70	20	80	69.1	3.0	4.3	3.9	5.6

#### Correlation

A number of correlation studies of the HemoCue system have been performed.

The results of the comparison studies between HemoCue Urine Albumin and other urine albumin methods are summarized in the table below.

Study	No of tests	Min mg/L	Max mg/L	Regression Line	Correlation coefficient
A	135	5.0	147.0	y= 1.10 x -3.6	0.975
B	137	7.6	150.8	y= 1.07 x -7.5	0.975
C	71	12	149.0	y= 0.936 x -2.16	0.963
D	80	5.0	156.0	y= 0.969 x +1.53	0.974
E	59	8.4	167.0	y= 0.98 x +0.09	0.991

A= Beckman Coulter Immage (nephelometry)

B= Roche Cobas Integra (turbidimetry)

C= Beckman Synchron LX (turbidimetry)

D= Roche Hitachi Modular (turbidimetry)

E= Beckman Array (nephelometry)

### Patent

The HemoCue Urine Albumin System is protected by US patent no US 5,674,457, US 6,468,807, US 6,333,007 and US 433,150.

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- HemoCue Urine Albumin Operating manual
- Evaluation of precision performance of clinical chemistry devices; approved Guideline NCCLS

### Symbols used

	Attention, see instructions for use		Temperature limitation
	In vitro diagnostic medical device		Catalogue number
	Do not reuse		Batch code
	CE mark		Use by
	Date of opening		

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