

# Afinion™ ACR – A new point of care test for determination of urine Albumin/Creatinine Ratio – Two assays combined in one test cartridge

## Albumin/Creatinine Ratio (ACR)

The Afinion™ ACR assay is a test for determining low urine albumin concentrations, corrected for variable urine volume by simultaneously measuring the creatinine concentration. The Albumin/Creatinine Ratio (ACR) is then calculated.

Albumin excretion rate in urine is a predictor for incipient diabetic nephropathy, risk of cardiovascular diseases, elevated blood pressure and pre-eclampsia. Microalbuminuria is the earliest stage of diabetic nephropathy, a serious complication of diabetes mellitus.

Urine albumin levels vary with diuresis, whereas creatinine levels correlate well with diuresis, as creatinine is almost entirely filtered by the glomeruli. Measuring the Albumin/Creatinine Ratio (ACR) therefore provides a more accurate evaluation of albumin excretion rate.

### ACR values:

- Normal: < 2.5 mg/mmol
- Microalbuminuria: 2.5 - 25 mg/mmol
- Clinical albuminuria: 26 - 300 mg/mmol

## Afinion™ ACR Assay System

The Afinion™ AS100 Analyzer, a compact, bench-top, multi-assay analyser, and the Afinion™ ACR Test Cartridge together constitute the Afinion™ ACR assay system (Figure 1).

A simple sampling procedure and no manual calibration or chemistry handling, make the Afinion™ ACR assay system suitable for point of care testing.

The Afinion™ ACR Test Cartridge (Figure 2) contains all the reagents necessary for simultaneously analysing a urine sample for both albumin and creatinine. The assay time is 5½ minutes.

The concentrations of albumin, creatinine and ACR are displayed on the Afinion™ AS100.



Figure 1. Afinion™ ACR Assay System

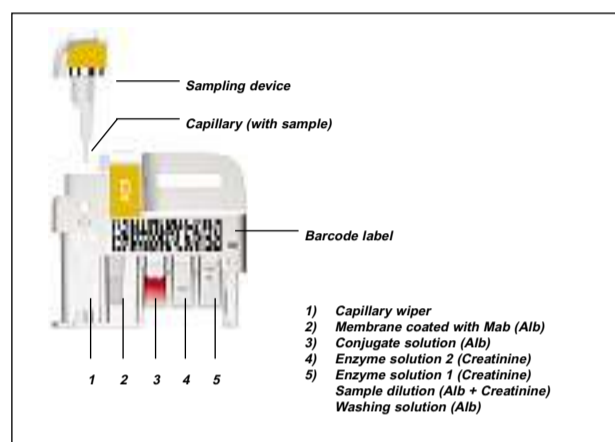


Figure 2. Afinion™ ACR Test Cartridge

## Step-by-step procedure



## Test principle

### The albumin assay

The albumin measurement is based on an immunometric membrane flow-through principle (Figure 3), utilizing a monoclonal antibody-coated membrane, sample dilution liquid, monoclonal antibodies conjugated with ultra-small gold particles and washing solution.

The Afinion™ ACR Test Cartridge is automatically processed within the Afinion™ AS100 Analyzer in which diluted antigen, conjugate, and washing buffer are sequentially soaked through the membrane. Finally, the colour intensity of the membrane is read by a digital camera.

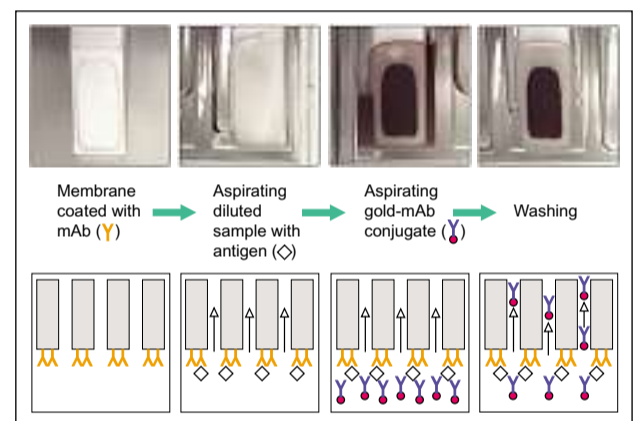


Figure 3. Principle of the membrane flow-through immunoassay for albumin (photos from prototype of the membrane tube)

### The creatinine assay

Creatinine is quantified using an enzymatic colourimetric test involving four enzymatic steps (Figure 4). A coloured end product is measured by transmission in one of the cartridge wells.

Transmission is read by a digital camera. The test requires two distinct enzyme solutions and preincubation of the sample before starting the reaction. Measurement of creatinine and albumin occur simultaneously.

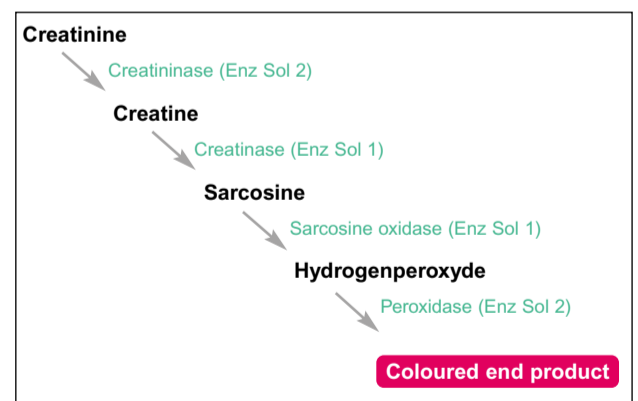


Figure 4. Principle of the enzymatic colourimetric creatinine assay

**E. Dworsky, C. Kvam, A. Campbell, E. Jernberg, H. Tøn, K. Hansen, J.R. Karlson, J. Holtlund, A.L. Faaren, A.K. Nordhei and F. Frantzen.**  
Axis-Shield PoC, Oslo, Norway

## Linearity

### Measuring range

Albumin: 5.0 – 200.0 mg/L  
Creatinine: 1.5 – 30.0 mmol/L  
ACR: 0.1 – 140.0 mg/mmol

### Linearity

The linearity of Afinion™ ACR was studied by analysing two native urine samples; one with high albumin (191 mg/L) and creatinine (29.7 mmol/L) and one with low albumin (3.6 mg/L) and creatinine (2.4 mmol/L), and 8 intermediate samples made by intermingling the two samples.

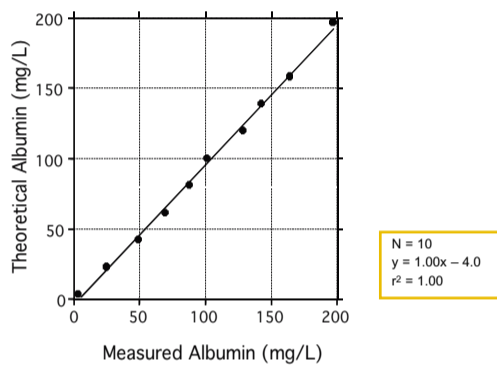


Figure 5.1. Theoretical versus measured values of albumin.

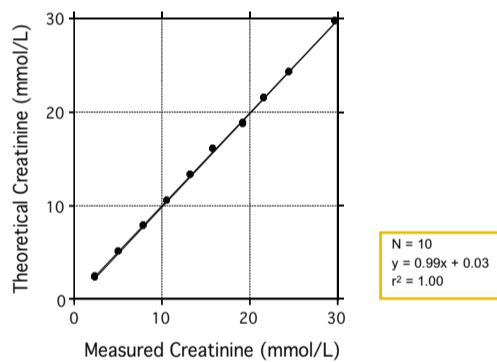


Figure 5.2. Theoretical versus measured values of creatinine.

## Precision

Within-day precision was performed with three urine samples, with 20 replicates of each sample analysed during one day. Between-day and total precision was performed with the three urine samples tested twice a day over 20 days. The studies were performed according to NCCLS guideline EP5-A.

Within-day precision						
Sample	Albumin (mg/L)		Creatinine (mmol/L)		ACR (mg/mmol)	
	Mean	CV%	Mean	CV%	Mean	CV%
Urine S1	176.7	3.9	4.7	3.3	38.0	5.2
Urine S2	55.7	4.6	14.5	3.9	3.9	3.8
Urine S3	13.0	3.5	31.1	3.3	0.4	0.0

Between-day precision						
Sample	Albumin (mg/L)		Creatinine (mmol/L)		ACR (mg/mmol)	
	Mean	CV%	Mean	CV%	Mean	CV%
Urine S1	174.9	2.0	4.5	0.0	38.5	2.8
Urine S2	55.3	0.0	14.4	0.0	3.9	0.6
Urine S3	12.6	1.2	30.8	0.6	0.4	0.0

Total precision						
Sample	Albumin (mg/L)		Creatinine (mmol/L)		ACR (mg/mmol)	
	Mean	CV%	Mean	CV%	Mean	CV%
Urine S1	174.9	5.0	4.5	3.8	38.5	6.0
Urine S2	55.3	4.8	14.4	2.7	3.9	4.6
Urine S3	12.6	5.5	30.8	3.0	0.4	4.8

## Method comparison

Afinion™ ACR was compared with one automatic laboratory method and one point of care method. A panel of 95 urine samples were analysed for albumin and creatinine with Afinion™ ACR and by the comparison methods. The results were compared separately by Passing/Bablok analysis (Figures 6 and 7).

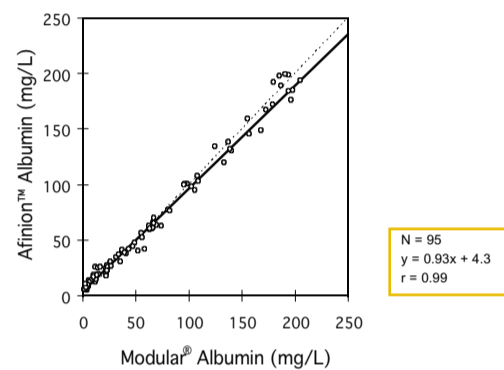


Figure 6.1. The albumin assay of Afinion™ ACR compared with Modular®.

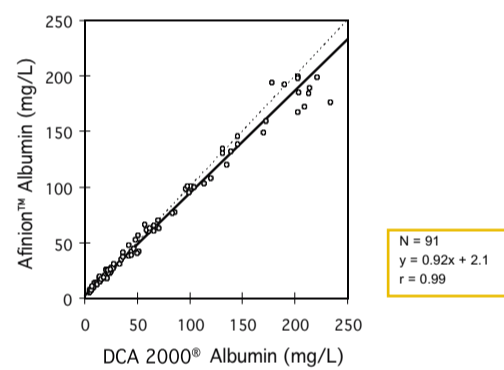


Figure 6.2. The albumin assay of Afinion™ ACR compared with DCA 2000®.

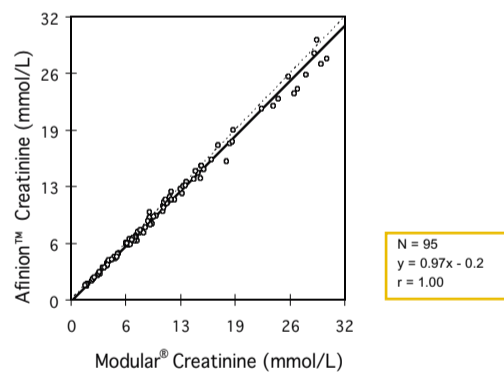


Figure 7.1. The creatinine assay of Afinion™ ACR compared with Modular®.

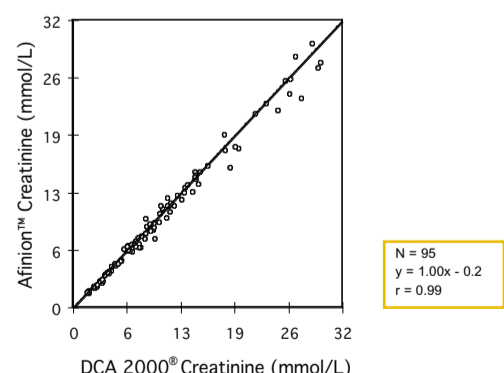


Figure 7.2. The creatinine assay of Afinion™ ACR compared with DCA 2000®.

## Substances tested for interference

Afinion™ ACR was tested for several possible interfering substances, including common urine components, drugs and their metabolites. The maximum concentrations of the substances which resulted in no significant interference are presented below.

Common urine components		
Acetoacetate	840 mg/L	7.8 mmol/L
Acetone	800 mg/L	13.8 mmol/L
Ascorbic acid	3000 mg/L	16.7 mmol/L
Bilirubin	3.5 mg/dL	0.06 mmol/L
Creatine	520 mg/L	4 mmol/L
Glucose	45 mg/mL	250 mmol/L
β-OH-butyric acid	5.9 mg/mL	46.8 mmol/L
IgG	20 mg/L	
Human β-2-microglobulin	20 mg/L	
Human myoglobin	20 mg/L	
Urea	30 mg/mL	500 mmol/L

Drugs / metabolites		
Acetaminophen	0.2 mg/mL	1.3 mmol/L
Acetaminophen glucuronide	10.5 mg/mL	30 mmol/L
Glyburide	14.8 µg/mL	30 µmol/L
Ibuprofen	2 mg/mL	10 mmol/L
Metformin (biguanid)	4 mg/mL	24.2 mmol/L
Salicylic acid	2 mg/mL	14.5 mmol/L
Salicylic acid *	1 mg/mL	5 mmol/L

\*Salicylic acid is the most important urine metabolite from acetylsalicylic acid. Administration of more than 1.2 g acetylsalicylic acid per day may result in falsely low creatinine measurements, and thus falsely high ACR results.

## Conclusions

The new Afinion™ ACR test provides a reliable, precise and convenient point of care method for the simultaneous determination of albumin, creatinine and ACR in only 3.5 µL urine.

The assay time is 5½ minutes using the Afinion™ AS100 Analyzer.

The Afinion™ AS100 Analyzer is easy to use, rapid and fully automated.

**Please note:** The results presented in this poster are obtained after reformulation of one of the reagents, and may differ slightly from the results in the initial abstract submitted for EUROMEDLAB 2007.