

Development of an ACR assay for the

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Axis-Shield PoC, Oslo, Norway

Abstract

Introduction

Diabetic nephropathy is one of the most serious and most frequent secondary complications of diabetes mellitus, resulting in increased morbidity and mortality. Microalbuminuria is the earliest stage of diabetic nephropathy and is characterized by a persistent and significant elevation of urinary albumin excretion. As creatinine is produced at an approximately constant rate, measurements of creatinine are used to correct for varying diuresis when quantifying urine proteins.

Method

We have developed the Afinion™ ACR in vitro diagnostic test for quantitative determination of albumin, creatinine, and albumin/creatinine ratio (ACR) in human urine using the Afinion™ AS100 Analyzer. The Afinion™ ACR Test Cartridge contains all the reagents necessary for determining albumin and creatinine in urine. Urine is sampled using a sampling device integrated into the Test Cartridge. The Afinion™ AS100 Analyzer is a compact, bench-top multi-assay analyzer for Point of Care (PoC) testing, combining advanced immunoassay and opto-mechanical technology, with an integrated camera, computer and LCD display. Albumin is quantified using an immunometric membrane flow-through assay, utilizing an antibody-coated membrane and specific monoclonal antibodies conjugated with ultra-small gold particles. The gold-antibody conjugate binds to the immobilized albumin on the membrane, which will turn red-brown. The Afinion™ AS100 Analyzer measures the color intensity of the membrane. Creatinine is quantified using an enzymatic colorimetric test that involves four enzymatic steps. A colored end product is measured by transmission in one of the cartridge wells. The concentration of albumin, the concentration of creatinine, and the calculated ACR are displayed on the Afinion™ AS100 Analyzer.

Results

The measurement range for albumin is 5-200 mg/L, 16-340 mg/dL for creatinine, and 1-1250 mg/g for ACR. The albumin and creatinine assays are linear over the whole dynamic range ($r = 1.0$, slope = 1.0). The method comparison for Afinion™ ACR versus Bayer DCA2000 using 95 samples resulted in a regression slope of 0.92, a linear correlation coefficient (r) of 0.99, and an intercept of 2.1 mg/L for albumin. A slope of 1.00, $r = 0.99$, and an intercept of -3.2 mg/dL for creatinine. A slope of 1.01, $r = 0.99$, and an intercept of 0.7 mg/g for ACR. Within day precision is $\leq 4.6\%$ for albumin, $\leq 3.8\%$ for creatinine and $\leq 5.2\%$ for ACR. Total precision is $\leq 5.5\%$ for albumin, $\leq 3.8\%$ for creatinine and $\leq 6.0\%$ for ACR. No significant interference was observed for elevated concentrations of bilirubin, glucose, urea, human myoglobin, human beta-2-microglobulin, IgG, creatine, acetacetate, acetone, ascorbic acid, beta-hydroxybutyric acid, metformin, glyburide, acetaminophen-glucuronide, ibuprofen, acetosalicylic acid and salicylic acid.

Conclusion

The new Afinion™ ACR test from Axis-Shield PoC provides a reliable, precise, and convenient PoC method for the simultaneous determination of albumin, creatinine and ACR. Assay time is 5½ minutes using the Afinion™ AS100 Analyzer. The 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 AACC 2007.

Albumin/Creatinine Ratio (ACR)

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

Albumin excretion rate in urine can be used to predict 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 level varies with diuresis, whereas creatinine levels correlate well with diuresis as creatinine is almost entirely filtered by the glomeruli. Therefore, determining the Albumin/Creatinine Ratio (ACR) will provide a more accurate evaluation of the albumin excretion rate.

ACR values:

- Normal : < 30 mg/g
- Microalbuminuria : 30 – 300 mg/g
- Clinical albuminuria : > 300 mg/g

Ref. American Diabetes Association. *Diabetes Care*, Vol. 25, Suppl. 1, January 2002.

Afinion™ ACR Assay System

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

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

The Afinion™ ACR Test Cartridge (Figure 2) contains all the reagents necessary for simultaneously analyzing a 3.5 µL urine sample for the two analytes, 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

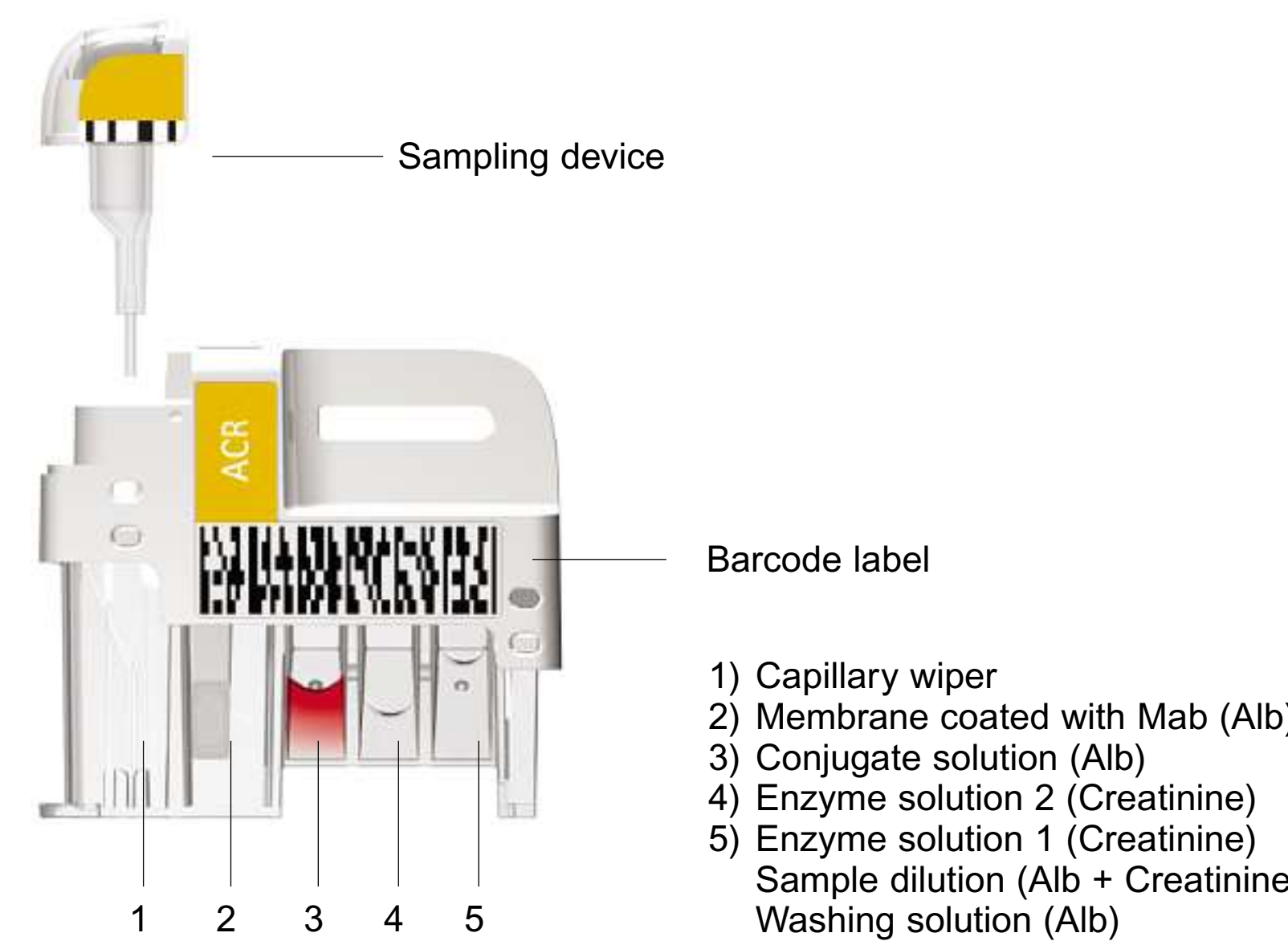


Figure 2. Afinion™ ACR Test Cartridge

Step-by-step procedure



Test principle

The albumin assay

The albumin measurement is based on an immunometric membrane flow-through principle, utilizing a monoclonal antibody-coated membrane, sample dilution liquid, monoclonal antibodies conjugated with ultra-small gold particles, and a 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 absorbed through the membrane (Figure 3). Finally, a digital camera reads the color intensity of the membrane.

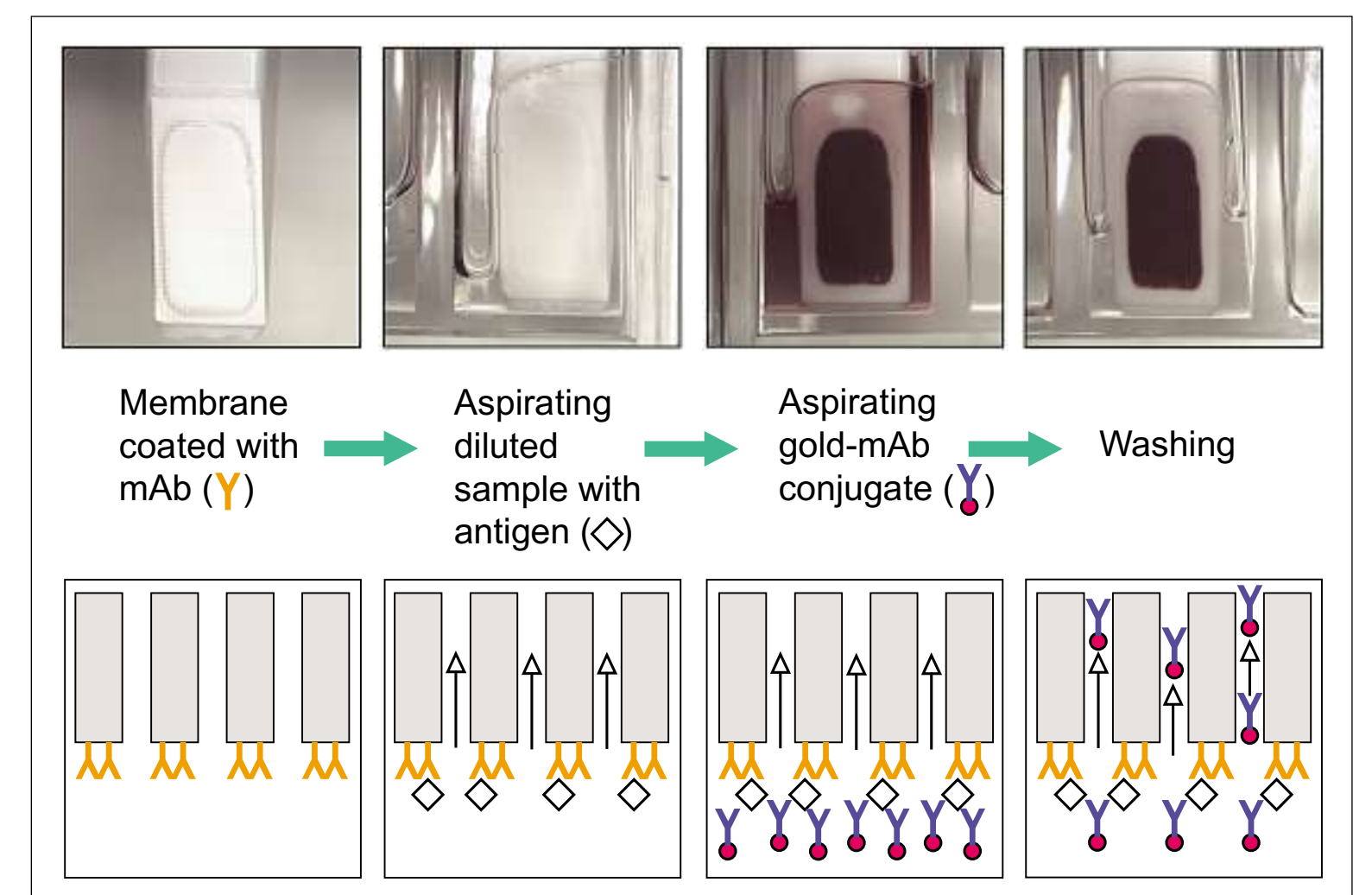


Figure 3. Principle of the membrane flow-through immunoassay for albumin.



Light from green LEDs illuminate the membrane (Figure 4). A camera then measures the reflected light (Figure 5), and the corresponding albumin concentration is displayed on the Analyzer screen.

Figure 4. The colored test membrane.

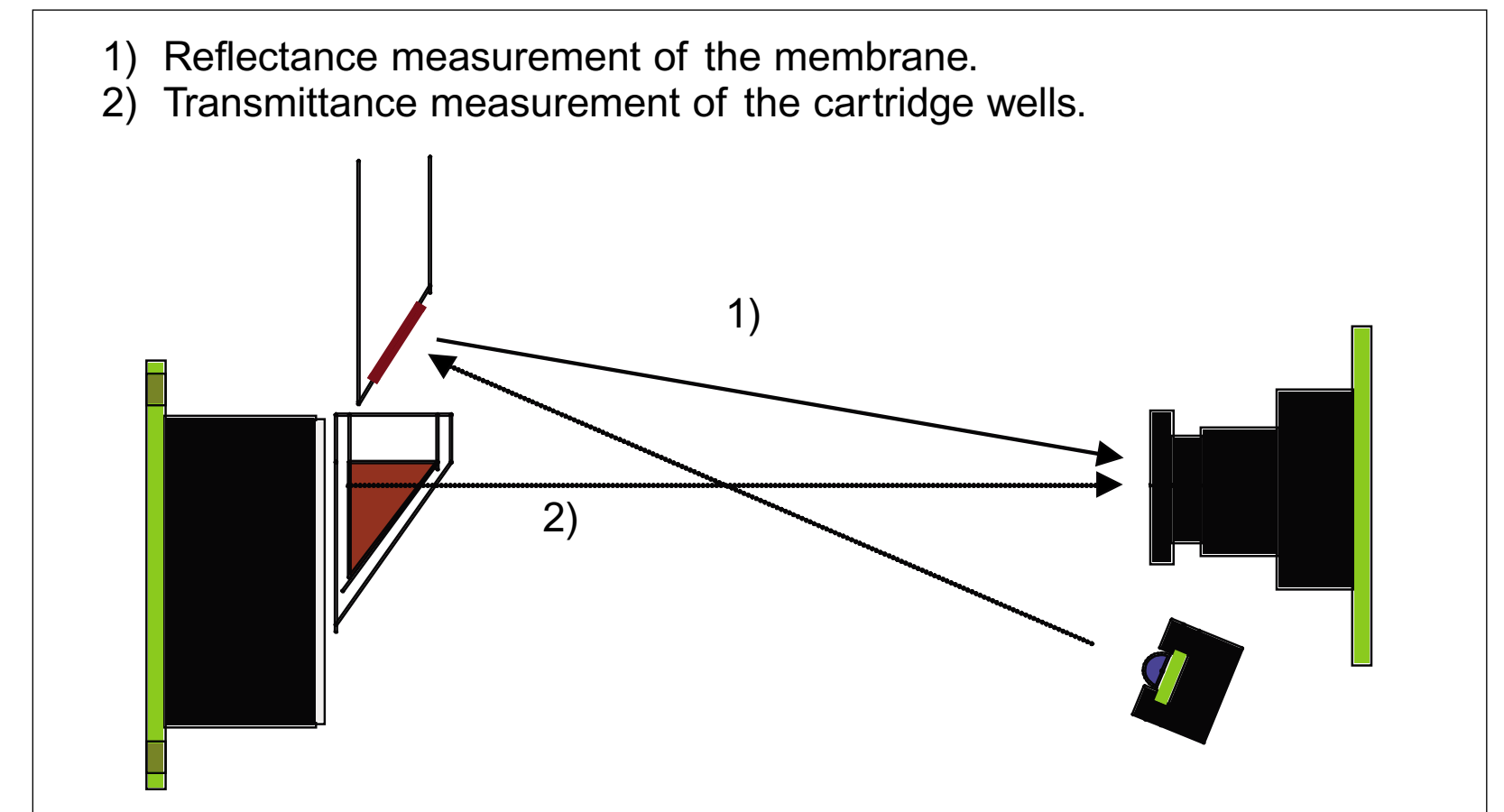


Figure 5. Color measurement.

The creatinine assay

Creatinine is quantified using an enzymatic colorimetric test involving four enzymatic steps (Figure 6). The digital camera measures transmittance of the colored end product in one of the test cartridge wells. The test requires two distinct enzyme solutions and pre-incubation of the sample before starting the reaction. Creatinine reaction and measurement takes place during albumin processing.

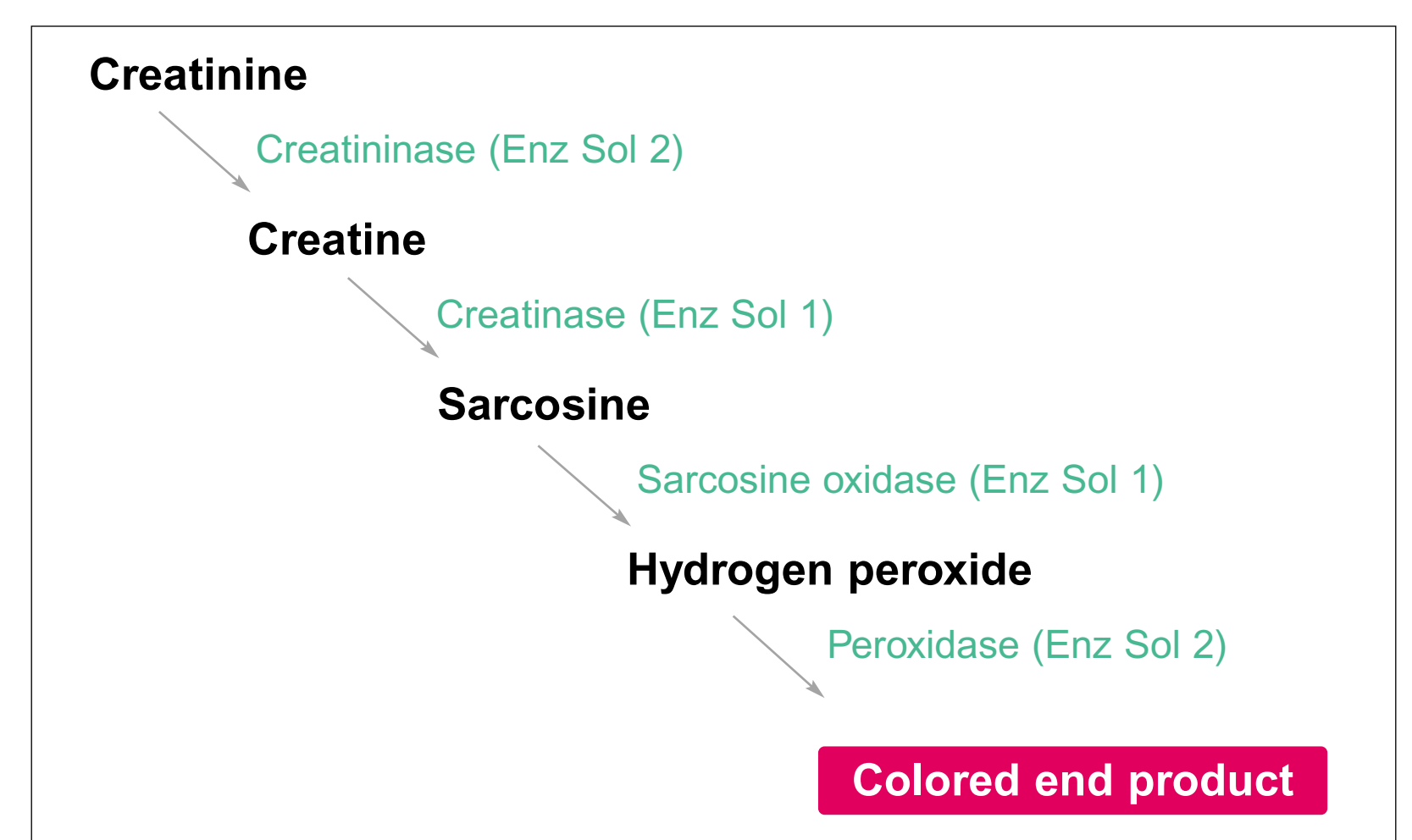


Figure 6. Principle of the enzymatic colorimetric creatinine assay

Afinion™ AS100 Analyzer

Linearity

Measuring range

Albumin: 5.0 – 200.0 mg/L
Creatinine: 16.4 – 339.9 mg/dL
ACR: 1.0 – 1250.0 mg/g

Linearity

The linearity of Afinion™ ACR was studied by analyzing two native urine samples; one with high albumin (191 mg/L) and creatinine (336.0 mg/dL), and one with low albumin (3.6 mg/L) and creatinine (27.1 mg/dL), and 8 intermediate samples prepared by intermixing the two samples.

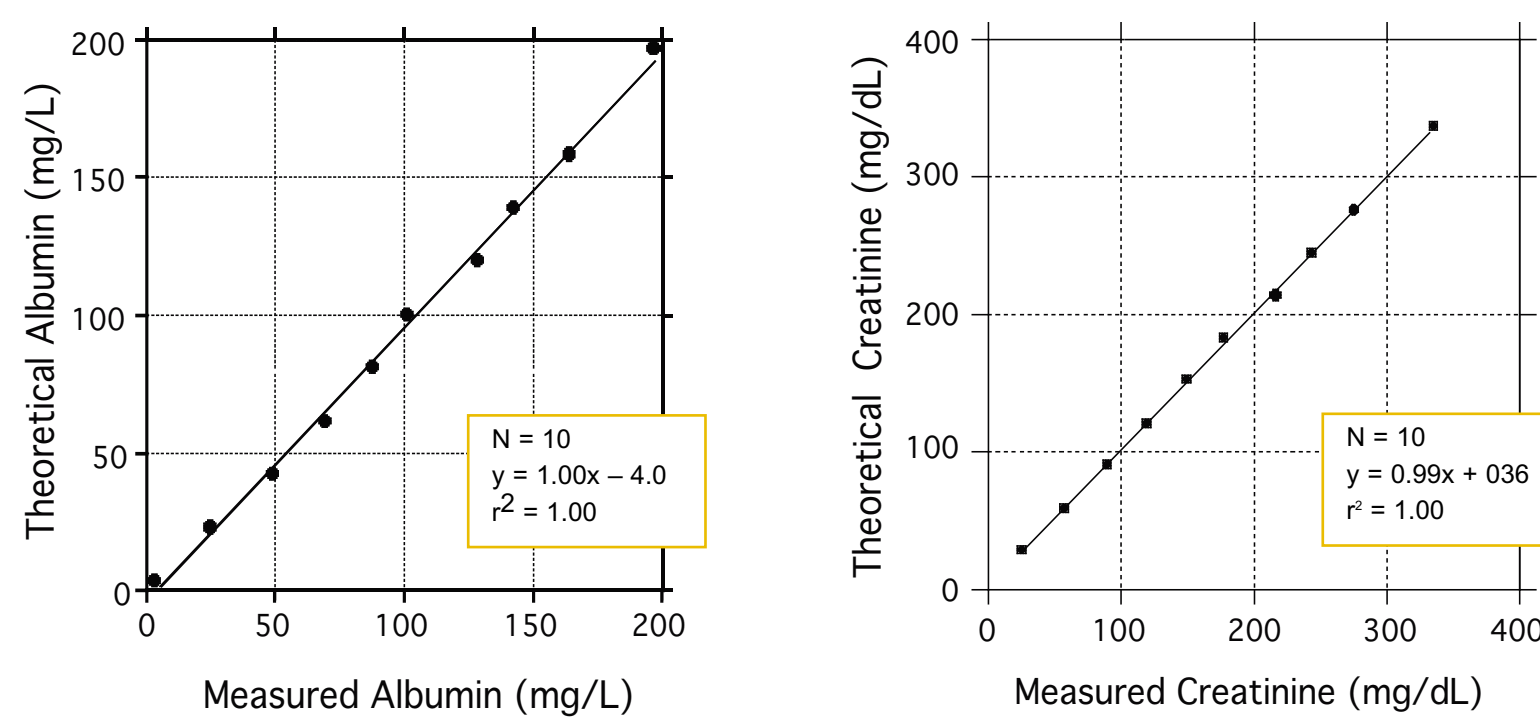


Figure 7. Theoretical versus measured values of albumin and creatinine.

Precision

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

Within-day precision						
Sample	Albumin (mg/L)		Creatinine (mg/dL)		ACR (mg/g)	
	Mean	CV %	Mean	CV %	Mean	CV %
Urine S1	176.7	3.9	52.7	3.2	335.8	5.2
Urine S2	55.7	4.6	163.7	3.8	34.1	4.2
Urine S3	13.0	3.5	351.4	3.3	3.7	4.0

Between-day precision						
Sample	Albumin (mg/L)		Creatinine (mg/dL)		ACR (mg/g)	
	Mean	CV %	Mean	CV %	Mean	CV %
Urine S1	174.9	2.0	51.4	0.0	340.6	2.8
Urine S2	55.3	0.0	162.3	0.0	34.1	0.7
Urine S3	12.6	1.2	348.1	0.6	3.6	0.0

Total precision						
Sample	Albumin (mg/L)		Creatinine (mg/dL)		ACR (mg/g)	
	Mean	CV %	Mean	CV %	Mean	CV %
Urine S1	174.9	5.0	51.4	3.8	340.6	6.0
Urine S2	55.3	4.8	162.3	2.8	34.1	4.6
Urine S3	12.6	5.5	348.1	3.0	3.6	6.0

The between instrument precision study was performed with 5 Afinion™ AS100 Analyzers in one operating day, with 10 replicates of each of the 3 urine samples being processed with each Analyzer.

Between instrument precision						
Sample	Albumin (mg/L)		Creatinine (mg/dL)		ACR (mg/g)	
	Mean	CV %	Mean	CV %	Mean	CV %
Urine S1	12.5	2.0	280.6	2.1	4.4	3.3
Urine S2	101.9	3.5	54.8	1.4	186.3	2.4
Urine S3	30.4	3.2	157.8	3.6	19.3	3.1

Method comparison

Afinion™ ACR was compared with one automatic laboratory method and one PoC method. A panel of 95 urine samples was analyzed for albumin and creatinine with Afinion™ ACR and with the comparison methods. The results were compared separately by Passing/Bablok analysis (Figures 8 and 9).

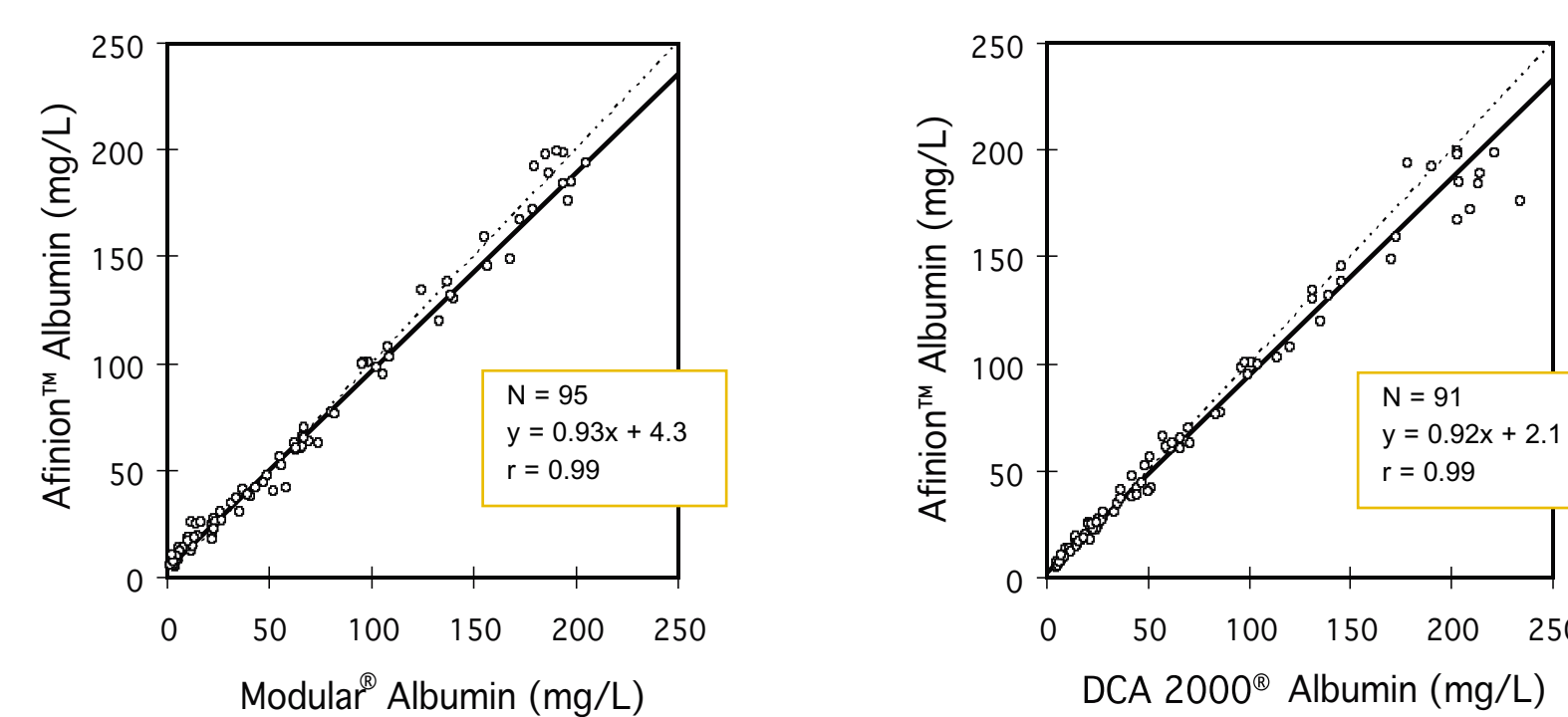


Figure 8. The albumin assay of Afinion™ ACR compared with Modular® and DCA 2000®.

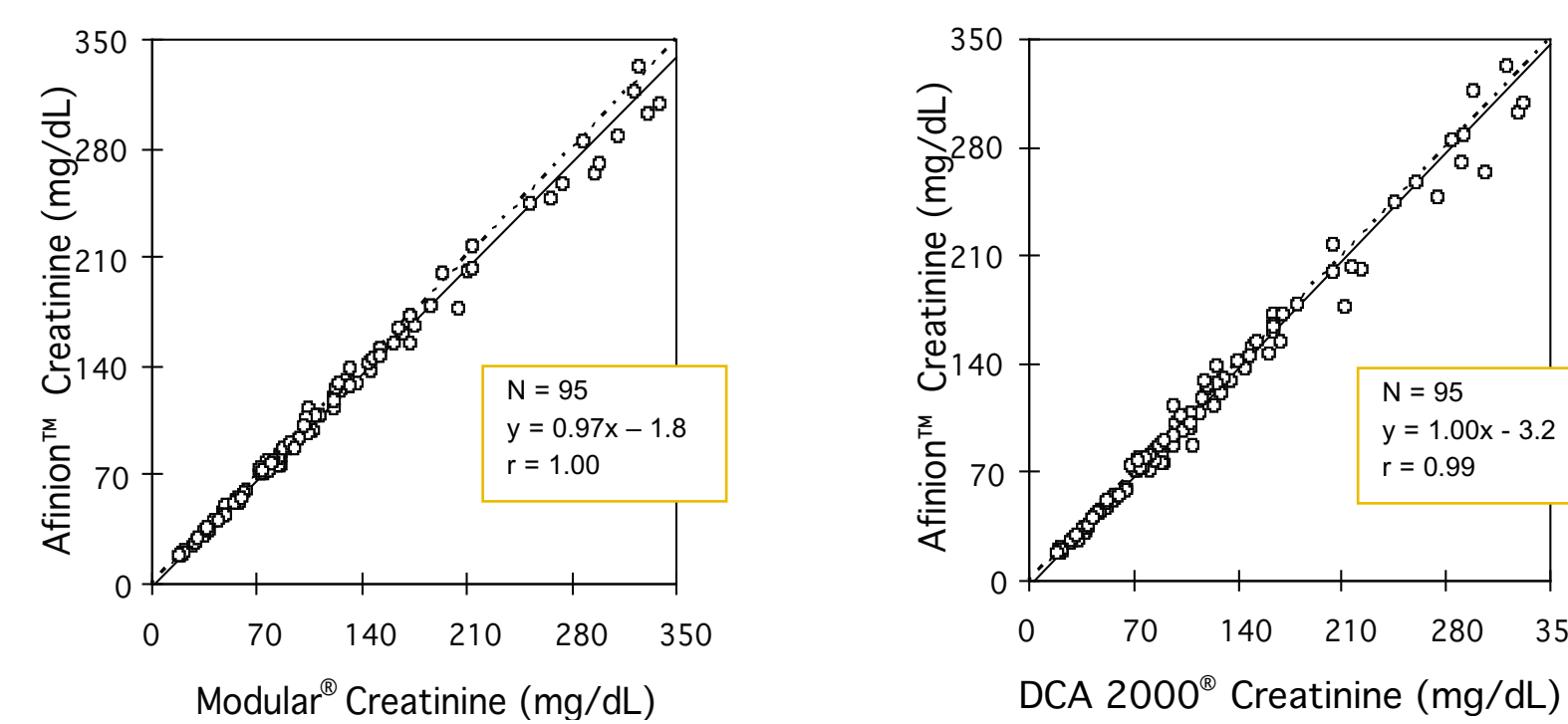


Figure 9. The creatinine assay of Afinion™ ACR compared with Modular® and DCA 2000®.

Sample dilution

Dilution table for preparation of samples for the sample dilution study.

Sample dilution	Saline volume (µL)	Sample volume (µL)	Theoretical value Albumin (mg/L)	Theoretical value Creatinine (mg/dL)
S1	0	300	206.9	416.2
S2	150	150	103.5	208.1
S3	200	100	69.0	138.7
S4	225	75	51.7	104.1

The results from this study show that albumin (Figure 10) and creatinine (Figure 11) in the samples can be properly recovered when diluted with saline (0.9 % NaCl).

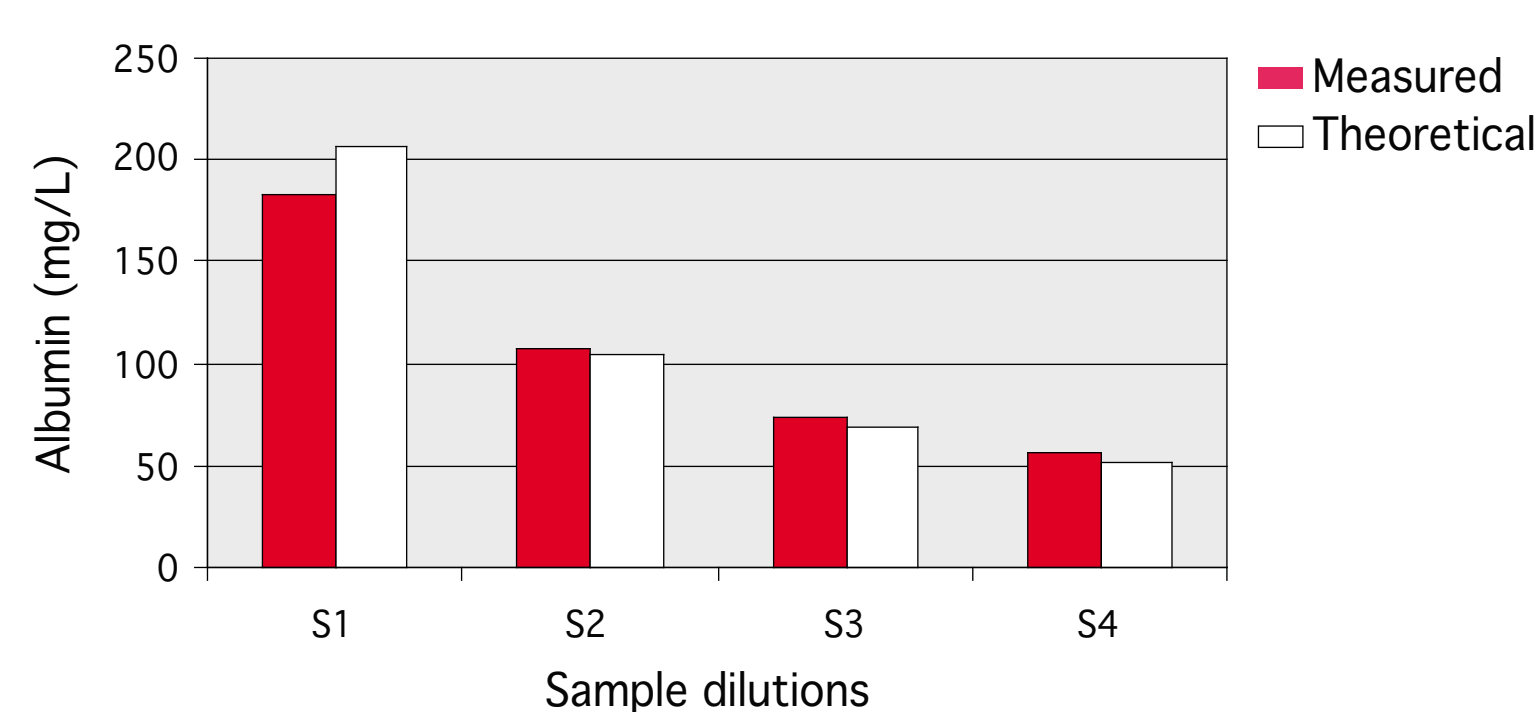


Figure 10. Sample dilution showing measured and theoretical albumin values.

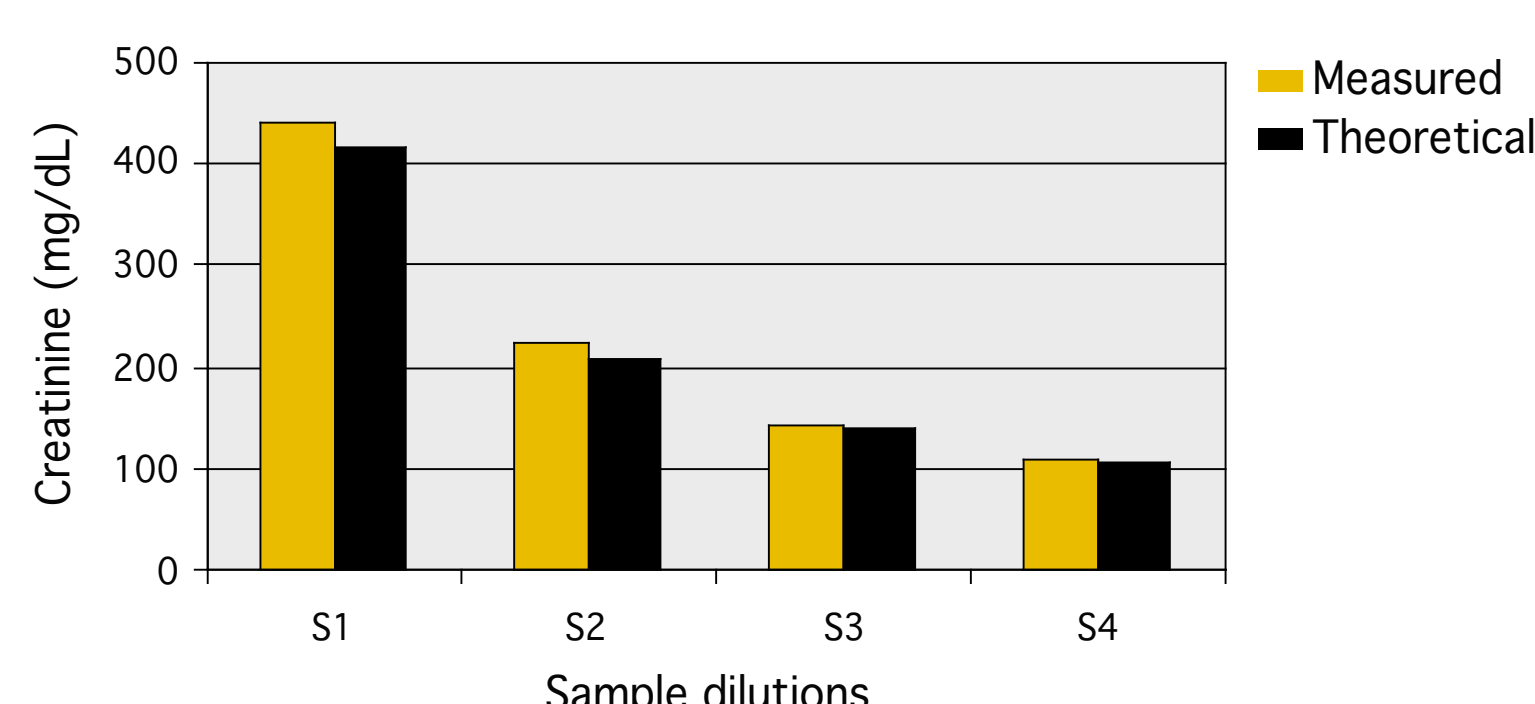


Figure 11. Sample dilution showing measured and theoretical creatinine values.

Excess albumin or creatinine

Excess albumin

The purpose of the study was to determine if a urine sample with excess albumin will result in a falsely low albumin measurement when using the Afinion™ ACR assay analyzed with the Afinion™ AS100 Analyzer. Spiked urine samples with up to 5000 mg/L albumin were tested.

The goal for this study was to show that samples with albumin above 200 mg/L will give results stated as "Albumin > 200.0 mg/L" on the display.

The study showed that excess of albumin in urine samples will not give falsely low albumin results. "Albumin > 200.0 mg/L" was stated on the display. Hence, no "hook effect" was seen.

Excess creatinine

The purpose of this study was to determine if excess creatinine will result in a falsely low creatinine measurement when using the Afinion™ ACR assay with the Afinion™ AS100 Analyzer. Spiked urine samples with up to approximately 1000 mg/dL creatinine were tested.

The goal for this study was to show that samples with creatinine above 339.9 mg/dL will give results stated on the display as "Creatinine > 339.9 mg/dL".

The study showed that excess of creatinine in urine samples will not give falsely low creatinine results. The result will be given as "Creatinine > 339.9 mg/dL".

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 giving 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 thereby falsely high ACR results.

Conclusions

The Afinion™ AS100 Analyzer is a compact desk-top analyzer, easily operated from the touch screen. The Analyzer measures both reflection and transmission with the integrated camera. The Analyzer is easy to use, rapid, and fully automated.

The Afinion™ ACR Test Cartridge contains all the reagents necessary for measuring the albumin and creatinine concentrations in urine samples. The new Afinion™ ACR test provides a reliable, precise, and convenient point of care method for the simultaneous determination of albumin, creatinine, and ACR (Albumin Creatinine Ratio). The assay time is 5 1/2 minutes using the Afinion™ AS100 Analyzer.

The user requirements for simplicity, robustness, security, and convenience are met by the simple sampling procedure, the automated analysis of the Afinion™ ACR Test Cartridge, the touch screen, and the error detection system.