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Afinion™ CRP – A new point of care test for determination of C-reactive protein in human serum/plasma and whole blood

Afinion™ CRP Assay System

Afinion™AS100 Analyzer is a small, bench top, multi-assay analyser for *in vitro* diagnostic point of care testing, launched by Axis-Shield PoC in May 2005.

The Afinion™ technology enables application of different assay principles, which allows for analysis of a variety of biochemical parameters in human blood or urine. Afinion™ CRP, for the quantitative determination of C-reactive protein (CRP) in human whole blood, serum and plasma, is one of the first tests developed for the Afinion™AS100 Analyzer.

The Analyzer with the single packed CRP Test Cartridges constitutes the Afinion™ CRP assay system (Figure 1). Assayed Afinion™ CRP Control materials are available for routine quality control.

Figure 1. *Afinion™ AS100 Analyzer System.*

CRP – a typical acute phase protein

C-reactive protein (CRP) is one of the acute phase proteins, normally found in plasma at concentrations below 5 mg/L. Cytokines stimulate de novo synthesis of CRP and the basic level is exceeded within four to eight hours after an acute inflammatory event, with values increasing up to 500 mg/L. The measurement of CRP is useful for the detection and evaluation of infection, tissue injury, inflammatory disorders and associated diseases.

Standardization and lot calibration

Afinion™ CRP is standardized against the IFCC/BRC/CAP protein reference material ERM® - DA470 (CRM 470).

Test specific calibration data are established for each lot of CRP Test Cartridges and then stored in the barcode label. When the Cartridge enters the Analyzer, the integrated camera reads the barcode. The calibration data for the actual lot are transferred to the Analyzer and used for calculating the results. Calibration by the operator is thus not required.

Assay principle

Afinion™ CRP is based on an immunometric membrane flow through assay. The Test Cartridge contains all the reagents necessary for one sample analysis (Figure 2), and is to be disposed after use. The blood sample is collected with the integrated sampling device before the cartridge is placed in the Analyzer. Test and lot specific information is read by the Analyzer from the barcode label of the Test Cartridge. The sample is then automatically diluted with a liquid that also lyses the blood cells. The sample mixture is aspirated through a membrane coated with anti-CRP antibodies and the CRP in the sample is concentrated onto this membrane. A solution containing anti-CRP antibodies conjugated with ultra-small gold particles is then aspirated through the membrane. The gold-antibody conjugate binds to the immobilized CRP on the membrane, which will turn red-brown. Excess gold-antibody conjugate is removed from the membrane by a washing solution (Figure 3). The colour intensity of the membrane is proportional to the amount of CRP in the sample.

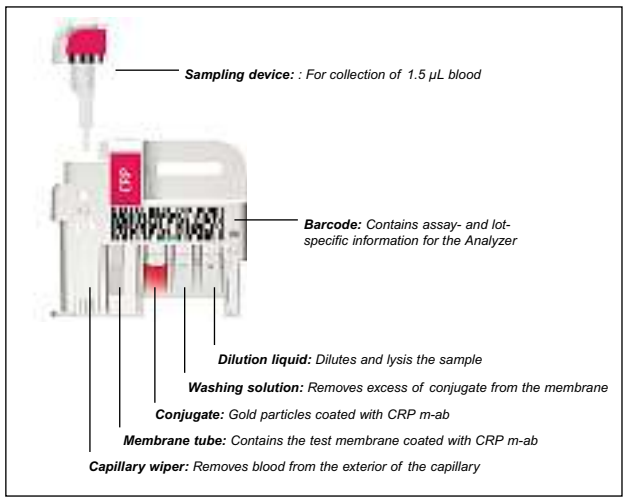


Figure 2. *Afinion™ CRP Test Cartridge.*

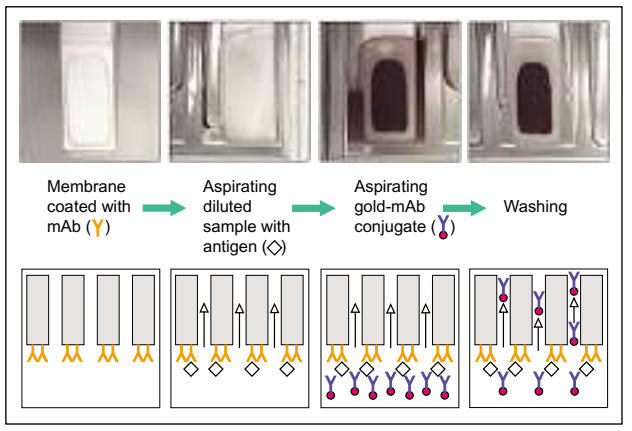
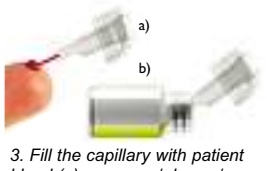


Figure 3. *Principle of the membrane flow-through immunoassay for CRP*

Step-by-step procedure

Collect sample	Analyse sample
 *1. Open foil pouch.*	 *5. Insert the Test Cartridge.*
 *2. Pull up the sampling device.*	 *6. Close the lid manually. Enter Patient ID.*
 *3. Fill the capillary with patient blood (a), or serum/plasma/control (b).*	 *7. Record the result when it appears on the screen.*
 *4. Replace the sampling device.*	 *8. Remove and discard the cartridge.*

Principle of measurement

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

Figure 4. *The coloured test membrane*

1) Measuring of reflection on the membrane.
2) Measuring of transmission of the wells with liquids.

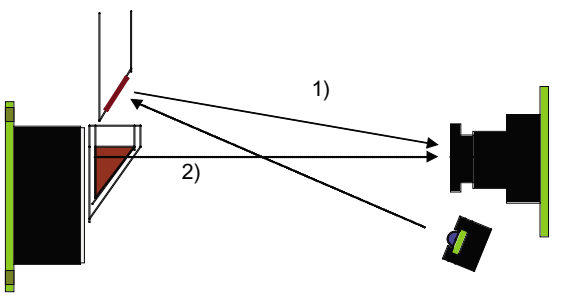


Figure 5. *Colour measurement*

Presented at the 17th IFCC – FESCC European Congress of Clinical Chemistry and Laboratory Medicine (EUROMEDLAB 2007), Amsterdam 3-7 June 2007.

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Linearity

CRP measuring range
5 - 160 mg/L (serum or plasma)
8 - 200 mg/L (whole blood)

Automatic correction for hematocrit (Hct)
When whole blood is used as sample material, the measured CRP value is automatically corrected according to the Hct of the sample (limited to range 20-60%). If the Hct value is outside the range 20-60%, no CRP test result will be reported and an information code will be displayed.

Linearity
The linearity of Afinion™ CRP was studied by analysing two native whole blood samples with high CRP (226 mg/L) and low CRP (3 mg/L) and eight samples with concentrations 3-226 mg/L prepared by mixing the two. The results are shown in Figure 6. Afinion™ CRP is linear in the whole dynamic measuring range.

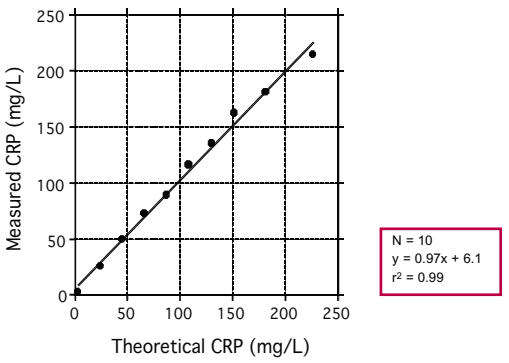

Figure 6. *Graph of theoretical CRP values versus measured values.*

Precision

Within-day precision
Within-day precision was performed with two serum samples and one whole blood sample, 20 replicas of each sample in one day. The results are shown in table 1.

Table 1. Mean CRP (mg/L) and CV (%) of within-day precision.

Sample	N (replicas)	Mean CRP (mg/L)	SD (mg/L)	CV (%)
Serum 1	20	15	0.4	2.5
Whole blood	20	35	1.4	4.0
Serum 2	20	68	2.7	4.0

Within-run, between-day and total precision
Within-run, between-day and total precision were performed according to NCCLS guideline EP5-A. Three patient samples, one whole blood and two serum samples were analysed in duplicates twice a day over 20 days. The two daily runs were separated by minimum two hours. The results are shown in table 2.

Table 2. Mean CRP (mg/L) and CV (%) of within-run, between-day and total precision for 3 samples calculated according to NCCLS guideline EP5-A.

Sample	N (days)	Mean CRP (mg/L)	Within-run CV (%)	Between-day CV (%)	Total CV (%)
Serum 1	20	15	4.7	2.2	5.2
Whole blood	20	35	5.7	2.3	6.2
Serum 2	20	68	4.0	1.7	4.4

Between instruments precision
Between instruments precision was performed with five samples analysed ten times per each of ten instruments. The results are shown in table 3.

Table 3. Mean CRP (mg/L) and CV (%) of between instruments precision for 5 samples analysed with 10 instruments, 10 times per each instrument.

Sample	No. of instrument	Mean CRP (mg/L)	CV (%)
Serum 1	10	7.8	2.9
Serum 2	10	19.3	1.6
Serum 3	10	77.3	1.6
Serum 4	10	141.0	1.6
Whole blood	10	41.0	2.3

Method comparison

Afinion™ CRP is compared with two automatic laboratory methods, both immunoturbidimetric assays. A panel of up to 84 serum samples was analysed by both methods and compared. The results are shown in Figure 7.

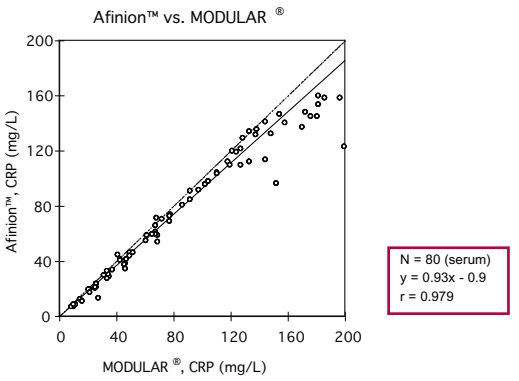

Figure 7.1. *Method comparison (Passing/Bablok regression) between Afinion™ CRP and Modular® using samples within measuring range for Afinion™ CRP.*

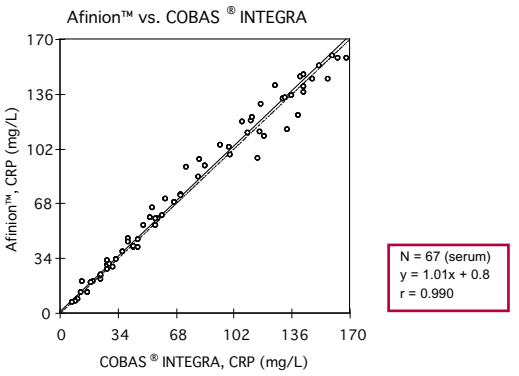

Figure 7.2. *Method comparison (Passing/Bablok regression) between Afinion™ CRP and Cobas® Integra using samples within measuring range for Afinion™ CRP.*

Substances tested for interference

Afinion™ CRP was tested for several possible interfering substances. Tested substance and maximum concentrations with no significant interference are shown in table 4.

Table 4. Maximum concentrations of substances giving no significant interference.

Substance	Results
Bilirubin	510 µmol/L (30 mg/dL)
Triglycerides	8 mmol/L (700 mg/dL)
Cholesterol	10 mmol/L (400 mg/dL)
Rheumatoid Factor	760 IU/mL
Leukocytes	30 x 10^9/L
High CRP concentration	No “Hook effect” up to 2000 mg/L
HAMA	913 ng/mL
Anticoagulants	EDTA and heparin at concentrations normally used in blood collection tubes.

Conclusions

The Afinion™ AS100 Analyzer is a compact desk-top analyser, easily operated from the touch screen. Once loaded with the Test Cartridge, the Afinion™ AS100 Analyzer represents a true walk-away system.

The Afinion™ CRP Test Cartridge contains all reagents necessary for measuring the CRP concentration in whole blood, serum or plasma. If the sample is identified by the Analyzer as whole blood, the hemoglobin concentration is also measured, the hematocrit calculated and the CRP value automatically corrected.

Afinion™ CRP is standardized against the ERM®-DA470 reference preparation. The Afinion™ CRP method correlates well to automated laboratory methods, and demonstrates good precision in the hands of trained and non-trained users.

The sample volume is 1.5 µL and assay time < 4 minutes. The Afinion™ CRP assay system has proven to fulfil user requirements for simplicity, speed, robustness, and reliability.

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