C Reactive Protein (CRP) – Turbidimetric method

Instructions for Use (IFU)

IVD CE

ORDERING INFORMATION

	Codice	Composizione
OPEN KONELAB INDIKO	REF B78182268	n° 3 vials x 60 mL R.A n° 2 vials x 3 mL R. B
CHEMILAB	REF B82181001	n° 1 vial x 29 mL R.A n° 1 vial x 2 mL R. B
	REF B82181002	n° 5 vials x 28 mL R.A n° 1 vial x 5 mL R. B

INTENDED USE

Immunoturbidimetric diagnostic test for the quantitative determination of C-Reactive Protein in human serum and plasma. All results must be interpreted in relation to the clinical context. FOR PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE

C-Reactive Protein (CRP) is present in serum at the onset of inflammatory processes of both infective and non-infective origin. The determination of this protein is of great diagnostic and prognostic importance because it is associated with numerous diseases (septic and aseptic inflammation, myocardial infarct, malignant tumours, rheumatic diseases, rheumatoid arthritis, senile vascular diseases, etc.) as a probable consequence of tissue degeneration; this parameter is considered even more specific than the erythrosedimentation rate (ESR).

However, while the ESR may remain constant even during the remission phase, CRP appears earlier at the beginning of the inflammatory phase and disappears more rapidly during recovery; for this reason, determination of the CRP level is of prognostic importance regarding evolution of the disease.

CRP is of particular interest in the differential diagnosis between myocardial infarct, where it is always present, and coronary insufficiency where it is generally absent.

In tumors, positive CRP rate is in a higher percentage in malignant rather than in benign forms. In conclusion, determination of the CRP is useful in rheumatic diseases to monitor the evolution of the disease itself, rather than as a diagnostic aid.

PRINCIPLE OF THE METHOD

Turbidimetric method. The CRP contained in the test sample reacts with the specific antibodies, forming immunocomplexes, causing a degree of turbidity which can be detected photometrically and is proportional to the CRP concentration in the sample. The quantitative analysis is obtained by interpolation of this photometric value with those found by testing known concentrations of CRP.

Storage and Stability

= **Storage temperature 2-8 °C**

If stored at 2-8°C avoiding direct light, the intact reagents remain stable until the expiration date, printed on the label. Do not freeze. Slight variations in composition among batches will not affect test results.

Concentration

Reagent A			
		Conc.	**U.M.**
CRP Buffer	TRIS	0.05	mol/L
	PEG	5	%
	NaN_3	< 0.1	%
Reagent B			
CRP Goat antiserum	NaN_3	< 0.1	%

Materials included in the kit

Reagent as described above.

Necessary materials not included in the kit

Controls, calibrators and pipettes with adequate volume.

PRECAUTIONS and WARNINGS

1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting of serious incidents

Please inform the manufacturer (through your distributor) and the competent authority of the member state of the European Union in which the user and/or patient is established, of cases of serious incident that has occurred in relation to the device. For other jurisdictions, reports of serious incidents must be made in accordance with the regulatory requirements of the home Member State. By reporting serious incidents, you help provide more information about the safety of your in vitro medical diagnostic device.

PROCEDURE

Quality Control

Use the Sclavo Diagnostics Int. ASO - CRP - RF Control Low B47182278 and High B47182279 for your quality control purposes at least once a day. Repeat the analysis also after calibration. Obtained values must be within the range of acceptability.

Calibration

For calibration, use the Sclavo Diagnostics Int. CRP Single Level Calibrator B47182290, according to dilutions reported on the calibrator value sheet

Traceability

The CRP value has been harmonized according to the ERM using the reference material ERM-DA 474/IFCC.

SAMPLE

Sample types and storage

Serum or plasma obtained by normal medical techniques can be used. No special preparation of the patient is necessary. Test samples can be stored for 3 days at 2-8°C or 12 months if frozen. Defrost samples at room temperature and mix carefully by turning upside down before testing. Avoid repeated freezing and thawing of samples.

The samples do not require predilution before analysis.

Strongly lipemic samples or those which present a high degree of turbidity or precipitates must be clarified by centrifugation (10 min. at 15,000xg), before testing.

PREPARATION OF THE REAGENT

The Reagents are liquid, ready for use. After opening, the Reagents are stable until the expiry date if kept as indicated in "Storage and stability".

Automation

The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R1/ R2/C. Validated applications are available for Sclavo Konelab® - Indiko® and CHEMILAB instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD

The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

Reaction conditions

Wavelength: 340 nm
Temperature: 37°C
Reaction: Two point-end

Dispense as follows for the blank, for each calibrator and unknown samples

	Volume (µL)
Buffer R1	250
Test Sample	25
Mix and incubate at 37°C for at least 3 minutes, read the absorbance (Abs-1) of the calibrators and samples, then add:	
CRP Reagent (R2)	12
Mix and incubate at 37°C. After 5 minutes, read the absorbance (Abs-2) of the calibrators and samples.	

Note: **The reaction volumes can be varies proportioning without altering the results.**

Results

The concentration of C Reactive Protein is obtained as following: Generate the calibration curve with the ΔAbs values and concentration of the single calibrators. Calculate the analytical result expressed in "mg/L", from the calibration curve. All samples with a C Reactive protein concentration higher than the highest calibration point and/or giving a signal denoting an excess of antigen (in the automated instruments) must be diluted and retested. It is advisable to use doubling dilutions in saline.

Control of the calibration curve

The calibration curve is valid for at least one month. However, its validity should be checked periodically using the SCLAVO Diagnostics Control ASO-CRP-RF Low

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B47182278 High B47182279. The validity is confirmed if the values obtained are within the concentration range of the controls reported on the vial labels.

Automation

The results are automatically calculated by the analyzer based on the calibration line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration line is calculated automatically by the different instruments.

REFERENCE RANGE

The typical reference range is ≤ 10 mg/L.

Since gender, age, geographic location, and other factors can affect population normal ranges, each laboratory should determine the mean and pathological normal ranges for the population in its catchment area for this test.

CHARACTERISTICS/PERFORMANCE

Analytical Range – Antigen excess

The analytical range was tested using a strongly positive sample and serial dilutions of this serum in saline solution. The method guarantees a correct response throughout the minimal detectable measurement range and the calibrator higher concentration. The present method does not show Antigen Excess until 1000 mg/L. Heterophyl antibodies and monoclonal immunoglobulins (or parts of these proteins) can interfere with the test. Results must be evaluated carefully in the case that patients' sera could contain these antibodies.

Accuracy

Commercial control sera were tested with the present kit and the data obtained with the analyzer are reported in the table below (mean of three tests).

IRMM ERM® - DA474			Sclavo
Sample	Mean (mg/L)	Range	Mean (mg/L)
Low	15.0	11.2 – 18.7	17.6
High	44.5	33.4 – 55.6	48.4

The protein concentrations are calculated in reference to the European Reference Standard for Proteins in Human Serum ERM®- DA474, It is produced and certified under the responsibility of the IRMM (Institute for Reference Materials and Measurements) in accordance with the principles formulated in the technical guidelines of the ERM "European Reference Materials® in the cooperation agreement between BAM-IRMM-LCQ.

Specificity

The method is 100% specific for human C Reactive Protein. The antiserum used is monospecific for human CRP if tested in immunoelectrophoresis against a pool of human serum or whole plasma, concentrated 2X.

Interferences

Interfering	Limit
Bilirubin	**50 mg/dL**
Ascorbic acid	**50 mg/dL**
Hemoglobin	**500 mg/dL**
EDTA	**10 mM**
Sodium citrate	**1000 mg/dL**
Heparin sodium	**40 mg/mL**
Triglycerides	**2%**
rheumatoid factor	**2000 UI/ml**

**No appreciable interference was found in any case, and the variations observed were within the expected precision range. Higher concentrations were not tested.*

However, in view of the wide heterogeneity of potentially interfering substances and pharmaceuticals, for diagnostic purposes the results of this test must always be taken into consideration in conjunction with the clinical history of the patient, other clinical tests and medical investigations.

Precision of the method

The Precision of the analytical results has been determined as Repeatability and Total Precision according to the CLSI EP15-A2 guideline, using commercial control sera. The data obtained are shown in the following table (confidence interval 95%).

Within-run Precision – Repeatability				
Level	**Replicates**	**Mean (mg/L)**	**DS**	**CV%**
Low	10	19.6	0.381	1.94
Medium	10	62.6	0.977	1.56
High	10	124.5	2.303	1.85
Total Precision (Within-lab Precision)				
Level	**Replicates**	**Mean (g/L)**	**DS**	**CV%**
Low	10	20.8	0.461	2.21
Medium	10	64.4	1.431	2.22
High	10	128.6	3.370	2.62

Limits of sensitivity

The Sensitivity limit has been measured using serial dilutions of a high concentrated serum. The smallest measurable concentration is 2.3 mg/L.

Comparison between methods

The method was compared with another commercially available, analyzing 173 human sera. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	4.463
Slope	1.0594
Correlation Coeff. (R)	0.9947

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

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