CRM® *diagnostic systems*

CRP/hs (Ultrasensitive C- Reactive Protein)

For the quantitative determination of C-reactive protein (CRP) **at low concentrations** in human serum by immunoturbidimetry.

Diagnostic Relevance

C-reactive protein (CRP) is one of the acute phase proteins being synthesised by hepatocytes. Complexed CRP activates the classical complement pathway, initiating a range of process including phagocytosis and lysis of invading cells. It is thought to recognise potentially toxic substances released from damaged tissues, bind and detoxify them, removing them from the blood. Serum CRP levels can increase up to 1000 fold after tissue injury. CRP levels can also distinguish the degree of disease activity with high levels indicating a poor outcome. CRP is distinguished by the speed of response to injury (4-6 hours). Serum CRP levels increase following surgery, myocardial infarction, trauma, infection and neoplastic proliferation.

New evidence has shown an inflammatory and, perhaps infections contribution to the atherosclerotic process. CRP may be useful in detecting this process and providing important prognostic information about patients with cardiovascular events.

Principle

This CRP test is based upon the reactions between C reactive protein (CRP) and latex-covalently bound antibodies against human CRP. CRP values are determined turbidimetrically using fixed-time measurement with sample blank correction. The relationship between absorbance and concentration permits a multipoint calibration with a measuring range between 0 and 50 mg/L. The measuring temperature is 37ºC. The assay can be performed on all instruments allowing turbidimetric measurements at 500 to 600 nm.

Reagents

Each CRP kit contains:

A.- Buffer - 30 ml of TRIS buffer (0.05), pH: 7.2, containing polyethyleneglycol and 0.09 % sodium azide as preservative.

B.- Latex reagent – 5.1 ml of Polystyrene particles (0.5%) coated with goat antibodies anti-human-CRP serum in a glycine buffer (0.1 M, pH: 8.2), containing NaCL (0.15 M) and bovine serum albumin (0.5%). Preservative: Sodium azide 0.075%

Precautions

For in vitro diagnostic use only. Do not pipette by mouth. Reagents containing sodium azide must be handled with precaution. Sodium azide can form explosive azides with lead and copper plumbing. Since absence of infectious agents cannot be proven, all specimens and reagents obtained from human blood should always be handled with precaution using established good laboratory practices.

Disposal of all waste material should be in accordance with local guidelines.

As with other diagnostic tests, results should be interpreted considering all other test results and the clinical situation of the patient.

Materials required but not provided

Automatic analyzer.
Saline solution.
Calibrator.
Controls.

Storage and Stability

The CRP reagents should be stored tightly capped at +2..+8ºC when not in use. **Do not freeze**. Reagents in the original vials are stable to the expiration date on the vial label when capped and stored at +2...+8ºC. Immediately following the completion of an assay run, the reagent vials should be capped until next use in order to maximize curve stability. Once opened the reagent can be used within 1 month if stored tightly closed at +2...+8ºC after use.

The CRP buffer reagent should be clear and colourless. Any turbidity may be sign of deterioration and reagent should be discarted.

The CRP latex reagent should have a white, turbid appearance free of granular particulate. Visible agglutination or precipitation may be a sign of deterioration, and the reagent should be discarted.

Specimens

Fresh or deep frozen serum. CRP remain stable for 8 days at +2 to +8ºC. If the test should be performed later, it is recommended to freeze the serum. Avoid successive freezing and thawing. Discard haemolysed or contaminated samples.

Heavily lipaemic sera and turbid frozen serum samples must be cleared with a delipidating agent. Delipidation of samples do not affect the results of CRP in serum samples. The cleared patient serum sample must be used on the same day, as turbidity may reoccur.

Procedure

The reagents are ready to use as supplied. Latex reagent should be gently shaken before each use.

Follow the instructions of the operator's manual to load the cartridge, technique programation, calibration, sample measurement and control.

Calibration. Quality control

Standardization: use CRM® *diagnostic systems* Calibrator. The method was standardized against the CRM 470 international standard

The CRP concentration of the Standard and Control is given on the label. Prepare the following dilutions of the standards using saline solution:

1; $^1/_2$; $^1/_4$; $^1/_8$; $^1/_{16}$, $^1/_{32}$; $^1/_{64}$

The standard dilutions are to be used for measurement within 4 hours.

This curve is stored in memory by the analyzer and recalled for later use. Calibration curves are stable for up to 14 days, after which a new curve must be generated. Additionally, recalibration must be performed whenever reagent lots are changed.

For quality control use CRM® *diagnostic systems* Control or other suitable control material. The control intervals and limits must be adapted to the individual laboratory requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Control must be assayed and evaluated as for patient samples.

Calculation

The turbidimetric analysers automatically calculate the CRP concentration of each sample.

Conversion: mg/L = µg/ml.

Reference Values

Although recently, it has be found that base-line plasma CRP concentrations are higher among individuals who went or to have cardiovascular events than among those individuals without these events. Until present, values < 6 - 8 mg/L are considered within the normal range. Each laboratory should establish an expected range for the geographical area in which it is located.

Assay range and Sensitivity

The assay range is established from 0 to 50 mg/L. Manually dilute samples having higher concentration with 0,9 % NaCl. Multiply the result by the appropriate factor.

Calculating the mean plus 3SD of twenty replicates of zero standard resulted in a lower limit of detection of 0,18 mg/L

Specificity

The assay is specific for CRP determination.

Due to the assay measuring principle (wavelength 550 nm), no endogenous interference by billirubin (up to 350 µmol/L), haemoglobin (up to 15 g/L) and intralipid (up to 0,5%) occurred.

As is well known, rheumatoid factors may cause falsely high values in the nephelometric or turbidimetric assays for CRP. Therefore, CRP values in RF positive samples must be carefully interpreted. Samples with very high RF activity could easily be identified by a RF-assay (i.e. CRM® *diagnostic systems* RF) should be treated with equal volumes of a 10-mmol/L dithiothreitol solution for 30 min. This pre-treatment abolished the RF activity completely (Clin Chem 1986; 32: 124).

Other substances can interfere. For a comprehensive review of interfering substances, refer to the publication by Young.

Precision

The precision of the method in the cut-off value of decision (1,8 - 2 mg/L) is less than 5,5%.

Assay Linearity

Linearity was evaluated using serial dilutions, prepared with saline solution, of three pooled samples, which contained values of CRP in the range of analysis ranging from 0,7 to 44 mg/L. Linear regression values of CRP mg/L vs concentration yielded correlation coefficients, r> 0.999, for all samples. Within the assay's measuring range, the deviations of measurement from theoretical values did not exceed the 10 % level. In addition, the system did not show prozone phenomenon at least up to 120 mg/L.

Method comparison

25 samples were correlated with a fixed-time nephelometric commercial procedure. When comparing the results by lineal regression the result was: y=1,003x – 1,6 and r=0,996.

Analytical characteristics have been obtained in a single experiment in a Cobas-Mira plus analyser. As is well known the analytical characteristics of a clinical chemistry reagent depend on both the reagents and the instrument used. Multicenter studies indicate important differences in analytical characteristics among similar instruments. Therefore, the data expressed in the present document should be interpreted as a guide example.

Bibliography

Ridker PM et al. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation 1998; 97: 2007-11.

Linzzo G et al. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. N. Engl. J. Med. 1994; 331: 417-24.

Kuller LH et al. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Am. J. Epidemiology 1996; 144: 537-47.

Young DS. Effects of Drugs on Clinical Laboratory Test. 5th Edition, AACC Press, 2000.

Sonderdruck aus DG Klinische Chemie Mitteilungen 1995; 26: 207 – 224.

Significados de los símbolos indicados en las etiquetas. Explanation of symbols used on labelling. Explication des symbols figurant sur les etiquettes. Spiegazione dei simboli utilizzati sull'eticheta. Significado dos símbolos indicados nas etiquetas. Erläuterung der symbole auf den etiketten.

Fecha de Caducidad Expirate Date Date de Péremption Data di Scadenza Data Expiração Verwendbar bis	Temperatura de almacén Storage Temperature Temperature de Conservation Temperatura de Conservazione Temperatura de Conservação Lagertemperatur	LOT Número de Lote Lot Number Número de Lot Numero di Lotto Número de Lote Chargen-Nr	IVD Para Diagnóstico In Vitro For In Vitro Diagnostic Usage In Vitro Per Uso Diagnostico In Vitro Utilizar em Diagnostico In Vitro In Vitro Diagnosticum	REF Número de catálogo Catalog Number Numéro de catalogue Numero di catalogo Número de catálogo Katalognummer	CE Conformidad Europea European Conformity Conformité aux normes européennes Conformità europea Comformidade com as normas europeias CE-Konformitätskennzeichnung	Fabricado por Manufactured by Fabriqué par Fabbricato da Fabricado por Hergestellt	REAG Reactivo Reagent Réactif Reagenti Reagente Reagenz	CAL Calibrador Calibrator Calibrateur Calibratore Calibrador Kalibrator	Buffer Tampón Buffer Tampon Tampone Buffer Puffer	LYOPH Liofilizado Lyophilised Lyophilisé Liofilo Liofilizado Lyophilisiert	Conc. Concentración Concentration Concentration Concentrazione Concentraçao Koncentration	Control H / Control L Control Alto / Control Bajo Control High / Control Low Contrôle élevé / Contrôle Bas Controllo Alto / Controllo Basso Controlo Alto / Controlo Baixo Kontrolle Hoch / Kontrolle Niegrid

BL, S.L. C/Calahorra 4, 26006 Lo- Spain.

2011/09

Ultrasensitive C-Reactive Protein / Proteína C Reactiva Ultrasensible Procedure for / Procedimiento para COBAS MIRA PLUS

GENERAL	
Measurement mode:	Absorb
Reaction mode:	D-R-S-SR1
Calibration mode:	LOGIT/LOG4
Reagent blank:	REAG/DIL
Cleaner:	NO
Wavelenght:	550 nm
Decimal position:	1
Units:	mg/L
ANALYSIS	
DILUENT NAME	Saline
FACTOR	NO
TIME	NO
STD	MAIN INDIRECT
MAIN STD POS**	mg/L*
FACTOR STD-1: 1.0	2: 2.0
3: 2.0	4: 2.0
5: 2.0	6:2.0
7: 0.0	8: NO
POST DIL. FACTOR:	2.00
CONC. FACTOR:	NO
SAMPLE	Cycle:3 Volume: 3.0 µL
DILUENT	Name: H_2O Volume: 10.0 µL
REAGENT	Cycle: 1 Volume: 250 µL
START R1	Cycle: 1 Volume: 50.0 µL
DILUENT	Name: H_2O Volume: 10.0 µL

CALCULATION	
Sample limit:	NO
Reaction Direction:	INCREASE
Check:	ON
Antigen excess	NO
Conversion factor:	1.00000
Offset:	0.00000
Normal range low:	NO
Normal range high:	NO
Number of steps:	1
Calc. Step A:	ENDPOINT
Reading first:	2
Reading last:	15
CALIBRATION	
Calibr. Interval:	ON REQUEST
Reagent blank:	
Reag. Range low: NO	High: NO
Blank range low: NO	High: NO
STANDARDS	
1: *	2:
3:	4:
5:	6:
7:	8: NO
REPLICATE: SINGLE	
Deviation:	NO
Correction std:	NO

(*) Standard value on the label / insert
(**) Select one position on the standard rack

Revision 2011/09