Audit Diagnostics

CE

CRP-ULTRA

CRP-ultrasensitive

Latex turbidimetry

Quantitative determination of low levels of C-Reactive Protein

IVD

Store 2 - 8ºC.

PRINCIPLE OF THE METHOD

The CRP-ultrasensitive is a quantitative turbidimetric test for the measurement of low levels of C- reactive protein (CRP) in human serum or plasma.
Latex particles coated with specific anti- human CRP are agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance change, dependent upon the CRP contents of the patient sample that can be quantified by comparison from a calibrator of known CRP concentration.

CLINICAL SIGNIFICANCE

CRP is an acute-phase protein present in normal serum, which increases significantly after most forms of tissue injuries, bacterial and virus infections, inflammation and malignant neoplasia. CRP may be also useful in detecting atherosclerotic process and providing important prognostic information about patients with asymptomatic heart disease, unstable angina, and myocardial infarction. Recent studies in apparently healthy people show that CRP concentration in serum rise long before traditional symptoms of heart and vascular diseases are noticed.

REAGENTS

Diluent-ultra (R1)	Tris buffer 20 mmol/L, pH 8.2. Preservative.
Latex-ultra (R2)	Latex particles coated with goat IgG anti- human CRP, pH 7.3. Preservative.
U-CRP CAL	Liquid Calibrator. C-Reactive protein concentration is stated on the vial label.
Optional	CRP Ultra Control.

PRECAUTIONS

Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

CALIBRATION

Use CRP Ultra Calibrator .
The sensitivity of the assay and the target value of the calibrator have been standardized against the Reference Material ERM-DA 472/IFCC.
Recalibrate when control results are out of specified tolerances, when using different lot of reagent and when the instrument is adjusted.

PREPARATION

CRP Calibrator: Ready for use.
Calibration curve: Prepare the following CRP calibrator dilutions in NaCl 9 g/L. Multiply the concentration of the CRP calibrator by the corresponding factor stated in table bellow to obtain the CRP concentration of each dilution.

Calibrator dliution	1	2	3	4	5	6
CRP Calibrator (µL)	--	5	10	25	50	100
NaCl 9 g/L (µL)	100	95	90	75	50	--
Factor	0	0.05	0.1	0.25	0.5	1.0

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not use reagents over the expiration date.
Do not freeze; frozen Latex or Diluent could change the functionality of the test.
Reagent deterioration: Presence of particles and turbidity.

ADDITIONAL EQUIPMENT

- Thermostatic bath at 37ºC.
- Spectrophotometer or photometer thermostatable at 37ºC with a 540 nm filter (530-550).

SAMPLES

Fresh serum. Stable 7 days at 2-8ºC or 3 months at –20ºC.

The samples with presence of fibrin should be centrifuged before testing.
Do not use highly hemolized or lipemic samples.

PROCEDURE

1.Bring the reagents and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:
   Wavelength: 540 nm (530-550)
   Temperature: 37ºC
   Cuvette ligth path: 1 cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

R1. Diluent (mL)	0.8
R2. Latex (mL)	0.2

5. Mix and read the absorbance (reagent blank).
6. Add the sample / calibrator.

	Blank	Sample/Calibrator
ClNa 9 g/L (µL)	10	--
Calibrator or sample (µL)	--	10

7. Mix and read absorbance after 4 minutes (A_2) of the sample/ calibrator addition.

Audit Diagnostics has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

CALCULATIONS

Calculate the absorbance difference (A_2-A_{blank}) of each point of the calibration curve and plot the values obtained against the CRP concentration of each calibrator dilution. CRP concentration in the sample is calculated by interpolation of its (A_2-A_{blank}) in the calibration curve.

QUALITY CONTROL

Control Sera are recommended to monitor the performance of manual and automated assay procedures. It should be used the Audit Diagnostics CRP Ultra Control .Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Below 3 mg/L is considered normal.
Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. **Linearity limit:** Up to 5 mg/L, under the described assay conditions. Samples with higher concentrations should be diluted 1/3 in NaCl 9 g/L and re-tested again. The linearity limit depends on the sample / reagent ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. **Detection limit:** Values less than 0.05 mg/L give non-reproducible results.
3. **Prozone effect:** No prozone effect was detected upon 100 mg/L.
4. **Sensitivity**: Δ 44 mA.mg/L.
5. **Precision:** The reagent has been tested for 20 days, using three different CRP concentrations in a EP5-based study.

EP5	CV (%)		
	+/- 0.28 mg/L	+/- 3.09 mg/L	+/- 5.95 mg/L
Total	7.7%	2.7%	3.0%
Within Run	4.5%	1.7%	1.4%
Between Run	4.7%	1.9%	2.7%
Between Day	4.1%	0.7%	0.0%

6. **Accuracy:** Results obtained using this reagent (y) were compared to those obtained using a commercial reagent (x) with similar characteristics. 23 samples of different concentrations of CRP were assayed. The correlation coefficient (r) was 0.81 and the regression equation y=0.517x+0.916.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Bilirubin (20 mg/dL), lipemia (10 g/L) and rheumatoid factors (75 IU/mL), hemoglobin (10 g/L), do not interfere. Other substances may interfere[8].

NOTES

Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

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