TURBITEST AA LINE

PCR

Immunoturbidimetric method for quantitative determination of C Reactive Protein (CRP)

SUMMARY

C Reactive Protein (CRP) a protein synthesized in liver, is one of the most sensitive acute phase reactants. Its levels are increased in response to acute or chronic infectious and inflammatory process, or when tissue tissue damage occurs. Since the increase in CRP levels and its duration are closely related to the severity and activity of the inflammatory disease, CRP determination is useful for the diagnosis and treatment of inflammatory condition.

PRINCIPLE

The CRP reacts with the specific antibody producing insoluble immune complexes. The turbidity caused by these immune complexes is proportional to the CRP concentration in sample and can be measured spectrophotometrically.

PROVIDED REAGENTS

A. Reagent A: saline buffer solution, pH 7.6.
B. Reagent B: monospecific anti-CRP antibodies.

NON-PROVIDED REAGENTS

- Saline solution
- Wiener lab.'s **PCR Calibrador en serie Turbitest AA**

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.

WARNINGS

Reagents are for “in vitro” diagnostic use.
All samples from patients should be handled as capable of transmitting infection.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable at 2-10ºC until the expiration date stated on the box. Do not freeze.

SAMPLE

Serum
a) Collection: obtain in the usual way.
b) Additives: not required.
c) Known interfering substances: do not use hemolyzed, lipemic or contaminated samples. Before testing, particles in samples should be removed by centrifugation.
No interferences are observed from bilirubin up to 22 mg/dl (220 mg/l), nor rheumatoid factor up to 500 IU/ml.
See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: sample should be preferably fresh. In case it cannot be processed immediately, the sample can be kept for 2 months at 2-10ºC or up to 3 years frozen (-20ºC). Avoid repeated freezing and thawing.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer.
- Square spectrophotometric cuvettes.
- Micropipettes and pipettes for measuring the stated volumes.
- Kahn or hemolysis tubes.
- Water bath at 37ºC.
- Stopwatch.

ASSAY CONDITIONS

- Wavelength: 340 nm
- Reaction temperature: 37ºC
- Reaction time: 10 minutes
- Sample volume: 80 ul
- Final reaction volume: 1.28 ml

Sample and reagents volumes may be proportionally changed without affecting the calculation factors.

PROCEDURE

CALIBRATION CURVE
In Kahn tubes duly labeled, place:

PCR Calibrador en serie (1; 3; 5; 6; 7; 8)	80 ul
Reagent A	1000 ul

Homogenize and incubate for 5 minutes at 37ºC. Read each Calibrator’s absorbance at 340 nm (OD_1) adjusting the instrument to zero OD with distilled water.
Then add:

Reagent B	200 ul

Homogenize. Incubate for 5 minutes at 37ºC and immediately measure absorbance at 340 nm (OD_2), adjusting the instrument to zero with distilled water.
Calculate the difference in absorbance ($\Delta A = OD_2 - OD_1$) for each Calibrator. Plot the difference in absorbance (ΔA) in graph paper against CRP concentration in mg/l.

PROCEDURE FOR SAMPLES
In duly marked Kahn tubes, place:

Sample	80 ul
Reagent A	1000 ul
Homogenize and incubate for 5 minutes at 37°C. Measure absorbance at 340 nm (OD_1) adjusting the instrument to zero with distilled water. Then add:	
Reagent B	200 ul
Homogenize and incubate for exactly 5 minutes at 37°C and immediately read the absorbance at 340 nm (OD_2) taking the instrument to zero with distilled water.	

CALCULATIONS

Calculate the absorbance difference ($\Delta A = DO_2 - DO_1$) corresponding to each sample assayed. Interpole this ΔA into the calibration curve to determine the CRP concentration (mg/l) of the sample under study.

The samples with absorbances that are higher than the last calibration point should be diluted (1:2 or 1:4) with saline solution and processed one more time. Multiply the obtained result by the dilution performed.

QUALITY CONTROL METHOD

Control Inmunológico nivel 1 Turbitest AA.
Control Inmunológico nivel 2 Turbitest AA.
The Controls should be processed in the same manner as the samples.

REFERENCE VALUES

0-5 mg/l

Each laboratory should set its own reference values. Two or more periodic determinations should be performed to monitor the development of the disease.

PROCEDURE LIMITATIONS

- See Known interfering substances under SAMPLE.
- It is recommended to perform a complete recalibration when changing Reagent lot or when suggested by Quality Control.
- During inflammation processes the CRP may reach levels 1000 times higher than the normal level. If severe inflammation processes are suspected or elevated results are obtained it is recommended to dilute the samples 1:5 or 1:10.
- Avoid contamination to preserve integrity of the reagents. Only use thoroughly clean and dry micropipettes for measurements.

PERFORMANCE

a) Reproducibility: evaluated by a modification of protocol EP5-A from CLSI. Thus, two samples with different CRP levels were tested. The intra-assay and total precision were calculated with the obtained data.

Intra-assay precision

Level	S.D.	C.V.
11.9 mg/l	± 0.28 mg/l	2.4 %
40.6 mg/l	± 0.49 mg/l	1.2 %

Total precision

Level	S.D.	C.V.
11.9 mg/l	± 0.72 mg/l	6.0 %
40.6 mg/l	± 1.30 mg/l	3.2 %

b) Detection limit: is the minimum analyte amount capable of being detected as a sample, different than zero, and corresponds to the concentration of 0.5 mg/l CRP.
c) Assay range: corresponds to the exactly quantifiable interval of values and ranges from 2 mg/l to the last calibration point (200 mg/l CRP approximately).
d) Prozone effect: not noted until 1000 mg/l CRP.

The performance results were obtained using Konelab 60i autoanalyzer. Therefore, such values may differ whenever another autoanalyzer or manual technique is used.

PARAMETERS FOR AUTOANALYZERS

Refer to the specific applications of each autoanalyzer.

WIENER LAB. PROVIDES

60 ml: 1 x 50 ml Reagent A
1 x 10 ml Reagent B
(Cat. Nº 1683267)

REFERENCES

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- Tietz Textbook of Clinical Chemistry - Burtis, C.; Ashwood, E. (5º Edition) WB Saunders, 2001.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 5th ed., 2000.
- EP5-A (Vol.19 - Nº2) Evaluation of precision Performance of Clinical Chemistry Devices; Approved Guideline - NCCLS.
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Symbols

The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Conteins sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Xn — Harmful

Corrosive / Caustic

Xi — Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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