



In vitro diagnostic kit

hsCRP ELISA



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CE

Example Version

hsCRP ELISA

REF 740011

IVD

Enzyme Immunoassay for the Quantitative High Sensitive Determination of C-Reactive Protein in Human Serum and Plasma.

C-Reactive Protein (CRP) is an acute-phase protein, produced exclusively in the liver. Interleukin-6 is the mediator for the synthesis by the hepatocytes of CRP, a pentamer of approximately 120.000 Daltons. CRP is present in the serum of normal persons at concentrations ranging up to 5 mg/l.

A series of prospective studies provide consistent data documenting that mild elevation of baseline levels of CRP among apparently healthy individuals is associated with higher long-term risk for future cardiovascular events. This predictive capacity of CRP is independent of traditional cardiovascular risk factors and offers a prognostic advantage over measurement of lipid alone. Inflammatory markers specifically hsCRP may help to identify those who would benefit most from these pharmacological intervention. hsCRP is the novel and evolving biomarker which provides a most useful predictive indicator for subsequent cardiovascular events.

This test should not be used for assessment of acute inflammation but should be ordered to evaluate CVD (Cardiovascular Disease) risk in apparently healthy individuals who have not had recent infection or other serious illness.

For the assessment of acute inflammation the CRP ELISA ref 740001 from Advanced Practical Diagnostics can be used.

PRINCIPLE OF THE hsCRP ELISA

Microtiter strips coated with anti-CRP antibody are incubated with diluted standard sera and patient samples. During this incubation step CRP is bound specifically to the wells. After removal of the unbound serum proteins by a washing procedure, the antigen-antibody complex in each well is detected with specific peroxidase-conjugated antibodies.

After removal of the unbound conjugate, the strips are incubated with a chromogen solution containing tetramethylbenzidine and hydrogen peroxide: a blue colour develops in proportion to the amount of immunocomplex bound to the wells of the strips. The enzymatic reaction is stopped by the addition of 0.5M H₂SO₄ and the absorbance values at 450 nm are determined.

A standard curve is obtained by plotting the absorbance values versus the corresponding standard values. The concentration of CRP in patient samples is determined by interpolation from the standard curve.

REAGENTS

1. Coated Microtiter strips – **MTP**

12 x 8-well strips coated with monoclonal antibodies to human CRP.

2. Standard Sera – **CAL N**

5 vials, each containing 1/10 prediluted CRP standard solutions (0.2 ml), N having following values:

CAL 0: 0 µg/ml; CAL 0.4: 0.4 µg/ml; CAL 1: 1 µg/ml; CAL 5: 5 µg/ml;

CAL 10: 10 µg/ml. Contains 0.09 % NaN₃.

Calibrated against the NIBSC 1st International Standard, 85/506 (specification 90-110 % agreement).

3. Conjugate – **CONJ**

1 vial, containing peroxidase conjugated monoclonal anti-human CRP antibodies (12 ml). Contains antimicrobial agents and an inert red dye.

4. Specimen Dilution Buffer – **DIL 5x**

1 vial, containing 40 ml dilution buffer 5x concentrated. Contains 0.09 % NaN₃ and an inert green dye.

5. Washing Solution – **WASH 20x**

1 vial containing 50 ml 20x concentrated phosphate buffered washing solution.

6. Chromogen Solution – **CHROM**

1 vial, containing 15 ml of a solution containing H₂O₂ and tetramethylbenzidine.

7. Stopping Solution – **STOP**

1 vial, containing 12 ml of 0.5M H₂SO₄.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision micropipettes and standard laboratory pipettes.
2. Clean standard laboratory volumetric glassware.
3. Clean glass or plastic tubes for the dilution of the samples.
4. A microtiter plate reader capable of measuring absorbance at 450 nm.

WARNINGS AND PRECAUTIONS FOR USERS

1. For in vitro diagnostic use only.
2. For professional laboratory use.
3. Human blood components used in the preparation of the standard sera have been tested and found to be nonreactive for hepatitis B surface antigen and HIV I. Since no known method can ever offer complete assurance that products derived from human blood will not transmit hepatitis or other viral infections, it is recommended to handle these standard sera in the same way as potentially infectious material.

4. Dispose of patient samples and all residual products, containers and residues from tests using these reagents as if contaminated with potentially infectious substances. Safe disposal of residual products and their containers or packaging and residues from tests using these reagents must be in accordance with hospital policies and local and/or national legislation.

5. Do not mix reagents or coated microtiter strips from kits with different lot numbers.

6. Chromogen Solution contains the hazardous ingredient N-Methyl-2-pyrrolidone at a concentration > 0,3 %. It is classified as a Reproductive Toxicant Category 1B.

Following hazard statements are applicable:

H360D: May damage the unborn child.

Following precautionary statements are applicable:

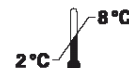
P280: Wear protective gloves/protective clothing/eye protection/face protection.

P308+P313: If exposed or concerned: Get medical advice/attention.

7. Some kit components contain sodium azide as a preservative. In order to prevent the formation of potentially explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.

8. Serious incidents related to the hsCRP ELISA must be reported to the manufacturer and to the Competent Authority of the EU member state(s) where the incident has occurred.

STORAGE CONDITIONS



1. Store the microtiter strips in their original package with the desiccant until all the strips have been used. Opened components should be stored at 2-8°C until next use and can be maintained for 6 months.

2. Never use any kit components beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

Human serum and plasma may be used in this assay. Remove serum from clot as soon as possible to avoid haemolysis. Lipemic and/or haemolysed samples can cause false results. Transfer the serum to a clean storage tube. Specimens may be stored at 2-8 °C for a few days (3 days)⁽¹⁾, or they can be stored frozen for a longer period of time (6 months at -20 °C, indefinitely at -70 °C)⁽¹⁾. Avoid repeated freezing and thawing.

ASSAY PROCEDURE

General Remarks :

1. Use a separate disposable tip for each sample transfer to avoid cross-contamination.
2. All reagents must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
3. Once the assay has been started, all steps should be completed without interruption.
4. Absorbance is a function of the incubation time and temperature. Therefore the size of the assay run should be limited. It is suggested to run no more than 20 patient samples with one set of Reference Standards in duplicate.
5. If an ELISA Washer is used, adaptation of the washing step might be necessary to obtain optimal results.

Reconstitution of the Reagents

Washing Solution: dilute 50 ml of concentrated Washing Solution (5) to 1000 ml with distilled water. Reconstituted solution can be stored at least 1 month, store at 2 – 8 °C.

At higher temperatures, the concentrated Washing Solution (5) may appear cloudy without affecting its performance. Upon dilution, the solution will be clear.

Specimen Dilution Buffer: dilute 40 ml of the concentrated Specimen Dilution Buffer (4) to 200 ml with distilled water. Reconstituted solution can be stored at least 3 months or as long as solution remains clear. Store at 2 – 8 °C.

Assay Procedure

1. The 10x prediluted standard sera (2) are diluted 1:100 as follows : pipette 10 µl of each calibrator into separate glass or plastic dilution tubes. Add 990 µl of diluted Specimen Dilution Buffer and mix carefully.

2. The patient samples are diluted 1:1000 in two consecutive steps: pipette 10 µl of each patient sample into separate glass or plastic dilution tubes and add 990 µl of diluted Specimen Dilution Buffer. Mix thoroughly. Add 450 µl of diluted Specimen Dilution Buffer to 50 µl of these 100x prediluted samples. Mix thoroughly.

Warning: do not store the diluted samples for more than 8 hours.

3. Pipette 100 µl of the diluted calibrators and samples into each of a pair of adjacent wells (1).

4. Incubate the covered microtiter strips for 30 ± 2 min at room temperature.

5. Wash the microtiter strips three times with Washing Solution. This can either be performed with a suitable microtiter plate washer or by briskly shaking out the contents of the strips and immersing them in washing solution. During the third step, the washing solution is left in the strips for 2-3 min. Change washing solution for each cycle. Finally empty the microtiter strips and remove excess fluid by blotting the inverted strips on adsorbent paper.

6. Add 100 µl of Conjugate solution (3) and incubate the covered microtiter strips for 30 ± 2 min at room temperature.

7. Repeat the washing procedure as described in 5.

8. Add 100 µl of Chromogen Solution (6) to each well.

9. Incubate for 10 ± 2 min at room temperature. Avoid light exposure during this step.

10. Add 50 µl of Stopping Solution (7) to each well.

11. Determine the absorbance of each well at 450 nm or at 450 nm with reference filter 600-650 nm within 30 min following the addition of acid.

RESULTS

The average absorbance value of each calibrator is plotted against the corresponding CRP-value and the best calibration curve (e.g. linear/linear, log/linear) is constructed. Use the average absorbance of each patient sample obtained in the hsCRP ELISA to determine the corresponding value by simple interpolation from the curve.

Depending on the experience and/or availability of computer capability, other methods of data reduction may be used.

When using the sample dilution 1:1000, results are directly read by interpolation from the standard curve, and values are expressed as µg/ml.

When using a higher dilution, results must be multiplied by a factor equal to the ratio to 1000. Example, if a 1:2000 sample dilution is used, results must be multiplied by 2 and values are expressed as µg/ml.

When using a lower dilution, results must be divided by a factor equal to the ratio to 1000. Example, if a 1:500 sample dilution is used, results must be divided by 2 and values are expressed as µg/ml.

PERFORMANCE CHARACTERISTICS

EXAMPLE OF TYPICAL OD VALUES

CALIBRATOR µg/ml	OD
0	0.015
0.4	0.112
1	0.354
5	1.370
10	1.954

NORMAL VALUES

Serum and plasma samples from 360 healthy Belgian blood donors were tested with the hsCRP ELISA from Advanced Practical Diagnostics BV. Following distribution of CRP levels has been found:

Concentration Range CRP µg/ml	Number of donors
≤ 1.0	187
1 - 2.9	118
3 - 10	44
> 10	11

Interpretation of the results

The following criteria are commonly found in the literature for the relation between the CRP values and the risk for developing CVD.

CRP values < 1.0 mg/L = Low risk for CVD

CRP values 1.0 – 2.9 mg/L = Intermediate risk for CVD

CRP values > 3.0 mg/L = High risk for CVD

PRECISION

Precision has been determined by assaying three spiked samples in a study based on CLSI EP05-A3 guidance document (ANOVA analysis).

Repeatability (replicate-to-replicate variability) and within laboratory precision (run-to-run and day-to-day variability) has been assessed in a 20 x 1 x 5 study design (20 days, 1 run/day, 5 replicates/run (n = 100 for each sample)), performed by one operator for one batch of the hsCRP ELISA.

Obtained results:

Sample	Mean (µg/ml)	Repeatability		Within-Laboratory Precision	
		SD (µg/ml)	CV (%)	SD (µg/ml)	CV (%)
Level 1	0.92	0.080	8.7	0.121	13.2
Level 2	3.02	0.233	7.7	0.374	12.4
Level 3	6.57	0.388	5.9	0.716	10.9

Reproducibility (site-to-site and instrument-to-instrument variability) has been determined based on a 3 x 5 x 1 x 5 study design (3 sites, 5 days, 1 run/day, 5

replicates/run (n = 75 for each sample)) performed by three operators, with three instruments, at three sites for one batch of the hsCRP ELISA.

Obtained results:

Sample	Mean (µg/ml)	Reproducibility	
		SD (µg/ml)	CV (%)
Level 1	0.95	0.136	14.3
Level 2	3.15	0.400	12.7
Level 3	6.90	0.725	10.5

ANALYTICAL SPECIFICITY

Cross-reactivity

The hsCRP ELISA recognizes natural and recombinant human CRP. No cross-reactivity was observed with following factors, prepared at 1 µg/ml in sample diluent: human pentraxin 2; human pentraxin 3; human monomeric CRP; rat CRP.

Endogenous Interference

Potential interference of following endogenous factors was investigated, according to the CLSI guideline EP-07. No interference was found for hemoglobin (5 mg/ml) lipids (intralipid, 2.5 mg/ml), bilirubin (0.2 mg/ml) and RF (250 IU/ml).

DETECTION CAPABILITY

The characteristics Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) have been determined according to the CLSI Guideline EP17-A2, whereby for LoB and LoD determination the classical approach has been followed and for LoQ determination the precision profile approach.

Following results are obtained:

LoB: 0.010 µg/ml

LoD: 0.044 µg/ml

LoQ: 0.080 µg/ml

METHOD COMPARISON

A clinical sample panel (26 serum samples) has been analysed on the hsCRP ELISA and a commercial assay on the market (CRP Vario, Sentinel Diagnostics). Pearson r correlation was 0.988 (CI 95%: 0.973-0.995). Passing-Bablok regression analysis revealed Y-intercept -0.29 (CI 95%: -0.36 - -0.16) and slope 0.95 (CI 95%: 0.898 - 0.988).

TEST VALIDITY

The following specifications must be met for each run to be valid:

OD value for the zero calibrator: < 0.080

OD value for the highest value calibrator: > 1.000

If one of the specifications is not met, the test run should be repeated.

TROUBLE SHOOTING

In case of high background signal, the washing was insufficient. Repeat the test with more vigorous washing (increased number of cycles, soak time).

REFERENCES

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Version history

Version number	Description
hsCRP10-18	Previous version
hsCRP11-21	Writing in full the manufacturer name, according to its legal status: Advanced Practical Diagnostics BV instead of the abbreviation apDia. Assay procedure: reading of OD values at 450nm may equally well be done using reference filter 600-650 nm.
hsCRP06-22	Adding information for waste disposal. Specifying professional laboratory use. Adding information on incident reporting. Defining specimen stability in days or months. Adding additional curve fitting type. Including information on storage after first opening of the kit. Updating precision data obtained according to CLSI guideline. Including additional performance data on accuracy, specificity, detection capability and method comparison.

Example Version