Cholestech LDX®

hs-CRP

High Sensitivity C-Reactive Protein Test Cassette

REF **12-807**

IVD CE

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inverness medical

Refer to the CD in the Cholestech LDX® Analyzer package for instructions in English. The instructions are also available from your local distributor.

Le CD contenu dans l'emballage de l'analyseur Cholestech LDX inclut les directives d'utilisation en français. Le mode d'emploi est également disponible auprès du distributeur local.

Anweisungen auf Deutsch befinden sich auf der CD in der Verpackung des Cholestech LDX-Analysegeräts. Die Anleitung ist auch von Ihrem Händler erhältlich.

Fare riferimento al CD nella confezione dell'analizzatore Cholestech LDX per istruzioni in italiano. Le istruzioni sono disponibili presso il distributore di zona.

Consulte el CD incluido en el envase del analizador Cholestech LDX para obtener instrucciones en español. También puede pedir las instrucciones a su distribuidor local.

Consulte o CD no pacote do Analisador LDX Cholestech para instruções em Português. As instruções também estão disponíveis junto do seu distribuidor local.

Der henvises til den vedlagte CD i Cholestech LDX-analysatorpakken for instruktioner på dansk. Instruktionerne fås også hos lokale forhandler.

Se CD:n i Cholestech LDX-analysatorförpackningen beträffande instruktioner på svenska. Instruktionerna finns också att få hos din lokala återförsäljare.

Hvis du vil ha flere instruksjoner, kan du se CDen som følger med i Cholestech LCX-analysatorpakken. Instruksjonene fås også hos din nærmeste forhandler.

Türkçe Talimatlar için Cholestech LDX Analizörü paketindeki CD'ye başvurun. Bu talimatları yerel distribütörünüzden de alabilirsiniz.

Ανατρέξτε στο CD στη συσκευασία του Αναλυτή Cholestech LDX για οδηγίες στα Ελληνικά. Οι οδηγίες είναι διαθέσιμες από τον τοπικό διανομέα σας.

Pokyny v angličtine získate z disku CD, ktoré sa nachádza v balíku analyzátora. Pokyny môžete získať od miestneho distribútora.

INTENDED USE

Cholestech LDX high sensitivity C-reactive protein (hs-CRP) is an *in vitro* diagnostic test for the quantitative determination of C-reactive protein in whole blood or serum. Measurement of CRP is useful as an aid in the detection and evaluation of infection, tissue injury, inflammatory disorders, and associated diseases.

SUMMARY AND EXPLANATION

CRP is an acute phase reactant that responds as a sensitive, though nonspecific, marker of systemic inflammation. The pentameric, globular protein is synthesized by the liver in response to stimuli from circulating inflammatory cytokines. CRP has traditionally been used as a systemic marker of infection and tissue injury.[1] An expanding body of research now indicates that CRP likely plays a direct, active inflammatory role in blood vessels, leading to the development of atherosclerosis.[2]

Within 24–48 hours of an infectious or noninfectious stimulus, CRP levels may rise up to 3,000-fold over the circulating levels seen in apparently healthy individuals, which is typically less than 10 mg/L.[1] CRP levels in conditions characterized by chronic inflammation, such as rheumatoid arthritis and certain other rheumatic disorders, are likewise characterized by significant elevations. Conventional CRP assays have therefore been optimized to facilitate measurement of dynamic increases in concentration. But this is achieved at the expense of sensitivity to detect low level increases due to more subtle causes of inflammation.

Low level increases in CRP have been reported in various conditions and disease states that are thought to be associated with inflammation.[3-6] The most prominently studied utility has been for cardiovascular disease, where CRP has been reported to predict cardiovascular outcomes independently of other conventional markers of risk.[4,7,8] Increases in CRP are nonspecific, however, and should be interpreted in the context of a complete clinical evaluation. Apparently healthy individuals with an elevated hs-CRP value should have the test repeated to rule out a recent response to undetected infection or tissue injury.[8]

PRINCIPLES OF THE PROCEDURE

The Cholestech LDX System is a desk-top analyzer that utilizes dry chemistry cassettes and reflectance photometry to quantify substances in blood. Samples used for testing can be whole blood from a fingerstick (collected in a lithium heparin coated capillary tube), serum, or anticoagulated whole blood collected by venipuncture. The sample is applied to a Cholestech LDX hs-CRP cassette. The cassette is then placed into the Cholestech LDX Analyzer where a unique system on the cassette separates the plasma from the blood cells. Plasma is then incubated with a colloidal gold anti-CRP conjugate. A lateral flow system transfers the gold conjugate through an anti-CRP antibody capture zone. Gold conjugate containing CRP is captured by the antibody while the rest of the gold conjugate is washed away. The signal in the capture zone is measured by the Cholestech LDX Analyzer. A brown (magnetic) stripe on each cassette contains the calibration information required for the Cholestech LDX Analyzer to convert the reflectance reading (% R) to hs-CRP concentration in mg/L.

REAGENTS AND MATERIALS

Materials Provided

Cholestech LDX hs-CRP Cassettes
Each cassette contains

CRP antibody gold conjugate	Titered
CRP Antibody	Titered

Nonreactive Ingredients: buffers and stabilizers

Materials Required But Not Provided

- Cholestech LDX Analyzer and power supply
- Alcohol swabs and gauze for cleaning puncture site
- Lancets for capillary blood collection
- Cholestech LDX 50 µL Capillary Tubes with green mark (with lithium heparin anticoagulant only)
- Cholestech LDX Capillary Plungers
- Gloves
- Biohazard waste containers
- Quality control material
- Cholestech MiniPet pipettes and tips, or micropipetter that will deliver 50 µL for use with whole blood venipuncture samples and 40 µL for use with serum or plasma, and control, calibration verification, or proficiency testing materials
- Vacuum collection tubes, needles, tube holders and sample tubes, if the sample is to be collected by venipuncture

PRECAUTIONS

For professional *in vitro* diagnostic use only.

All blood samples, containers, capillary tubes and materials that have come in contact with blood should be handled as if capable of transmitting infectious disease and discarded into a biohazardous waste container after use.

STORAGE AND HANDLING

Cassette Storage and Stability

Cassettes must be stored in the sealed foil pouches.

Place cassettes in the refrigerator after receipt. Cassettes may be used until the date printed on the pouch when stored in a refrigerator (36–46°F / 2–8°C).

The cassettes may be stored for up to 30 days at room temperature (48–86°F / 9–30°C). The new expiration date is the date the cassettes are placed at room temperature plus 30 days. Write the new expiration date on the side of the cassette box in the space provided.

IMPORTANT: Once the cassettes have been stored at room temperature, they should not be returned to the refrigerator.

- Do not use a cassette beyond the printed expiration date.
- Do not use a cassette that has been stored at room temperature for more than 30 days.
- Do not reuse cassettes.

Cassette Handling

Cassettes should sit at room temperature for 10 minutes before opening the pouch. Use the cassette as soon as the pouch is opened.

SPECIMEN COLLECTION AND HANDLING

Sample Type

- Fingerstick or venous whole blood, serum, or plasma

Sample Requirement

- Sample Volume: 50 µL of whole blood, or 40 µL or serum or plasma.

Fingerstick whole blood

- Collect the sample from a fingerstick into a Cholestech LDX 50 µL Capillary Tube. (See the Fingerstick Procedure below).
- Place the blood into the cassette within 5 minutes after collection.
- Blood from the fingerstick should flow freely. Too much squeezing of the finger may produce inaccurate results.

Venous blood

- Collect blood into a green-top tube (sodium or lithium heparin anticoagulant) or a serum tube without additives.

IMPORTANT: Do not use a tube with any other additives because it may cause inaccurate results.

- Use a pipette and tip to place blood into the cassette.
- Whole blood should be used within 30 minutes. Blood sample may be taken directly from the tube after mixing.
- Serum samples should be allowed to clot for 30 minutes. Serum should be separated from the blood cells immediately, and stored refrigerated in a tightly sealed sample tube at 2–8°C (36–46°F) until testing is performed.
- Samples should be at room temperature for testing.
- Mix all samples by inverting gently 7–8 times before testing.

TEST PROCEDURE

Calibration

No calibration is performed by the user. Test information is encoded on the brown magnetic stripe of the cassette. The magnetic stripe is read by the Cholestech LDX Analyzer each time a cassette is run.

An Optics Check should be run on the Analyzer each day that patient samples are tested. See the Cholestech LDX User Manual for instructions.

IMPORTANT: A warm hand and good blood flow from the puncture site are essential in order to collect a good capillary sample.

WARNING: Squeezing the finger excessively may cause inaccurate test results.

Fingerstick Procedure

1. The patient should sit quietly for five minutes before the blood sample is collected.
2. Put a capillary plunger into the end of a Cholestech LDX 50 µL Capillary Tube with the **green mark**. Set aside.
3. Choose a spot that is on the side of **one of the center fingers** of either hand. The fingers and hands should be warm to the touch. To warm the hand, you can:
   a. Wash the patient's hand with warm water, or…
   b. Apply a warm (not hot) compress to the hand for several minutes, or…
   c. Gently massage the finger from the base to the tip several times to bring to blood to the fingertip.
4. Clean the site with an alcohol swab. Dry thoroughly with a gauze pad **before pricking the finger.**
5. Firmly prick the selected site with a lancet.
6. Squeeze the finger gently to obtain a large drop of blood. Wipe away this first drop of blood as it may contain tissue fluid.
7. Squeeze the finger gently again while holding it downward until a second large drop of blood forms. **Do not milk the finger**. The puncture should provide a free-flowing drop of blood.
8. Hold the capillary tube horizontally or at a slightly descending angle by the end with the plunger. Touch it to the drop of blood without touching the skin. The tube will fill by capillary action to the black mark. **Do not collect air bubbles**. If it is ecessary to collect another drop of blood, wipe the finger with gauze then massage again from base to tip until a large drop of blood forms.
9. Fill the capillary tube within 10 seconds.
10. Wipe off any excess blood from the finger and have the patient apply pressure to the puncture until the bleeding stops.

Using the Cholestech MiniPet Pipette

Use 50 µL for venous whole blood. Use 40 µL for serum or plasma, or control, calibration verification, or proficiency testing materials. Use this procedure to apply a venous blood sample, or control, calibration verification or proficiency testing materials to the cassette. Any pipette that can deliver the correct volume may be used.

1. Firmly attach the pipette tip to the end of the appropriate MiniPet Pipette. Use a new tip for each sample.
2. To fill the pipette, push the plunger down as far as you can. Place the pipette tip midway into the sample and **slowly** release the plunger. Confirm that no air bubbles are in the pipette tip.
3. Place the pipette tip into the cassette sample well. Dispense the sample into the cassette sample well by pressing the plunger down. Move the pipette tip out of the sample well before releasing the plunger again.
4. Remove the pipette tip and throw it away in a biohazard waste container.

IMPORTANT: If the plunger is released before the pipette tip is out of the sample well, it will remove the sample just dispensed.

IMPORTANT: Keep the cassette horizontal at all times after applying the sample.

Running a Test

1. If the cassettes have been refrigerated, allow them to come to room temperature (at least 10 minutes) before opening.
2. 2. Make sure the Analyzer is plugged in and has warmed up.
3. Remove the cassette from its pouch. Hold the cassette by the short sides only. Do not touch the black bar or the magnetic stripe. Place the cassette on a clean, hard, non-absorbent flat surface.

IMPORTANT: Gloves should be worn whenever working with blood samples

IMPORTANT: When running fingerstick or venous whole blood, the configuration menu must be set for whole blood. When running serum or plasma, or control, calibration verification, or proficiency testing materials, the configuration menu must be set for "serum". See Setting the Configuration Menu in the Cholestech LDX System User Manual.

4. Press RUN. In a few seconds the screen will display:

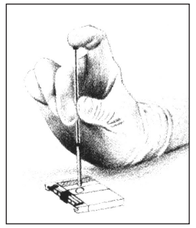
```
Selftest
running.
```

```
Selftest OK
```

5. The cassette drawer will open. The screen will display:

```
Load cassette
and press RUN
```

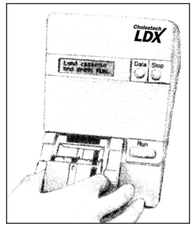
6. Place the sample into the cassette's sample well. Use a Cholestech LDX 50 µL Capillary Tube for fingerstick samples. Use the Cholestech 40 µL MiniPet Pipette for serum or plasma, or control, calibration verification or proficiency testing materials.



IMPORTANT: Do not touch the white material at the end of the sample well with the tip of the capillary tube or pipette.

IMPORTANT: Fingerstick samples must be applied within five (5) minutes or the blood will clot.

7. Keep the cassette flat after the sample has been applied. ***Immediately*** place the cassette into the drawer of the Analyzer The black reaction bar must face toward the Analyzer. The brown magnetic stripe must be on the right.
8. **DO NOT PUSH IN THE DRAWER.** Immediately Press **RUN**. The drawer will close. During the test the screen will display:




```
hs-CRP
Running***
```

9. Put everything that touched the blood samples or control, calibration verification, or proficiency testing materials into a biohazardous waste container.
10. When the test is complete, the Analyzer will beep, and the screen will display:

```
hs-CRP=###
```

11. When the results are outside the measuring range, the screen will display:

Fingerstick or Whole Blood	Serum
hs-CRP < 0.30	hs-CRP < 0.30
or	or
hs-CRP > 10.0	hs-CRP > 8.00

12. If there is a problem with the test, a message will appear on the screen. See the Troubleshooting section of the Cholestech LDX User Manual if this happens.

Please call Technical Service at 1.877.441.7440 or 1.321.441.7200 to report any problems or if you have questions about the operation of the Cholestech LDX System.

13. When the drawer opens, remove the cassette, and put it in a biohazardous waste container. Leave the Analyzer drawer empty when not in use.
14. Record the hs-CRP results on the appropriate form.
15. To run another cassette, press **RUN**. The screen will display:

```
Load cassette
and press RUN
```

IMPORTANT: If you do not want to run another test and the drawer is open, press STOP to close the drawer.

16. Repeat step 3, and steps 6 through 14.
17. Otherwise, after four minutes a beep will sound and the screen will display:

```
System timeout
RUN to continue
```

18. If necessary, press the **DATA** button to view the results from the last cassette used.

IMPORTANT: Pressing the RUN button will erase the previous result.

QUALITY CONTROL

External quality control material should be run routinely to show that your system is giving accurate results. We recommend the following quality control procedures for the Cholestech LDX System.

Choice of Materials

Liquid Level 1 and Level 2 controls that work well with the Cholestech LDX System are available. If you use other controls, you will need to establish ranges for the Cholestech LDX System.

Handling

- Follow the instructions that come with your controls.
- Check the expiration date before use. Do not use if expired.
- See "Running a Test" for the procedure.

External Quality Control

External control material should be used to demonstrate that the reagents and the assay procedure perform properly. Good Laboratory Practice principles suggest that controls should be run whenever the laboratory director has any question about test system integrity, reagent storage conditions, or the reliability of any test result. If the controls do not perform as expected, repeat the test or contact Technical Service before testing patient samples.

Controls should be tested:

- With each new lot of cassettes;
- With every new shipment of cassettes, even if the lot has been received previously;
- When reagents may have been stored or handled in a way that can degrade their performance;
- As otherwise required by your laboratory's standard quality control procedures;
- As otherwise required by federal, state and local guidelines.

Record the results in a Quality Control Log.

The quality control results should be in range before testing patient samples. See the Cholestech LDX System User Manual if they are not. Please call Technical Service at 1.877.441.7440 or 1.321.441.7200 to report any problems or if you have any questions about quality control.

RESULTS

hs-CRP test results will be displayed on the screen when the test is complete.

LIMITATIONS

- The measuring range for hs-CRP using whole blood or fingersticks is 0.30–10.0 mg/L. Results outside this range will appear as <0.30 or >10.0.
- The measuring range for hs-CRP using serum or plasma is 0.30–8.00 mg/L. Results outside this range will appear as <0.30 or >8.00.
- Increases in CRP are nonspecific and should be interpreted in the context of a complete clinical evaluation. Apparently healthy individuals with an elevated hs-CRP value should have the test repeated to rule out a recent response to undetected infection or tissue injury.[8]
- Patient samples may contain heterophilic antibodies that could react in immunoassays to give a falsely elevated or depressed result. Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

The substances listed below were tested for interference with the hs-CRP test. Less than 10% interference was seen at the levels shown.

Substance Concentration

	mg/dL	mmol/L
Ascorbic Acid	3	0.17
Bilirubin	20	0.34
Creatinine	30	2.65
Ditaurobilirubin	20	0.24
Glucose	1200	66.61
Hemoglobin	120	0.02
Lactate	100	11.10
Potassium	39	10.00
Triglycerides	3000	33.88
Urea	500	83.25
Uric Acid	20	1.19
	g/dL	**g/L**
Protein (total)	9.0	90
Protein (albumin)	3.0	30
Protein (gamma globulin)	4.5	45

- Hematocrits between 30% to 55% do not affect results.

EXPECTED VALUES

hs-CRP values range between 0.28 and 8.55 mg/L in healthy men and between 0.19 and 9.14 mg/L in healthy women who are not taking hormone replacement therapy.[10] Apparently healthy individuals with an elevated hs-CRP value should have the test repeated to rule out a recent response to undetected infection or tissue injury.[8]

PERFORMANCE CHARACTERISTICS

Precision

A study was conducted according to NCCLS protocol EP5-A, Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (1999).[11]

	Commercial Control Material Level 1	Commercial Control Material Level 2	Frozen Serum Pool
$\bar{X}$ (mg/L) =	1.20	2.94	6.51
Within run CV (%) =	12.1%	11.7%	8.7%
Total CV (%) =	14.3%	11.5%	11.4%

	Whole Blood Within-Run Precision			
	Level 1	Level 2	Level 3	Level 4
$\bar{X}$ (mg/L) =	0.60	1.22	2.89	4.85
SD (mg/L) =	0.09	0.21	0.33	0.32
%CV =	15.0%	17.2%	11.4%	6.6%

ACCURACY (METHOD COMPARISON)

hs-CRP measured using the Cholestech LDX cassette was compared to a commercial nephelometric method.

Results

X = Commercial Method (serum)
Y = Cholestech LDX Analyzer

Sample Type	No. of Pairs	Slope	y-intercept	Correlation Coefficient	Range of Values
Venous Whole Blood	72	1.02	0.21	0.98	0.17–7.18
Serum	78	1.06	0.07	0.98	0.17–8.75
Fingerstick	78	1.08	0.00	0.98	0.17–8.75

REFERENCES

1. Pepys MB. C-reactive protein fifty years on. Lancet 1981; 1:653-7.
2. Szmitko PE, Wang CH, Weisel RD, de Almeida JR et al. New markers of inflammation and endothelial cell activation: Part I. Circulation 2003; 108:1917-23.
3. Wasunna A, Whitelaw A, Gallimore R, Hawkins PN, Pepys MB. C-reactive protein and bacterial infection in preterm infants. Eur J Pediatr 1990; 149:424-7.
4. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003; 107:363-9.
5. Spector TD, Hart DJ, Nandra D, Doyle DV et al. Low-level increases in serum Creactive protein are present in early osteoarthritis of the knee and predict progressive disease. Arthritis Rheum 1997; 40:723-7.
6. Schmidt R, Schmidt H, Curb JD, Masaki K et al. Early inflammation and dementia: a 25-year follow-up of the Honolulu-Asia Aging Study. Ann Neurol 2002; 52:168-74.
7. Danesh J, Whincup P, Walker M, Lennon L et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. BMJ 2000; 321:199-204.
8. Pearson TA, Mensah GA, Alexander RW, Anderson JL et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003; 107:499-511.
9. Ledue TB, Rifai N. Preanalytic and analytic sources of variations in C-reactive protein measurement: Implications for cardiovascular disease risk assessment. Clin Chem 2003; 49:1258-71.
10. Rifai N, Ridker PM. Population distributions of C-reactive protein in apparently healthy men and women in the United States: implication for clinical interpretation. Clin Chem 2003; 49:666-9.
11. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices: approved guideline. NCCLS document EP5-A 1999. Wayne, PA.