INSTRUCTION MANUAL

REF **4095**

April 01, 2014

CRP Latex

- 100 determinations -

CE

IVD *In vitro* diagnostic device

Latex agglutination test for the detection of C-reactive protein (CRP) in human serum

REF	Catalogue number	**LOT**	Batch code
L	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

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INTENDED USE

CRP Latex is used for the qualitative and semi-quantitative determination of C-reactive protein (CRP) in human serum.

C-reactive protein (CRP), the classic acute-phase of human serum, is synthesized by hepatocytes. Normally, it is present only in trace amounts in serum, but it can increase as much as 1.000-fold in response to injury or infection. The clinical measurement of CRP in serum therefore appears to be a valuable screening test for organic disease and a sensitive index of disease activity in inflammatory, infective and ischemic conditions (1,2). MacLeod and Avery found that antibody produced against purified CRP provided a more sensitive test than the C-polysaccharide assay (3). Since that time a number of immunological assays have been devised to measure CRP such as capillary precipitation, double immunodiffusion and radical immunodiffusion (4,5).

The CRP reagent kit is based on the principle of the latex agglutination assay described by Singer and Plotz (6). The major advantage of this method is the rapid two minute reaction time.

1. Pepys MB:. Lancet 1:653 (1981).
2. Werner M:. Clin.Chem. Acta 25:299 (1969).
3. MacLeod CM, et. al.: J. Exp. Med 73:191 (1941).
4. Wood HF, et. al.: J. Clin. Invest. 30: 616 (1951).
5. Mancini G, et. al.: Immunochemistry 2:235 (1965).
6. Singer JM, et. al.: Am. J. Med 21: 888 (1956).
7. Fischer CL, Gill CW: In Serum Protein Abnormalities. Boston, Little, Brown and Co., (1975).

PRINCIPLE OF THE TEST

CRP Latex is used for the determination of C-reactive protein (CRP) in human serum.

The CRP reagent kit is based on an immunological reaction between CRP antisera bound to biologically inert latex particles and CRP in the test specimen. When serum containing greater than 0.8 mg/dl CRP is mixed with the latex reagent, visible agglutination occurs.

TEST COMPONENTS for 100 determinations

A **LATEX**	**Latex reagent**, Latex particles coated with CRP antiserum		4.0 ml ready for use dropper bottle
P **CONTROL**	**Positive control** CRP positive human serum concentration on the label	**+**	1.0 ml ready for use dropper bottle
N **CONTROL**	**Negative control** CRP negative human serum	**-**	1.0 ml ready for use dropper bottle
	Agglutination slide		1 ready for use
	Disposable stirring sticks		50 ready for use

Materials required but not provided

- timer
- test Tubes and rack.
- serological pipettes
- high intensity light
- Glycine saline buffer (alternatively PBS)
- rocking shaker (optional)

Size and storage

CRP Latex has been designed for 100 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the CRP Latex have to be stored at 2 - 8 °C, preferably in the original kit box. The CRP Latex Reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal. **Do not freeze!**

Do not use the latex reagents if it is marked with turbidity as this may indicate reagent deterioration or contamination.

After opening all kit components are stable for at least 3 months, provided proper storage.

Agglutination slide should be thoroughly rinsed with water and wiped with lint-free tissue after each use.

PATIENT SAMPLES

Use fresh serum collected by centrifuging clotted blood.

If the test cannot be carried out on the same day, the serum may be stored between 2 - 8°C for no longer than 72 hours after collection. For longer periods the sample must be frozen.

As in all serological tests, hemolytic or contaminated serum must not be used. **Do not use plasma!**

ASSAY PROCEDURE

Qualitative evaluation

1. Allow all reagents and samples to reach room temperature prior to testing. Shake well all reagents before use.
2. Place **1 drop** (appr. 40 µl) of the positive control (P) on field no. 1 of the agglutination slide.
3. Place **1 drop** (appr. 40 µl) of the negative control (N) on field no. 2 of the agglutination slide.
4. Place **40 µl** of each undiluted patient sample to the following fields of the agglutination slide using different serological pipettes.
5. Gently resuspend the CRP Latex reagent (A) and add **1 drop** (40 µl) to each test field.
6. Mix well using separate stirring sticks.
7. Gently rock the slide for **2 minutes** by hand or use a rocking shaker (80-100 rpm).
8. Read immediately under direct light.

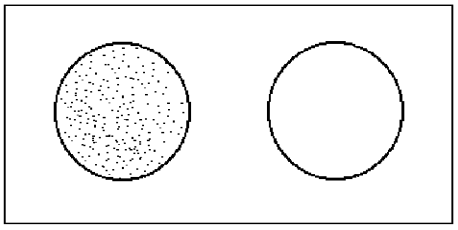
Semi-quantitative evaluation

1. Allow all reagents and samples to reach room temperature prior to testing. Shake well all reagents before use.
2. Set up at least five dilutions per patient sample: 1:2, 1:4, 1:8, 1:16, 1:32, etc. with glycine saline solution.
3. Place **1 drop** (appr. 40 µl) of the positive control (P) on field no. 1 of the agglutination slide.
4. Place **1 drop** (appr. 40 µl) of the negative control (N) on field no. 2 of the agglutination slide.
5. Place **40 µl** of each sample dilution (refer 2.) to the following fields of the agglutination slide using different serological pipettes.
6. Gently resuspend the CRP Latex reagent (A) and add **1 drop** (40 µl) to each test field.
7. Mix well using separate stirring sticks.
8. Gently rock the slide for **2 minutes** by hand or use a rocking shaker (80-100 rpm).
9. Read immediately under direct light.

EVALUATION OF RESULTS

POSITIVE
A positive reaction is indicated by any observable agglutination in the reaction mixture. The specimen reaction should be compared to the CRP Negative Control.

NEGATIVE
A negative reaction is indicated by a uniform milky suspension with no agglutination as observed with the CRP Negative Control.



Positive Negative

Semi-quantitative test evaluation

A positive reaction is indicated by any observable agglutination in the reaction mixture. Record the last dilution showing a positive reaction. The titer of the serum is the reciprocal of the highest dilution which exhibits a positive reaction. For example, if the last positive reaction is found in the 1:8 dilution, the titer of the sample is 8.

Test validity

CRP Positive and Negative Control should be included in each test batch.

Acceptable performance is indicated when a uniform milky suspension with no agglutination is observed with the CRP Negative Control and agglutination with large aggregates is observed with the CRP Positive Control.

Expected values

CRP in healthy individuals is approximately 0.02 - 1.35 mg/dl. The mean value in adults is 0.047 mg/dl.

A weak positive correlation was found between CRP and age.

It is important to determine the level of CRP for monitoring patient progress. This is due to 1) the concentration of CRP is an index of tissue damage incurred and 2) increasing or decreasing levels of CRP (e.g. daily) indicate the progress of inflammatory process (7).

Limitations of the method

Reaction time is critical. If reaction time exceeds two minutes, drying of the reaction mixture may cause false positive results.

Freezing the CRP Latex Reagent will result in spontaneous agglutination.

Intensity of agglutination is not necessarily indicative of relative CRP concentration; therefore, screening reactions should not be graded.

A false negative can be attributed to a prozone phenomenon (antigen excess). It is recommended, therefore, to check all negative sera by retesting at a 1:10 dilution with glycine buffer.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed.
- Do not use or mix reagents from different lots. Do not use reagents from other manufacturers.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Sodium azide (0.095%) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Positive and negative controls prepared using human sera found negative for hepatitis B surface antigen (HBsAg) and antibodies to HIV (Human Immunodeficiency Virus) and HCV (Hepatitis C Virus) by FDA required test. However, handle controls as if potentially infectious.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

ASSAY CHARACTERISTICS

Sensitivity:

The analytical sensitivity is 0.8 mg/dl.

Comparison studies:

Qualitative Results:
A study performed using CRP Latex Reagent and a commercially available product yielded 98% accuracy. The discrepant results were obtained in samples with titers near the limit of sensitivity of the reagents.

Quantitative Results:
A panel of 10 known CRP positive serum samples were quantitated on three consecutive days. The results of the study indicated that CRP Latex Reagent has 100% precision.

Remarks: