CE IVD

SAA and CRP Rapid Test Kit (Colloidal Gold)

Instruction for Use

A rapid test for the diagnosis of inflammatory condition by detecting Serum Amyloid A (SAA) and C-reactive protein qualitatively in whole blood, serum or plasma.
For professional in vitro diagnostic use only.

PRODUCT NAME

SAA and CRP Rapid Test Kit(Colloidal Gold)

SPECIFICATION

25 tests/kit, 5 tests/kit, 1 test/kit

INTENDED USE

The SAA and CRP Combo Rapid Test is a rapid chromatographic immunoassay for the qualitative detection of human SAA and CRP in whole blood, serum or plasma as an aid in the diagnosis of inflammatory condition. The cutoff for SAA is 10 μg/mL and cut-off for CRP is 10 μg/mL.

SUMMARY

Serum amyloid A (SAA) proteins are a family of apolipoproteins associated with high-density lipoprotein (HDL) in plasma. Different isoforms of SAA are expressed constitutively (constitutive SAAs) at different levels or in response to inflammatory stimuli (acute phase SAAs). These proteins are produced predominantly by the liver.[1] The conservation of these proteins throughout invertebrates and vertebrates suggests that SAAs play a highly essential role in all animals.[2] Acute-phase serum amyloid A proteins (A-SAAs) are secreted during the acute phase of inflammation. These proteins have several roles, including the transport of cholesterol to the liver for secretion into the bile, the recruitment of immune cells to inflammatory sites, and the induction of enzymes that degrade extracellular matrix. Serum amyloid A (SAA) is also an acute phase marker that responds rapidly. Similar to CRP, levels of acute-phase SAA increase within hours after inflammatory stimulus, and the magnitude of increase may be greater than that of CRP. Relatively trivial inflammatory stimuli can lead to SAA responses. It has been suggested that SAA levels correlate better with disease activity in early inflammatory joint disease than do ESR and CRP. Although largely produced by hepatocytes, more recent studies show that SAA is produced by adipocytes as well, and its serum concentration is associated with body mass index.[3] C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via C1q.[4] CRP plays a role in innate immunity by binding to the phosphocholine expressed on the surface of dead or dying cells and some bacteria. This activates the complement system, promoting phagocytosis by macrophages, which clears necrotic and apoptotic cells and bacteria.[5]

PRINCIPLE

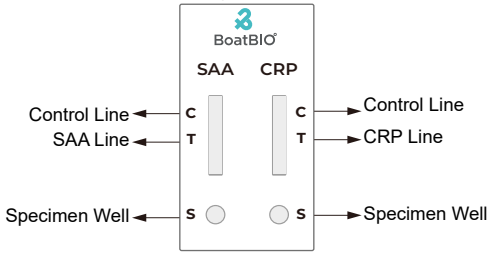
The SAA and CRP Combo Rapid Test Cassette has two parts. One part is for SAA and another one is for CRP. Both are qualitative, solid phase, two-site sandwich immunoassay for the detection of target analyte, i.e., SAA or CRP respectively in whole blood, serum or plasma.In two separate sections below, principles for both are described.

For SAA

The membrane is pre-coated with anti-SAA antibodies on the test line region of the cassette. During testing, SAA, if present in the specimen (whole blood, serum or plasma) above the cut-off level reacts with Colloidal Gold conjugated anti-SAA antibodies. The complex migrates upward on the membrane chromatographically by capillary action to react with anti-SAA antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

For CRP

The membrane is pre-coated with anti-CRP antibodies on the test line region of the cassette. During testing, CRP, if present the specimen (whole blood, serum or plasma) above the cut-off level reacts with the Colloidal Gold conjugated anti-CRP antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-CRP antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.



MAIN COMPONENTS

Materials Provided

Components	25 tests/kit	5 tests/kit	1 test/kit
Cassettes	25 cassettes with dependent sealed foil pouch	5 cassettes with dependent sealed foil pouch	1 cassette with dependent sealed foil pouch
Plastic tubes with buffer	1 bottle (10ml)	1 bottle (3ml)	300ul/tube
Capillaries	25 pcs	5 pcs	1 pcs
Droppers	25 pcs	5 pcs	1 pcs
Package insert	1 pcs	1 pcs	1 pcs

MATERIALS REQUIRED BUT NOT PROVIDED

- Specimen Collection Containers
- Pipette and Disposable tips (optional)
- Centrifuge
- Lancets
- Timer

WARNINGS AND PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after the expiration date.
- The test should be remained in the sealed pouch until use.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

STORAGE AND STABILITY

- The test can be stored at room temperature or refrigerated (2-30°C).
- The test cassette is stable through the expiration date printed on the sealed pouch.
- The test cassette must be remained in the sealed pouch until use.
- **DO NOT FREEZE.**
- Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

Preparation

Before performing the test, please make sure that all components are brought to room temperature (15-30℃).

1.Take a tube with buffer solution out of the kit. Document patients name or ID on it. Unscrew the cap.

Sample Collection

2.Collect the specimen according to standard procedures.

- Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be used within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by finger stick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- EDTA K2, Heparin sodium, Citrate sodium and Oxalate potassium can be used as ant-coagulants. The specimens collected with these anti-coagulants also need to follow the same step of dilution with buffer.

Sample Dilution / Sample Stability

3.Administer the blood-filled end-to-end capillary into the plastic tube with dilution buffer. Alternatively, the 10μL of specimen can be added directly with the micro pipette into the buffer

4.Close the tube and shake the sample vigorously for approximately 10 seconds to mix specimen and dilution buffer well.

5.Allow the diluted sample to homogenize for 1 minute. Do not shake it during this time.

6.The sample can then be used immediately or stored for up to 8 hours.

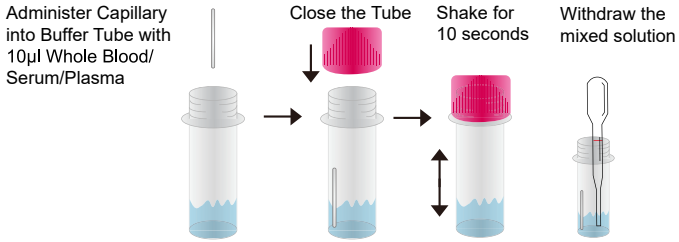
DIRECTIONS FOR USE

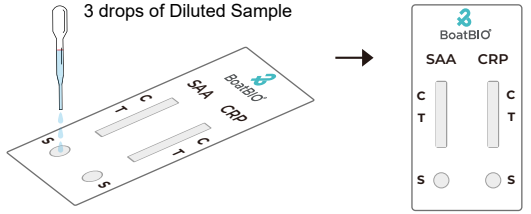
Allow the test cassette, specimen, buffer and/or controls to reach room temperature (15-30 °C) prior to testing.

1.Remove the Test Cassette from its sealed pouch, and place it on a clean, level surface. For best results, the assay should be performed within one hour.

2.Open the tube with the diluted sample .Transfer **3 drops of diluted sample (pproximately 120 μL)** to each sample well(S) Start the timer.

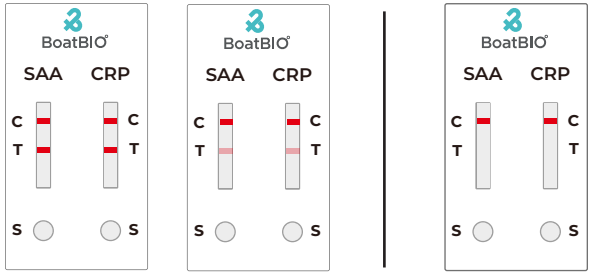
Wait for the colored lines to appear. The result should be read at **5 minutes**. Do not interpret the results after 10 minutes.

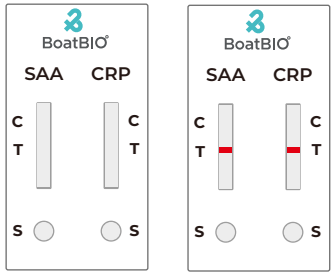




RESULT INTERPRETATION

POSITIVE:* Two lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).
***NOTE:** The intensity of the color in the test line region (T) will vary depending on the concentration of SAA antigen and/or CRP antigen present in the specimen. Therefore, any shade of color in the test line region (T) should be considered positive.
NEGATIVE: One colored line appears in the control line region (C). No line appears in the test line region (T).
INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.





QUALITY CONTROL

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is an internal valid procedural control. It confirms sufficient specimen volume and correct procedural technique.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1.The SAA and CRP Combo Rapid Test Cassette (Whole Blood/Serum/Plasma) is for professional in vitro diagnostic use, and should only be used for the qualitative detection of serum amyloid A protein and C-reactive protein.

2.The SAA and CRP Combo Rapid Test Cassette (Whole Blood/Serum/Plasma) will only indicate the presence of serum amyloid A protein and/or CRP antigen in the specimen and should not be used as the sole criteria for evaluating inflammatory conditions.

3.As with all diagnostic tests, all results must be considered with other clinical information available to the physician.

4.A faint line may be appeared when the concentration of Serum Amyloid A protein or CRP antigen in the specimen was close to 10μg/ml.

5.The hematocrit of the whole blood should be between 25% and 65%.

EXPECT VALUE

In a normal healthy individual without any marked inflammation, the level for both SAA and CRP should be 10μg/ml.

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity, Specificity and Overall Accuracy

The SAA and CRP Combo Rapid Test was compared with leading commercial immunoturbidimetry (ITM) tests; the results show that SAA and CRP Rapid Test (Whole Blood/Serum/Plasma) has a high sensitivity and specificity.

SAA Rapid Test

SAA Rapid Test	ITM		
BESTest	Positive	Negative	Total
Positive	97	4	101
Negative	2	233	235
Total	99	237	336
Relative Sensitivity: 98.0% (97.5%CI*: 92.9%-99.8%) Relative Specificity: 98.3% (95%CI*: 95.7%-99.5%) Accuracy: 98.2% (95%CI*: 96.2%-99.3%) *Confidence Interval Relative			

CRP Rapid Test

CRP Rapid Test	ITM		
BESTest	Positive	Negative	Total
Positive	103	4	106
Negative	2	247	249
Total	105	250	355
Relative Sensitivity: 98.1% (97.5%CI*: 93.3%-99.8%) Relative Specificity: 98.8% (95%CI*: 96.5%-99.8%) Accuracy: 98.6% (95%CI*: 96.7%-99.5%) *Confidence Interval Relative			

Analytical Sensitivity (Detection Limitation)

The SAA Rapid Test (Whole Blood/Serum/Plasma) can detect out Serum Amyloid A protein as low as 10μg/ml.

The CRP Rapid Test (Whole Blood/Serum/Plasma) can detect out C-reactive protein as low as 10μg/ml.

Precision

Intra-Assay

For SAA, within-run precision has been determined by using 3 replicates of the specimens containing negative, 10μg/ml SAA, 40μg/ml SAA and 100μg/ml SAA standard sample. The negative and positive values were correctly identified 99% of the time.

For CRP, within-run precision has been determined by using 3 replicates of the specimens containing negative, 10μg/ml CRP, 40μg/ml CRP and 80μg/ml CRP standard sample. The negative and positive values were correctly identified 99% of the time.

Inter-Assay

For SAA Between-run precision has been determined by using the same specimens of negative, 10μg/ml SAA, 40μg/ml SAA and 100μg/ml SAA standard sample in 3 independent assays. Three different lots of the SAA Rapid Test (Whole Blood/Serum/Plasma) has been tested over a 3-days period using negative, low positive and high positive specimens. The specimens were correctly identified 99% of the time.

For CRP Between-run precision has been determined by using the same specimens of negative, 10μg/ml CRP, 40μg/ml CRP and 80μg/ml CRP standard sample in 3 independent assays. Three different lots of the CRP Rapid Test (Whole Blood/Serum/Plasma) has been tested over a 3-days period using negative, low positive and high positive specimens. The specimens were correctly identified 99% of the time.

Cross-reactivity

The SAA and CRP Combo Rapid Test has been tested by HBsAg, anti-HIV, anti-HCV, anti-Syphilis, Rheumatoid factor (RF), anti-H. Pylori, anti-CMV IgG, anti-Rubella IgG and anti-TOXO IgG positive specimens. The results showed no cross-reactivity.

Interfering Substance

The following potentially interfering substances were added to SAA/CRP negative and positive specimens.

Acetaminophen: 20 mg/dL	*Caffeine: 20 mg/dL*
Acetylsalicylic Acid: 20 mg/dL	*Gentisic Acid: 20 mg/dL*
Ascorbic Acid: 2g/dL	*Albumin: 2 g/dL*
Creatin: 200 mg/dL	*Hemoglobin 1000mg/dL*
Bilirubin: 1g/dL	*Oxalic Acid: 60mg/dL*

None of the substances at the concentration tested interfered in the assay.

BIBLIOGRAPHY

1.Uhlar CM, Whitehead AS (October 1999). "Serum amyloid A, the major vertebrate acute-phase reactant". European Journal of Biochemistry.

2.Manley PN, Ancsin JB, Kisilevsky R (2006). "Rapid recycling of cholesterol: the joint biologic role of C-reactive protein and serum amyloid A". Medical Hypotheses.

3.Pincus MR; McPherson RA; Henry JB (2007). Henry's Clinical Diagnosis and Management by Laboratory Methods. Saunders Elsevier.

4.Thompson D, Pepys MB, Wood SP (Feb 1999). "The physiological structure of human C-reactive protein and its complex with phosphocholine".

5.Bray, Christopher (December 2016). "Erythrocyte Sedimentation Rate and C-reactive Protein Measurements and Their Relevance in Clinical Medicine"

INDEX OF SYMBOLS

Symbol	Used For	Symbol	Used For
	Use-by date		Consult instructions for use
LOT	Batch code	IVD	In vitro diagnostic medical device
	Temperature limit		Manufacturer
	Please don't reuse it		Keep away from sunlight
	Don't use the product when the package is damaged		Keep dry
	Date of manufacture	Σ	Tests per kit
CE	CE Mark		Biological Risks
EC REP	Authorized representative in the European Community		

BASIC INFORMATION

Ningbo BESTest Bio-technology Co.,Ltd.
Address: No.80 building, No.777, Qing Feng Road, Cicheng Town,Jiangbei District, Ning Bo, Zhejiang, China 315033
Tel: 0086 571 2799 8736

EC REP **CMC MEDICAL DEVICES & DRUGS S.L**
Address: C/Horacio Lengo Nº 18 CP 29006, Málaga-Spain
Tel: +34951214054
Email - info@cmcmedicaldevices.com