

cCRP

Canine C-Reactive Protein Reagent Kit TP-812-RGT

Polystyrene Immunoturbidimetric test for the quantitative In-Vitro determination of canine C-reactive protein in serum or plasma on photometric systems



Intended Use

This *in vitro* **veterinary** research-only assay is designed to quantitatively measure canine C-reactive protein (CRP) concentration in serum and plasma. Components of the package are available as a kit only.

Application

C-reactive protein (CRP) is a positive acute phase protein. The serum level of CRP rises in animals that suffer from different types of infection or inflammation and is generally correlated with degree and activity of the causative disease. For this reason measurements of serum CRP are used to aid the evaluation of the inflammation associated with infection, tissue damage and other clinical disorders. Increased levels of CRP have been described in dogs that suffered from pneumonia, pyometra, pancreatitis, parvovirus infection or trauma. Dogs with an increased number of leukocytes had significantly higher CRP levels than dogs with non-inflammatory leukograms. Increased CRP levels were also found in dogs with arthritis, thrombophlebitis or proctitis. Increased CRP levels were also observed in dogs infected with *Bordetella bronchiseptica* or *Ehrlichia canis*.

Test Principle

This test is a polystyrene microparticle enhanced immunoturbidimetric assay. The turbidity produced is proportional to the concentration of CRP present in the original specimen.

- A canine serum or plasma sample is mixed with cCRP buffer (R1), and incubated at 37°C.
- Addition of CRP antiserum reagent (R2) follows and incubation continues at 37°C.
- The agglutination reaction between CRP present in the standard, control or sample begins upon addition of second reagent (R2).
- If CRP is absent from the standard, control or sample, no agglutination will occur.
- If CRP is present in the sample, agglutination occurs and this leads to an increase in the absorbance of the mixture. The concentration of CRP present in a standard, control or sample will dictate the rate of increase in absorbance.
- The reaction is monitored at 600nm and the rate of agglutination is used to calculate CRP concentration from a delta absorbance vs CRP concentration calibration curve.

Reagent Materials Provided & Reagent Description

The cCRP kit contains three reagents:

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|---|-----------|
| • R1 cCRP Buffer | 2 x 25 ml |
| • R2 anti cCRP polystyrene microparticles | 2 x 5ml |
| • Calibrator/sample diluent (20X) | 1 x 10ml |

The cCRP Buffer (R1) consists of a Tris buffered solution containing polyethylene glycol (PEG) to enhance agglutination, sodium chloride and thimerosal as a preservative.

C-reactive protein antibody reagent (R2) consists of polyclonal antibody conjugated to polystyrene microparticles.

The sample/calibrator diluent (20X) is a Tris buffered saline solution, pH 7.4, 2% Tween 20 and thimerosal as preservative.

Warnings and Precautions

The test is for *in vitro* **veterinary** research use only. Do not use for determination of CRP concentrations in human specimens or for diagnostic use.

Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Avoid ingestion of reagents. Never pipette by mouth and wear disposable latex gloves and eye protection where appropriate.

Materials required but not provided

Canine CRP immunoturbidimetric calibrator kit (Tridelta, Cat no. TP-812-CAL), Canine CRP immunoturbidimetric control kit (Tridelta, Cat no. TP-812-CON), serum or plasma collection equipment, sample containers, distilled H₂O, vortex mixer, 20µl, 200µl and 1000µl pipettes, disposable tips, automated chemistry analyser and its requirements for use.

Reagent preparation and use

The R1 and R2 reagents are provided ready to use and require no preparation. The R2 reagent should be inverted several times gently prior to use to resuspend any settled microparticles. Inversion of R2 should be performed periodically for extended on-board use.

The 20X calibrator/sample diluent must be diluted to 1X by the addition of 19 volumes of deionised H₂O to 1 volume of diluent concentrate. Diluted diluent buffer is stable for up to two weeks when stored at 4°C.

Test Procedure

The chemistry parameters for this test are entered into the end-user's system as specified for each respective automated chemistry analyser. Cobas Mira chemistry parameters are as follows and should be used as a guideline:

Sample volume: 2µl

R1: 250µl

R2: 50µl

Cycle 1: Addition of R1 + sample

Cycle 7: Addition of R2

Read times: First 7: Last 27

Read wavelength: 600nm

Reagent blank: None

Calibration: LOGIT/LOG5

(Cobas Mira cycle time: 25 seconds)

Note: All patient samples and controls must be diluted in equal parts sample/control to equal parts 1X diluent buffer prior to assaying. If this function is not available onboard the analyzer it must be done manually.

Additional settings can be found on our website at www.trideltatd.com

Calibration

The Canine CRP calibrator is provided in a separate kit by Tridelta (Cat no. TP-812-CAL). Calibration should be performed when the C-reactive protein test is established on an automated chemistry analyser. This calibration curve is automatically stored on the automated chemistry analyser once the test has been assigned to a reagent position.

Recalibration is required after a change of reagent bottles, a change of critical components, if control results fall outside the acceptable and established ranges or if the instrument undergoes any cleaning or maintenance. Calibration curve stability on the analyzer has been determined to be up to 8 weeks when the proper conditions are met.

The calibrator is supplied as a 60µg/ml stock which must be serially diluted using 1X diluent buffer. This can be done manually or by analysers with this function. The recommended calibration curve points are 60µg/ml, 30µg/ml, 15µg/ml, 7.5µg/ml and 0µg/ml.

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Sample Handling

Use serum or lithium-heparin plasma. Serum and plasma samples may be stored for 24 hours at 2°C-8°C or for long-term at -20°C. It is important that frozen or refrigerated samples are brought to room temperature and mixed to ensure accurate determination of CRP concentration.

Measuring range

All samples and controls must be diluted with equal parts 1X diluent buffer prior to assaying. For samples returning values higher than the top calibrator (60µg/ml), the sample must be diluted 1 in 5 and reassayed.

Quality Control

Good laboratory practice suggests the use of control specimens to ensure proper assay performance. Controls results must fall within the established ranges, as determined by your laboratory. If results fall outside of the established ranges, assay results are invalid. Canine CRP immunoturbidimetric controls are provided by Tridelta in a separate kit (Cat no. TP-812-CON).

Storage instructions and stability

- **Unopened and stored at 2°C-8°C:** Unopened CRP reagents are stable until the last day of the month of the expiration date printed on the product label.
- **Opened and stored at 2°C-8°C:** Opened CRP reagents are stable for three months from date of first use of kit if kept closed in their original containers, free from contamination and at the recommended storage temperature.
- **On-board stability:** Opened CRP reagents have on-board stability at room temperature for up to forty-eight hours.
- **Protect all reagents from extreme heat or freezing.** In order to ensure maximum stability of the cCRP reagents on each automated chemistry analyser, it is important to use proper boats and anti-evaporation tray covers.

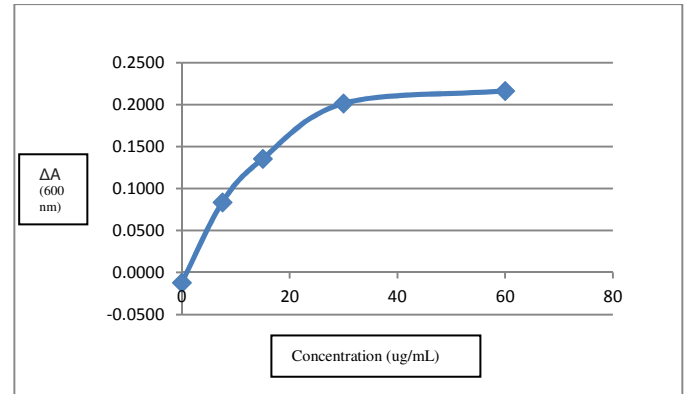
Waste management

Please refer to local legal requirements.

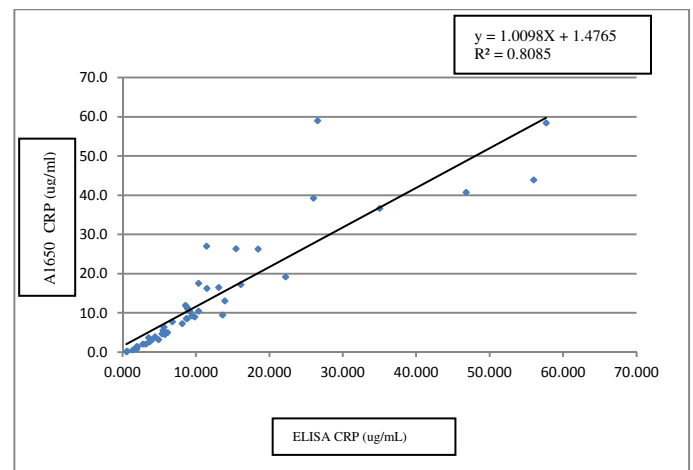
Performance Characteristics

Typical assay performance data provided has been established on a Cobas Mira and an Advia 1650. However, similar performance data should be attainable on other instruments.

- **Calibration Curve:** A typical 5-point calibration curve



- **ELISA (TP803) Vs Turbidimetric (Sample Comparison Evaluation)**



Additional performance characteristics are available from the Tridelta Development Ltd. website www.trideltaltd.com

Contact Details

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