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Canine C-Reactive Protein (cCRP) TurboReader™ Assay

Instructions For Use (IFU) manual- Version 2, October 2017

A quantitative point-of-care assay for cCRP in canine (dog) plasma or serum using the TurboReader™ instrument.

FOR VETERINARY AND RESEARCH USE ONLY.

1 INTENDED USE

The cCRP TurboReader™ assay is an immunoturbidimetric point-of-care immunoassay for the quantitative, *in vitro* determination of cCRP in dogs, which can be a useful tool for monitoring systemic inflammation.

Art No.		
2530-01	Test Cuvettes	20 pcs
	Dropper bottle R2	1 x 3.0 ml
	CRP Level 2 Control	1 x 0.05 ml
	Test instruction	1

2 GENERAL DESCRIPTION[1-4]

cCRP is a pentameric serum protein that consists of five 20 kDa subunits (of which two are glycosylated). The protein is a well-known acute phase reactant and its normal plasma concentrations in healthy dogs is <25 mg/l. cCRP is a real-time diagnostic marker for systemic inflammation with plasma concentrations increasing approximately 4 hrs after stimulation, peaking around 24 hrs and clearing between 48-72 hrs after cessation of inflammatory conditions. Measurement of cCRP has a large diagnostic window, increasing more than 10x the normal plasma concentrations during inflammatory activity. Clinical use of cCRP is not limited to monitoring systemic inflammation, but can also be used for determination if selected treatment is effective or monitoring post-operative conditions and surgery recovery.

3 ASSAY PRINCIPLE

The cCRP TurboReader™ assay is a quantitative immunoturbidimetric point-of-care immunoassay for the detection of cCRP in canine (dog) plasma or serum. The dropper bottle R2 contains polyclonal antibodies against cCRP. Upon mixing of reagents, the cCRP antigen present in the canine sample together with the R2 reagent forms a precipitation reaction which yields a turbid solution. The turbidity of the solution is measured photometrically and is directly proportional to the concentration of cCRP present in the canine sample.

4 COMPOSITION OF SUPPLIED REAGENTS

Contents	**Substance & Concentration**
Cuvette Assay Buffer	max 4% Polyethylene Glycol max 50 mM Tris buffer, pH 7.6 150 mM NaCl
Dropper bottle R2 (1502-36)	goat anti(CRP)serum
CRP Level 2 Control (1502-23)	80 ±15 mg/l
Test instruction (1810-03)	1 copy for laboratory

5 MATERIALS NEEDED BUT NOT SUPPLIED

- Sample collection device and/or pipette
- Disposable gloves
- NaCl solution, 0.9 % (w/v)
- TurboReader™ instrument

6 STORAGE & STABILITY

The cuvette assay buffer, dropper bottle R2 and the CRP level 2 control are supplied ready-to-use and are stable up to the expiry date when stored at +2-8 °C. They may not be frozen.

7 PRECAUTIONS

- FOR VETERINARY AND RESEARCH USE ONLY.
- Do not use after expiration date.
- Do not freeze any test reagents.
- Lipaemina, haemolytic samples or high levels of detergents in sample may interfere with assay results.
- Follow Good Laboratory Practices, wear a lab coat, use disposable gloves and keep laboratory area clean.
- After use, the test should be discarded according to local regulations regarding biological and hazardous material.
- Make sure to insert the cuvette into the TurboReader™ instrument in the correct orientation (the arrow on the cuvette wall and on instrument must align).

8 SAFETY & WASTE HANDLING

Only qualified laboratory personnel under appropriate laboratory conditions may use the reagents. CAUTION: kit components contain sodium azide (<0.1%) as preservative. Therefore, handle as hazardous material and wear disposable gloves, eye protection and a lab coat. Do not ingest! Avoid contact with skin, mucous membranes and eyes. If uncertain, consult expertise for help. Health and Data Sheets are available at request. Handling of waste should be done in accordance with national laws and local regulations.

9 SPECIMEN COLLECTION

Collect canine (dog) lithium heparin plasma or serum sample using a blood collection tube according to the manufacturer's instructions. Do not use EDTA collection tubes. The stability of cCRP serum is 2 weeks at +2-8 °C. For long-term storage, the specimen must be kept frozen (<-20°C). Repetitive freezing and thawing cycles is not recommended. The sample must be completely thawed, thoroughly mixed and at room temperature before testing can occur.

10 INSTRUMENT PARAMETERS

Recommended parameter settings for the TurboReader™ instrument:

- Volume S (sample): 20 µl
- Volume Dropper bottle R2: 3 drops
- Reaction time 1 (S): 1 min
- Reaction time 2 (S+R2): 3 min
- Multi-point calibration

11 PROCEDURE

Start TurboReader™ instrument and select NEW TEST followed by RUN on the instrument touch screen. Use a pipette to transfer 20 µl of the dog serum sample (or control) into an unused cuvette. Turn the cuvette slowly upside down 4 times (no bubbles should be introduced). Place the cuvette into the TurboReader™ and make sure it has the correct orientation (the arrow on the cuvette wall and on instrument must align). Select OK on the touch screen. After 1 minute the TurboReader™ will request you to remove the cuvette and add 3 drops of Dropper bottle R2 (keep the bottle completely vertical when adding so that remaining fluid is retracted back into the bottle when finger pressure is released). Turn the cuvette slowly upside down 4 times (no bubbles should be introduced). Place the cuvette into the TurboReader™ and make sure it has the correct orientation (the arrow on the cuvette wall and on instrument must align). Select OK on the touch screen. After 3 minutes the TurboReader™ will display the concentration of CRP.

13 CALIBRATION & QUALITY CONTROL

The TurboReader™ instrument is precalibrated (multi-point calibration) and therefore, no additional calibration is required.

In order to survey accuracy and precision, daily Quality Control is recommended with cCRP Level 2 Control (Art. No. 1502-23).

14 PERFORMANCE

Assay measuring range: The measuring range of the assay is 6 – 200 mg/l (up to 300 mg/l with reduced performance). Samples with cCRP levels larger than 200 mg/l should be diluted 1:4 with 0.9 % (w/v) NaCl solution and the result multiplied with 4.

Sensitivity: The minimum level of detection is approximately 6 mg/l.

Prozone limit: No prozone effect can be observed for cCRP concentrations of up to 600 mg/l (600 µg/ml).

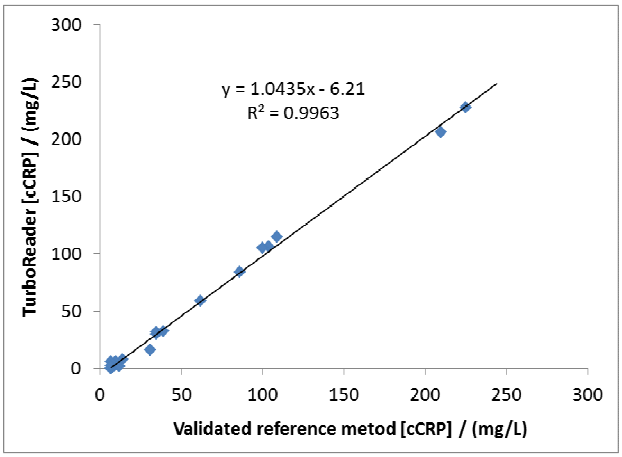
Specificity & Interference: The antiserum used is monospecific for canine CRP. It has not been shown to cross-react with other serum proteins under the conditions of the assay. However, the assay may be interfered by Lipaemina, Haemolytic samples or high levels of detergents in sample.

Precision: The precisions of the assay is given in tables below.

Intra Assay Precision (n=5)	Mean mg/L	SD mg/L	CV %
Dog sample	94.5	0.8	0.9

Inter Assay Precision (n=8)	Mean mg/L	SD mg/L	CV %
Dog sample	102	6	6

Correlation with validated method: The assay performance has been compared with a validated reference method on 19 dog serum samples (see graph below).



Normal ranges: The normal range of the cCRP concentration in healthy dogs is < 35 mg/L (35 µg/mL). Each laboratory should establish its own normal range which corresponds to local genetic and environmental factors.

• Repetitive measurement of cCRP can be used to determine if selective treatment is effective and for the monitoring of post-operative conditions and surgery recovery.

• cCRP results should be used with other clinical and diagnostic information for forming a diagnosis and for health management.

15 REFERENCES

1. Ganrot K., Plasma protein respons in experimental inflammation in dogs., Res. Exp. Med., 1973, 161(4), 251-261.

2. Hansson L.O., Lindquist L. C-Reactive protein: its role in the diagnosis and follow-up od infectious diseases. Curr. Opin. Infect. Diseases, 1997, 10:196-201.

3. Yamamoto S., Changes in serum C-reactive protein levels in dogs with various disorders and surgical traumas, Vet. Res. Com. 1993, 17:85-93.

4. Kjelgaard-Hansen M., Lundorff Jensen A.T., Evaluation of a commercially available Human C-Reactive Protein (CRP) turbidimetric immunoassay for determination of Canine Serum CRP concentration, Vet Clin Pathology, 2003, 32:2, 81-84.

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