Canine CRP

Gentian Canine CRP Reagent Kit

REF 1501

For *in vitro* diagnostic use by laboratory professionals.

This document describes the general use of the product above. For instrument specific settings, please refer to the application notes available upon request from marketing@gentian.com.

Intended Purpose

The Gentian Canine CRP Immunoassay is an in vitro diagnostic test for quantitative determination of canine CRP in dog serum and plasma. The measurement of canine CRP is used to detect and monitor inflammation activity in dogs.

Summary and Explanation of Test

C-reactive protein is an acute phase protein produced in the liver hepatocytes as response to inflammatory stimuli.[1,2] Increased production of canine CRP can be detected 4-6 hours after induction with a peak concentration within 24-48 hours. After elimination of the inflammation, CRP levels rapidly decrease due to the short the half-life of CRP.[1,2]

Calibrator Standardisation

No international standard is available for canine CRP. Gentian Canine CRP Calibrator values are established based on internal canine CRP reference material.

Assay Principle

The Gentian Canine CRP Immunoassay is a particle-enhanced turbidimetric immunoassay (PETIA). The canine plasma or serum sample is mixed with Canine CRP Immunoparticles. Canine CRP from the sample and the anti-CRP antibodies from the immunoparticles solution bind to form aggregates that increase the turbidity of the solution. The degree of turbidity is proportional to the concentration of CRP, which can be quantified via an established standard calibration curve.

Assay Kit Components

Products provided	
Gentian Canine CRP Reagent Kit • R1 Assay Buffer (45 mL) • R2 Immunoparticles (10.5 mL)	REF 1501
Products required, but not provided	
Gentian Canine CRP Calibrator Kit (6 levels x 0.5 mL)	REF 1551
Gentian Canine CRP Control Kit (2 levels x 0.5 mL)	REF 1519

All products are ready for use.

Composition

Reaction Buffer 1 (R1): Canine CRP Assay Buffer. R1 is a MOPS [3-(N-Morpholino)-propane sulfonic acid] buffered saline, containing avian proteins and preserved with ProClin® 950.
Reaction Buffer 2 (R2): Canine CRP Immunoparticles. R2 contains a purified immunoglobulin fraction directed against canine CRP, which is covalently attached to polystyrene nanoparticles. The solution is preserved with ProClin® 950.

Warnings and Precautions

1. Materials are not classified as hazardous.
2. Contains substances from animal origin and should be considered as potentially infectious material. Handle with caution and discard following local regulations.
3. Use protection googles, lab coat and gloves for personal protection.
4. Reagents (R1) containing MOPS/Tween can be irritating to eyes, respiratory tract and skin. Handle with due caution and do not ingest.
5. R1 contains avian proteins. Handle with due caution to avoid allergic skin reaction.

To obtain the SDS (safety data sheet), please contact Gentian at marketing@gentian.com.

Additional Handling Instructions

1. This test is for *in vitro* use only and must be handled by qualified personnel.
2. Use only validated and approved instrument applications.
3. Do not use products after the expiration date has passed.
4. Do not mix reagents of different lots or interchange caps of reagents, controls, calibrators, and lots.
5. Tighten caps carefully back on after use of reagents, calibrators, and controls to avoid evaporation.

Reagent Storage and Stability

All products provided for the Gentian Canine CRP Immunoassay must be stored at 2-8°C. The expiry date is printed on the labels. Using an Architect c4000 instrument (Abbott), the on-board stability of the Gentian Canine CRP Reagents was found to be at least 8 weeks in a study using a protocol based on the CLSI guideline EP25.

Specimen Collection and Handling

Recommended sample material is canine heparinized plasma or canine EDTA plasma, or canine serum. It is recommended to analyse the samples as fresh as possible. Sample stability testing using an Architect c4000 instrument (Abbott) showed that canine CRP was stable for 14 days at 4-22°C.[3] Mix samples well before analysing. The samples can be shipped without special cooling and must then be analysed within 14 days after shipment. Samples have been tested and shown to withstand up to 4 freeze and thaw cycles.[3]

Performance Characteristics

The results refer to the main validation of the Gentian Canine CRP Immunoassay on an Architect c4000 instrument (Abbott) at one instrument site with one lot of reagents, unless otherwise stated. For the instrument specific performance characteristics, please refer to the instrument specific application notes.

Measuring Range

The measuring range of the Gentian Canine CRP Immunoassay is approximately 10 - 300 mg/L, with a security zone of up to 1000 mg/L. The exact measuring range is calibrator- and instrument-specific, please refer to the analytical value sheet for the lot specific calibrator values and the instrument specific application notes.

Analytical Sensitivity

The analysis of the analytical sensitivity of the Gentian Canine CRP Immunoassay was tested in a study including 4 samples run in 12 replicates over 3 days using a protocol based on the CLSI guideline EP17. The limit of quantification (LoQ) is defined as the lowest concentration of an analyte that can be reliably detected and at which the total error meets the requirements for accuracy. The LoQ was measured as 5 mg/L. For the instrument specific LoQ, please refer to the instrument specific application notes.

Precision

The total precision of the Gentian Canine CRP Immunoassay was measured with a CV of <5% in a study using a protocol based on the CLSI guideline EP5. For the instrument specific total precision, please refer to the instrument specific application notes.

Analytical Specificity and Limitations

No interference is detected for this product with haemoglobin, intralipid, or bilirubin at the tested concentrations in a study using a protocol based on the CLSI guideline EP07. For the instrument specific interference, please refer to the instrument specific application notes. There is no interference detected with the drugs tested on the recommendations from Sonntag and Scholer.[4] As the antibodies in the Gentian Canine CRP Immunoassay are of avian origin, there is no interference due to Rheumatoid Factor in the samples.[5]

Method Comparison

Results obtained with the Gentian Canine CRP Immunoassay on the Architect c4000 instrument (Abbott) and on the AU400 instrument (Beckman Coulter) were compared using Passing-Bablok regression with results from Randox (canine) CRP assay on the Architect c4000 instrument (Abbott) in a study using a protocol based on the CLSI guideline EP09.

n	Range of samples [mg/L]	Term	Co-efficient	95% CI
Architect c4000				
45	26 - 439	Intercept	7.79	[5.81, 12.43]
		Slope	0.91	[0.87, 0.94]
AU400				
31	16 - 209	Intercept	-5.11	[-11.24, -0.32]
		Slope	1.22	[1.17, 1.30]

Instrument variation

Results obtained with the Gentian Canine CRP Immunoassay on Architect c4000* instrument were compared using Passing-Bablok regression with results from using the AU400 instrument (Beckman Coulter) in a study using a protocol based on the CLSI guideline EP09.

n	Range of samples [mg/L]	Term	Co-efficient	95% CI
33	16 - 254	Intercept	0.56	[-0.72, 0.94]
		Slope	1.05	[1.04, 1.06]

Assay Procedure

Application Notes

Applications of the Gentian Canine CRP Immunoassay have been established on several clinical chemistry analysers. Detailed, validated application notes describing the procedures for installation and analysis on specific instruments are available upon request from marketing@gentian.com. For instructions on how to install a new application, consult the instrument manual. Maintenance, operation and precautions must be handled in accordance with the specific instrument manual.

Reagent Preparation

The reagents are ready for use. Mix the reagents gently before placing them into the assigned reagent positions. The reagent bottles may fit directly into the instrument unless otherwise stated in the application notes.

Calibration Curve Establishment

The calibration curve can either be created from a dilution series that is automatically prepared by the instrument or by using a 6-point pre-diluted calibrator kit as specified in the instrument manual. Calibrator values are lot-specific, and calibration must be performed whenever a new lot is used. The values assigned to the calibrators are provided in the analytical value sheet. A new calibration should be performed every 4 weeks or when a new reagent lot is used.

QC Controls

The Gentian Canine CRP Controls should be assayed every day that the test is in use to validate the calibration curve. The controls have lot-specific concentration ranges that must be met before measuring samples. The assigned value ranges are given in the analytical value sheet. If the control values measured are not valid, repeat the control measurements. Recalibrate if necessary. If the calibration cannot be performed without error, or valid control values cannot be reproduced, contact the local distributor for support.

Measuring Patient Samples

When a valid calibration curve has been established and the control values are within the valid range, the plasma or serum sample may be measured. Ensure that the minimum sample volume is present in the sample cups/tubes and assay the samples according to the instructions given in the instrument manual.

Results

The results are calculated automatically by the instrument for all applications established for the Gentian Canine CRP Immunoassay. The results are presented in mg/L.

Reference Intervals

The canine CRP reference intervals were determined in a study using a protocol based on the CLSI guideline C28 on an Architect c4000 instrument (Abbott). The reference interval was determined from a population of healthy dogs of different breeds, examined by a veterinarian. A total of 40 samples from dogs (22 males, 18 females) ranging in age from 0.5 to 11 years were measured. The samples used were serum samples. The reference interval was calculated non-parametrically and was determined to be 0.2 - 4.9 mg/L. It is recommended that every laboratory should determine a local reference interval since values may vary depending on the population tested.

Clinical Decision Limits

The diagnostic specificity of canine CRP can be enhanced without seriously impairing diagnostic sensitivity by using a cut-off limit somewhat above the normal range.[6] Each laboratory should establish its own cut-off.

Symbols Key

2°C 8°C	Temperature Limit
	Use by Date
	Consult Instructions for Use
	Manufacturer
	Veterinary use
LOT	Lot Number
REF	Catalogue Number
CONTENTS	Contents
R1	R1 Assay Buffer
R2	R2 Immunoparticles

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References

1. Ceron *et al*. Vet Clin Pathol. 2005; 34: 85-99
2. Eckersall *et al.* Vet J.2010; 185 (1):23-27
3. Hillström *et al.* Vet Clin Pathol. 2014; 43(2):235-43
4. Sonntag O, Scholer A. Ann Clin Biochem 2001;38:376-85.
5. Larsson A, et al. Poultry Science 1993;72:1807-18
6. Kjelgaard-Hansen; PhD Thesis. 2004

Serious Incidents

Please notify your manufacturer and competent authority if any serious incidents have occurred in relation to the device.

Modification from the Previous Version

New layout. Updated Symbols Key list.

Date of issue

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