

# Gentian Canine CRP Immunoassay

## Application Note for cobas c 311\*

### Intended Use

The canine CRP immunoassay on cobas c 311 is an *in vitro* diagnostic test for quantitative determination of canine CRP in dog serum and plasma. The measurement of canine CRP is used in the diagnosis and treatment of inflammatory diseases in dogs [1,2,3,4,5].

### Measuring Range

The measuring range of the Gentian canine CRP immunoassay on cobas c 311 is 10 - 300 mg/L, with a security zone up to 600 mg/L.

### Normal Values

Healthy dogs have CRP concentrations <10 mg/L with the Gentian canine CRP method. An exact reference range cannot be determined as CRP concentrations in healthy dogs are below the LoQ of the Gentian canine CRP assay.

### Clinical Decision Limits

The diagnostic specificity of canine CRP can be enhanced without seriously impairing diagnostic sensitivity by using a cut-off limit somewhere above the normal range [2]. Each laboratory should establish its own cut-off.

### Assay Reagents

Materials Provided by Gentian	
Gentian Canine CRP Reagent Kit <ul style="list-style-type: none"><li>R1 Reaction buffer (45 ml)</li><li>R2 Immunoparticles (10,5 ml)</li></ul>	REF 1501 REF 1507 <sup>#</sup> REF 1514 <sup>#</sup>
Gentian Canine CRP Calibrator Kit (6 levels, 0,5 ml per level)	REF 1551
Gentian Canine CRP Control Kit <ul style="list-style-type: none"><li>Control low (0,5ml)</li><li>Control high (0,5ml)</li></ul>	REF 1519 REF 1520 <sup>#</sup> REF 1521 <sup>#</sup>

All materials are ready for use.

<sup>#</sup>Not available for individual sale.

### Calibrator Standardization

Gentian canine CRP calibrator values are established on the basis of internal canine CRP reference material. No international standard is available for canine CRP.

### Calibration Stability

The stability of the calibration curve has not been tested on cobas c 311. On Abbott's Architect c4000 and Beckman Coulter's AU400, the calibration curve has been shown to be stable for more than four weeks. Yet, recalibration every 4<sup>th</sup> week is generally recommended.

### Material Storage and Stability

All materials provided for the Gentian canine CRP test must be stored at 2-8°C. The expiry date is printed on the labels. Using an Abbott Architect c4000, the on board stability of the Gentian canine CRP reagents was found to be at least eight weeks.

### Sample Material

Recommended sample material is canine serum, canine heparinized plasma or canine EDTA plasma. Analyze the samples as fresh as possible, and mix them well in advance. Sample stability testing showed that canine CRP (in serum) was stable for 14 days at 4-22°C [6]. The samples can be shipped without any special cooling and must then be analyzed within 14 days after shipment. Samples have been tested and shown to withstand up to four freeze and thaw cycles [6].

### Assay Procedure

#### Assay Principle

The canine serum or plasma sample is mixed with canine CRP immunoparticles. Canine CRP from the sample and the immunoparticles' anti-canine CRP aggregate. The complex particles created absorb light, and turbidimetric measurements of absorption are related to canine CRP concentration via interpolation on an established standard calibration curve. Results are automatically calculated by cobas c 311.

#### Application Parameter Setup

The application must be installed with the instrument settings provided for the Gentian canine CRP method. For instructions on how to install a new application, consult the instrument manual.

\*Registered trademark of Roche Diagnostics.

## Reagent Preparation

The reagents provided are ready for use. Mix the reagents gently before adding them to the reagent cassette. The bottle fill volumes,  $V_{fillR1}$  and  $V_{fillR3}$ , are calculated as follows:

$$V_{fillR1} \text{ (mL)} = 1,09 * N * V_{pipR1} \text{ (}\mu\text{L)} / 1000 + 3,85$$

$$V_{fillR3} \text{ (mL)} = 1,06 * N * V_{pipR3} \text{ (}\mu\text{L)} / 1000 + 2,4$$

Here,  $N$  is the number of tests, while  $V_{pipR1}$  and  $V_{pipR3}$  correspond to the pipetting volume per test in  $\mu\text{L}$  for reagent R1 and R3, respectively. ( $V_{pipR1} = 180 \mu\text{L}$  and  $V_{pipR3} = 50 \mu\text{L}$ , as stated in the Instrument Settings below.) For instance, if the total number of tests wanted is  $N = 100$ , the cassette needs to be filled with the following reagent volumes:

$$V_{fillR1} = 1,09 * 100 * 180 / 1000 + 3,85 = \underline{23,5 \text{ mL}}$$

$$V_{fillR3} = 1,06 * 100 * 50 / 1000 + 2,4 = \underline{7,7 \text{ mL}}$$

Consult the cobas c pack MULTI system information for explanation and more detailed instructions.

## Calibration Curve Establishment

Use the Gentian canine CRP calibrator kit to establish a calibration curve as described in the instrument manual. A recalibration must be performed when a new calibrator lot and/or a new reagent kit lot is to be used. The assigned concentration values of the calibrators are lot dependent. The relevant values are stated in the Analytical Value Sheet provided with the calibrator kit.

## QC Controls

The Gentian canine CRP control samples should be assayed every day the method is in use, to validate the calibration curve. Each control has an assigned concentration value range that must be met before measuring regular samples. The relevant concentration ranges are given in the Analytical Value Sheet provided e control kit. If the measured concentrations are outside the valid range, 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 satisfactory calibration curve has been established and the control values are within the valid concentration range, canine serum or plasma samples may be measured. Check that minimum sample volume is present in sample cups/tubes and assay the samples according to the instructions given in the instrument manual.

## Results

The sample concentration of canine CRP is calculated automatically by the analyzer and presented in mg/L.

## Performance Characteristics cobas c 311

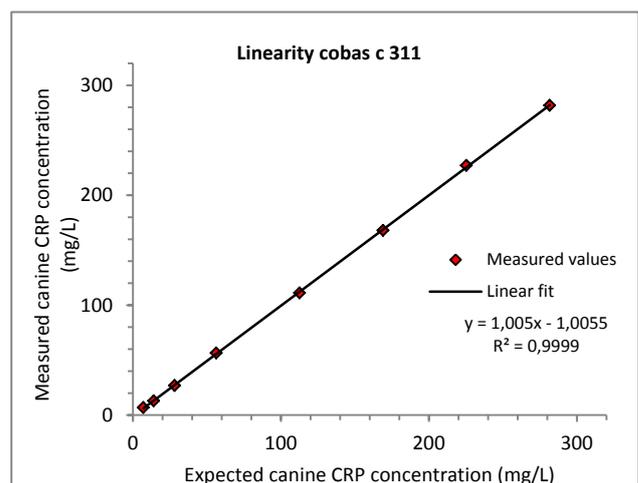
### Lower Quantification Limit

The lower quantification limit (LoQ) of canine CRP is defined as the lowest sample concentration that can be measured with a total error (TE) < 29,6 % [7]. Total error is calculated from the pooled standard deviation of the sample's measured concentration and the bias between the sample's mean and theoretical concentration value. A sample of 5,3 mg/L had a total error of 8,0 % with the Gentian canine CRP method on cobas c 311. Since this was the lowest concentration included in the validation, the LoQ of the Gentian canine CRP immunoassay on cobas c 311 is set to 5 mg/L.

### Linearity

Dilution of a high canine CRP serum was performed, and the concentration of the resulting samples was measured. Recovery from expected concentrations was calculated. The assay is considered linear down to the lowest concentration tested (7,0 mg/L). However, as users in general do not need concentration values below 10 mg/L, this will serve as the lower linearity limit.

Dilution factor	Expected concentration (mg/L)	Measured concentration (mg/L)	Recovery (%)
100 %	281,8	281,8	100,0
80 %	225,4	227,1	100,8
60 %	169,1	168,0	99,4
40 %	112,7	111,1	98,5
20 %	56,4	56,5	100,3
10 %	28,2	26,9	95,3
5 %	14,1	12,9	91,2
2,5 %	7,0	6,9	97,2



## Imprecision

Three samples were assayed in triplicate in three runs (with recalibration in between). Results were subjected to one way analysis of variance (one way anova). The total CV of the measured concentrations was below 2 % for all three samples.

Sample ID	Mean (mg/L)	Within run CV (%)	Between run CV (%)	Total CV (%)
Low	25,4	1,07	0,62	1,23
Medium	75,8	1,05	0	1,05
High	170,7	0,69	0	0,69

## Interference

Canine CRP samples of about 25 mg/L were spiked with 5 g/L hemoglobin or 10 g/L Intralipid. The samples were compared to control samples containing saline and water instead of hemoglobin and Intralipid, respectively. None of the spiked samples demonstrated interference – defined as more than 10 % difference between test sample and control sample.

	Control sample (mg/L)	Test sample (mg/L)	Recovery (%)
Hemoglobin (5 g/L)	23,9	23,7	99,2
Intralipid (10 g/L)	23,8	23,0	96,6

## Security Zone

Samples with a canine CRP concentration up to 600 mg/L return a value above 300 mg/L (upper limit of linearity range) and can be sent to automatic diluted rerun. An improved security zone may be obtained by a reduction in sample volume. Laboratories expecting samples of canine CRP concentration higher than 600 mg/L should therefore consider reducing the sample volume from 2,0 µL (as stated in the Instrument Settings below) to somewhere between 1,5 µL and 2,0 µL, although this has not been validated.

## Instrument Variation

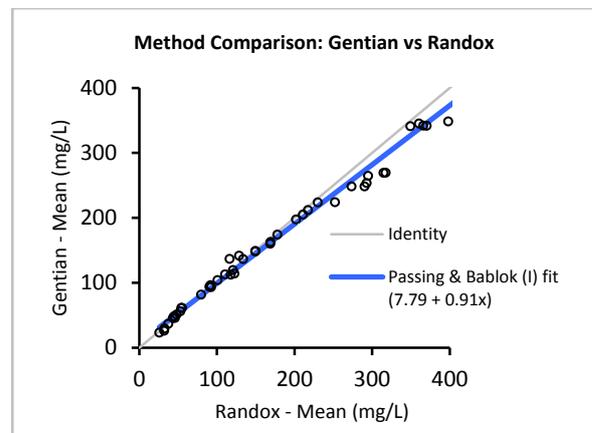
A set of nine samples was assayed on cobas c 311 with the Gentian canine CRP method. The measured concentrations were compared to the mean of corresponding values obtained with Beckman Coulter AU400 and Mindray BS-380. The recovery of the cobas c 311 values from the AU400 / Mindray BS-380 values was calculated.

Mean concentration AU400 / Mindray BS-380 (mg/L)	Mean concentration cobas c 311 (mg/L)	Recovery (%)
25,9	24,7	95,1
175,2	172,4	98,4
154,5	151,2	97,9
64,9	61,7	95,1
39,7	37,1	93,4
56,8	54,7	96,2
194,8	195,0	100,1
61,1	58,3	95,4
73,4	70,8	96,4
	Mean recovery (%)	96,4

## Method Comparison

The Gentian canine CRP test was compared to the Randox human CRP immunoassay, used for canine CRP applications (with canine CRP calibrators). A set of 45 serum samples was assayed on both methods at the Swedish University of Agriculture Sciences Animal Hospital, using an Abbott Architect c4000. Results were subjected to statistical analysis.

Method Comparison	N = 45
Bland Altman bias	-3,0 %
Bland Altman limits of agreement	-19,4 to 13,4 %
Passing Bablok slope	0,91
Passing Bablok intercept (mg/L)	7,79



## Shipping Damage

Please notify your distributor if the product received is damaged.

## Symbols Key

	Lot number
	Temperature limit
	Use by date
	Consult instructions for use
	Manufacturer
	Catalogue number



## **Manufacturer    Distributor**

Gentian AS, Bjornasveien 5 N-1509 Moss, Norway TEL: +47 99 33 99 05 FAX: +47 69 24 09 62 <a href="http://www.gentian.no">http://www.gentian.no</a>	scil animal care company GmbH Dina-Weissmann-Allee 6 68519 Viernheim, Deutschland Tel: +49 (0) 6204 7890 0 Fax: +49 (0) 6204 7890 200 E-Mail: info-de@scilvet.com
---	--

## **References**

- [1] Ceron *et al.* Vet Clin Pathol. 2005; 34: 85-99
- [2] Kjelgaard-Hansen; PhD Thesis. 2004
- [3] Kjelgaard-Hansen *et al.* Vet Clin Pathol. 2003; 32: 81-87
- [4] Yamamoto *et al.* Vet Res Comun. 1993; 17: 259-266
- [5] Eckersall *et al.* Vet J. 2010; 185 (1): 23-27
- [6] Hillström *et al.* Vet Clin Pathol. 2014; *in press*.
- [7] Kjelgaard-Hansen *et al.* Comp Clin Path. 2003; 12: 69-74

# Instrument Settings Canine CRP cobas c 311

TEST NAME	cCRP*
SAMPLE TYPE	Ser/Pl
<b>&lt;&lt; ANALYZE &gt;&gt;</b>	
ASSAY	2POINT END
REACTION TIME	10
MEASUREMENT POINT (1)	25
MEASUREMENT POINT (2)	50
MEASUREMENT POINT (3)	0
MEASUREMENT POINT (4)	0
WAVELENGTH (PRIMARY)	600
WAVELENGTH (SECONDARY)	CANCEL
S. VOLUME (NORMAL)	2.0
	0.0
	0
S. VOLUME (DECREASE)	25.0
	2.0
	75
S. VOLUME (INCREASE)	2.0
	0.0
	0
R. VOLUME (R1)	180
(DIL)	0
R. VOLUME (R2)	0
(DIL)	0
R. VOLUME (R3)	50
(DIL)	0
LINEARITY LIMIT (%)	0
	0
(ABS)	0
	0
PROZONE LIMIT	0
	0
(POINT)	
	INSIDE
(Minimum rate of Total)	0
(Minimum margin rate)	0
ABSORBANCE LIMIT	32000
	INCREASE
CELL DETERGENT	DETERGENT1
STIRRING LEVEL	1

## &lt;&lt; CALIBRATION &gt;&gt;

CALIBRATION METHOD	SPLINE
CALIBRATION POINT	6
SPAN POINT	6
WEIGHT (CALIB. PRIORITY NUM.)	0
UPDATE TYPE (POINT)	NONE
SD LIMIT	999
DUPLICATE LIMIT (%)	99
DUPLICATE LIMIT (ABS)	32000
SENSITIVITY LIMIT (LOW)	-99999
SENSITIVITY LIMIT (HIGH)	99999
S1 ABS (LOW)	-32000
S1 ABS (HIGH)	32000
AUTO MASKING	NO
CHANGE OVER (To LOT) (To CASSETTE)	CANCEL
AUTO CALIB. TIMEOUT	YES
LOT CALIB. (TIMEOUT) (DAYS)	CANCEL
CASSETTE CALIB. (TIMEOUT) (DAYS)	CANCEL
AUTO CALIB. QC VIOLATION (METHOD)	NO BLANK
(RULE)	1s
(CONTROL1)	0
(CONTROL2)	0
(CONTROL3)	0

## &lt;&lt; RANGE &gt;&gt;

UNIT	mg/L
REPORT NAME	cCRP*
DATA MODE	ACTIVE
AUTOMATIC RERUN	YES** / NO
TECHNICAL LIMIT (LOW)	10
TECHNICAL LIMIT (HIGH)	300
REPEAT LIMIT (LOW)	-99999
REPEAT LIMIT (HIGH)	999999
CONTROL INTERVAL	0
L	0
H	0
I	0

<< OTHERS >>		
STD(1)	(CALIB. CODE)	920*
	(CONCENTRATION)	0.0***
	(POSITION)	S024*
	(VOLUME)	2.0
		0.0
		0
STD(2)	(CALIB. CODE)	921*
	(CONCENTRATION)	8.5***
	(POSITION)	S025*
	(VOLUME)	2.0
		0.0
		0
STD(3)	(CALIB. CODE)	922*
	(CONCENTRATION)	28.7***
	(POSITION)	S026*
	(VOLUME)	2.0
		0.0
		0
STD(4)	(CALIB. CODE)	923*
	(CONCENTRATION)	75.8***
	(POSITION)	S027*
	(VOLUME)	2.0
		0.0
		0
STD(5)	(CALIB. CODE)	924*
	(CONCENTRATION)	150***
	(POSITION)	S028*
	(VOLUME)	2.0
		0.0
		0
STD(6)	(CALIB. CODE)	925*
	(CONCENTRATION)	300***
	(POSITION)	S029*
	(VOLUME)	2.0
		0.0
		0

\*Defined by user. (Suggestion/example provided.)

\*\*If YES, make sure the automatic rerun function is activated system-wide. Consult the operator's manual on how to do this.

\*\*\*Lot dependent. Find the relevant calibrator values in the Analytical Value Sheet provided with the calibrator kit.