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Gentian Canine CRP Immunoassay

Application Note for Siemens ADVIA* 1800/1650

Intended Use

The canine CRP immunoassay on Siemens ADVIA 1800/1650 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 Siemens ADVIA 1800/1650 is 10 - 300 mg/L, with a security zone up to 1000 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 • R1 Reaction buffer (45 ml) • R2 Immunoparticles (10,5 ml)	REF 1501 REF 1507* REF 1514*
Gentian Canine CRP Calibrator Kit (6 levels, 0,5 ml per level)	REF 1551
Gentian Canine CRP Control Kit • Control low (0,5 ml) • Control high (0,5 ml)	REF 1519 REF 1520* REF 1521*

All materials are ready for use.
**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 Siemens ADVIA 1800/1650. Recalibration every 4th 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 ADVIA.

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.

Reagent Preparation

The reagents provided are ready for use. Mix the reagents gently before placing them into the assigned reagents positions. The bottles provided can be used directly on Siemens ADVIA 1800/1650.

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 with the 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 Siemens ADVIA 1800/1650

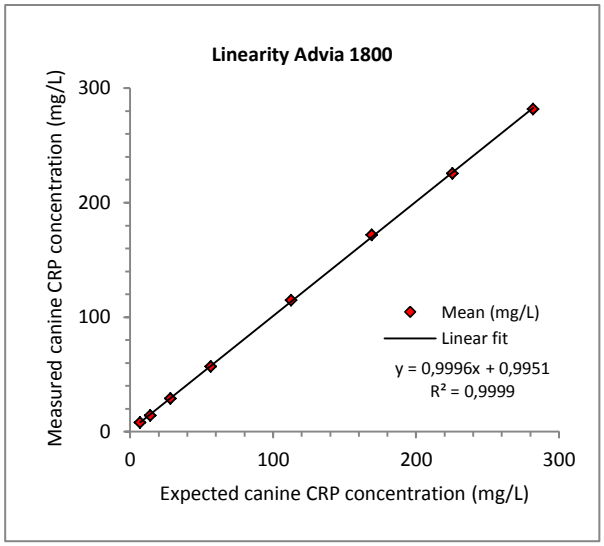
Lower Quantification Limit

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 5,4 mg/L had a total error of 17 % with the Gentian canine CRP method. The LoQ of the Gentian canine CRP immunoassay on Siemens ADVIA 1800/1650 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. Based on interpolation, the assay is considered satisfactory linear in the range 10 - 300 mg/L.

Dilution factor	Expected concentration (mg/L)	Measured concentration (mg/L)	Recovery (%)
100 %	281,9	281,9	100,0
80 %	225,5	225,7	100,1
60 %	169,1	172,0	101,7
40 %	112,8	114,8	101,8
20 %	56,4	57,1	101,3
10 %	28,2	28,9	102,5
5 %	14,1	14,2	100,4
2,5 %	7,1	8,1	114,9



Imprecision

Three samples were assayed in triplicate in three runs (with recalibration in between) on the same day.

Sample ID	Mean (mg/L)	Between run CV (%)	Within run CV (%)	Total CV (%)
Low	23,3	3,41	1,30	3,65
Medium	73,2	1,53	0,71	1,69
High	164,4	1,37	0,54	1,47

Interference

Canine CRP samples of about 30 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% differences between test sample and control sample.

	Control sample (mg/L)	Test sample (mg/L)	Recovery (%)
Hemoglobin (5 g/L)	29,03	28,93	99,7
Intralipid (10 g/L)	29,17	29,70	101,8

Security Zone

Samples with a canine CRP content up to 1000 mg/L return a value above 300 mg/L (upper limit of linearity range), and can be sent to automatic diluted rerun.

Instrument Variation

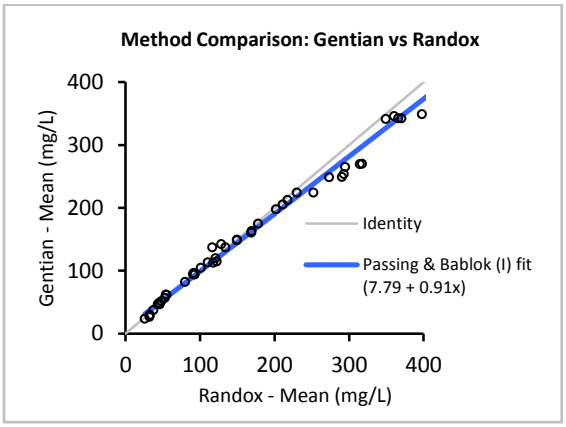
Nine samples were assayed on both Beckman Coulter AU400 and Siemens ADVIA 1800 with the Gentian canine CRP method. The recovery of the ADVIA 1800 values to the AU400 values was calculated.

Measured mean concentration AU400 (mg/L)	Measured mean concentration ADVIA 1800 (mg/L)	Recovery (%)
26,5	27,0	101,6
177,5	182,4	102,8
156,2	159,4	102,0
65,7	64,6	98,4
40,4	39,8	98,6
57,4	57,0	99,3
198,2	205,0	103,4
61,9	61,7	99,7
73,9	73,1	99,0
	Mean recovery (%)	100,5

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 — Lot number

Temperature limit

Use by date

Consult instructions for use

Manufacturer

REF — Catalogue number

Manufacturer

Gentian AS,
Bjornasveien 5
N-1509 Moss, Norway
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http://www.gentian.no

Distributor

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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 Advia 1800

Analytical conditions:

R1 volume:	135.0
R2 volume:	35.00
R1 dilute volume:	0.000
R2 dilute volume:	0.000
Sample vol. (Serum):	5.00
Dil. sample vol. (Serum):	30.0
Diluent vol. (Serum):	120.00
Diluent pos. (Serum):	C-0
Dilution type (Serum):	Standard
Reaction time:	10 min
R1 stir:	Weak
R2 stir:	Weak

Sub parameters:

Test name:	GCCRP*	*Defined by user.
Digit:	1	
Wavelength (main/sub):	596 / ---	
Method:	EPA	
Cal. type:	MSTD	
Qual. judge:	Not do	

Reanalysis conditions:

Sample vol. (Serum) (u/d):	1.25 / 25.0
Dil. sample vol. (Serum) (u/d):	0.00 / 0.00
Diluent vol. (Serum) (u/d):	0.000 / 0.000
Diluent pos. (Serum) (u/d):	C-0 / C-0
Diluent type (Serum) (u/d):	None / None

Standard settings:

FV:	1.00000
Abnormal (Serum) (H/L):	300.0 / 10.0

Calculation method settings:

Main DET.P l – m – n:	0 – 97 – 98
Sub DET.P p – r:	50 – 51
ABS. limit:	0.003
Variance:	10.00
Prozone type:	Not do
Prozone limit:	9.999
Prozone dir.	Upper
Judge limit:	9.999
Main DET.P m – n:	0 – 0
Sub DET.P p – r:	0 – 0

Calculation method settings / Reaction rate method:

Cycle:	3
Factor:	3.0
Reaction type:	Inc.
Max limit:	3.500
E2 corr.:	Not do
Blank (u/d):	9.9999 / -9.9999
Sample (u/d):	9.9999 / -9.9999
Check D.P. I:	0

Calculation method settings / Endpoint method:

Rerun ABS. (u/d)	1.06* / -9.999	* To be set by user for each lot and at each calibration.

Rerun conditions:

Flag (*):	No mark, no rerun
Absorbance (U):	With mark, rerun (U condition)
Absorbance (D):	With mark, no rerun
Absorbance limit (u):	With mark, rerun (U condition)
Absorbance limit (d):	With mark, no rerun
Cell Blank (N):	No mark, no rerun
Abnormal value (H):	With mark, rerun (U condition)
Abnormal value (L):	With mark, no rerun
Normal value (h):	With mark, no rerun
Normal value (l):	With mark, no rerun
Reagent shortage (r):	No mark, no rerun
Overflow (/):	No mark, no rerun
Safety (S):	No mark, no rerun
Prozone (P):	No mark, no rerun
Effect.nbr.o.pnts (n):	No mark, no rerun
Calibration (C):	No mark, no rerun
Rerun (R):	With mark, no rerun
Maximum ABS. limit (K):	With mark, rerun (U condition)
Calibration range High (j):	With mark, rerun (U condition)
Calibration range Low (k):	With mark, no rerun
Clot error (A):	With mark, no rerun
Mix error (M):	With mark, no rerun
LL Sensor error (Q):	With mark, no rerun
Crash (G):	With mark, no rerun
Temperature error (F):	With mark, no rerun
Calib. mismatch (c):	With mark, no rerun

Serum normal value and Formatted range for report:

Use default values.

Multipoint cal. settings:

Formula:	Spline correction
BLANK:	Blank is zero
Axis convert:	No convert
FV 0 / BLK:	See analytical value sheet standard 1
FV 1:	See analytical value sheet standard 2
FV 2:	See analytical value sheet standard 3
FV 3:	See analytical value sheet standard 4
FV 4:	See analytical value sheet standard 5
FV 5:	See analytical value sheet standard 6
Dilute method:	Standard
Dilute method 1:	Standard
Dilute method 2:	Standard
Dilute method 3:	Standard
Dilute method 4:	Standard
Dilute method 5:	Standard
Dil. sample vol.:	30.0
Dil. sample vol. 1:	30.0
Dil. sample vol. 2:	30.0
Dil. sample vol. 3:	30.0
Dil. sample vol. 4:	30.0
Dil. sample vol. 5:	30.0
Diluent vol.:	120.0
Diluent vol. 1:	120.0
Diluent vol. 2:	120.0
Diluent vol. 3:	120.0
Diluent vol. 4:	120.0
Diluent vol. 5:	120.0

Reagent blank settings:

Max. rep. dev.:	9.99999
Min. no of reps:	1
Max. RBL:	9.9999
Min. RBL:	-9.9999
Max. RBL dev.:	9.9999

Multi-standard settings:

Curve type:	Increasing
Max. rep. dev.:	9.99999
Max. rep. dev. 1:	9.99999
Max. rep. dev. 2:	9.99999
Max. rep. dev. 3:	9.99999
Max. rep. dev. 4:	9.99999
Max. rep. dev. 5:	9.99999
Min. no. of reps:	1
Min. ABS. delta Lhi - Llow:	0.0000
Max. fit dev.:	99999.99
Max. fit dev. 1:	5.00
Max. fit dev. 2:	10.00
Max. fit dev. 3:	20.00
Max. fit dev. 4:	30.00
Max. fit dev. 5:	60.00
Max. RMS of fit:	99999999.00