v02-Jan2014

Gentian Canine CRP Immunoassay

Application Note for ABX Pentra* 400

Intended Use

The canine CRP immunoassay on Horiba Medical's ABX Pentra 400 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 recommended measuring range of the Gentian canine CRP immunoassay on ABX Pentra 400 is 15 - 300 mg/L, with a security zone up to 700 mg/L.

Normal Values

Healthy dogs have CRP concentrations <15 mg/L with the Gentian canine CRP method on ABX Pentra 400. 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 ABX Pentra 400. 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 Pentra 400.

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 ABX Pentra 400.

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 ABX Pentra 400

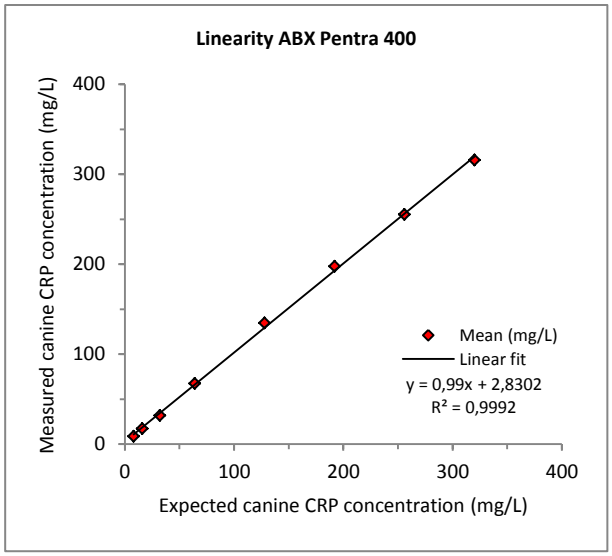
Lower Quantification Limit

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 7,7 mg/L had a total error of 18 % with the Gentian canine CRP method. The LoQ of the Gentian canine CRP immunoassay on ABX Pentra 400 is set to 8 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 %	320,1	315,7	98,6
80 %	256,1	255,4	99,7
60 %	192,1	197,8	103,0
40 %	128,0	134,5	105,1
20 %	64,0	67,4	105,2
10 %	32,0	32,0	99,9
5 %	16,0	17,3	108,3
2,5 %	8,0	8,6	107,9



Imprecision

Four samples (A-D) were assayed over eight days on an ABX Pentra 400 analyzer. Four other samples (E-H) were assayed on another Pentra 400 instrument. Each sample was measured in duplicate, twice a day.

Sample ID	Mean (mg/L)	Within run CV (%)	Between run CV (%)	Between day CV (%)	Total CV (%)
A	25,4	7,43	0[1]	3,24	8,10
B	63,8	2,08	1,65	2,97	3,98
C	121,1	1,34	1,56	2,61	3,32
D	257,8	0,76	0,41	2,00	2,18
E	7,2	12,13	7,84	6,14	15,7
F	58,4	1,85	2,14	4,57	5,38
G	103,9	0,85	0,88	1,79	2,16
H	272,1	0,68	1,83	1,05	2,22

1: According to statistical convention and EP5-A2, vol 24, No 25, the CV is set to zero, if a negative value is returned.

Interference

Canine CRP samples of about 30 mg/L were spiked with 10 g/L hemoglobin, 10 g/L Intralipid or 800 mg/L bilirubin. The samples were compared to corresponding control samples. None of the spiked samples demonstrated clinically significant interference – defined as more than 10 % difference between test sample and control sample.

	Control sample (mg/L)	Test sample (mg/L)	Recovery (%)
Hemoglobin (10 g/L)	29,0	29,1	100,5
Intralipid (10 g/L)	27,9	28,2	101,2
Bilirubin (800 mg/L)	28,7	30,4	105,8

Security Zone

Samples with a canine CRP concentration up to 700 mg/L return a value above 300 mg/L (upper limit of linearity range) and can be sent to automatic diluted rerun.

Analytical Recovery

The analytical recovery of the canine CRP assay was determined by adding fixed amounts of canine CRP to samples and calculating the recovery from theoretical concentrations.

Sample ID	Expected concentration (mg/L)	Measured concentration (mg/L)	Recovery (%)
1	64,0	63,8	99,7
2	124,8	129,0	103,4
3	187,8	186,6	99,4

Instrument Variation

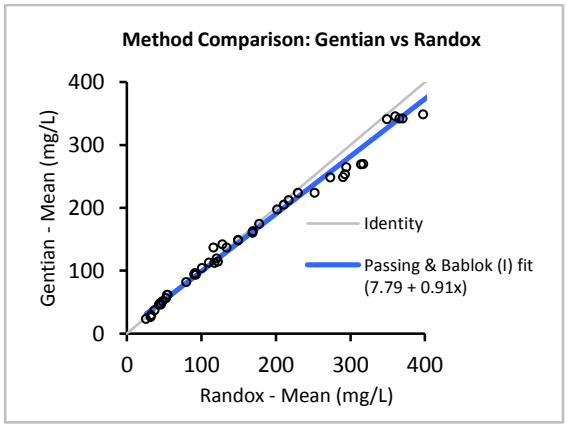
A total of 32 samples was assayed on both ABX Pentra 400, Abbott Architect c4000 and Beckman Coulter AU400 with the Gentian canine CRP immunoassay. A statistical analysis of the measured instrument variation was performed.

Instrument Variation – Pentra 400 vs. Architect c4000	
Bland Altman bias	1,4 %
Bland Altman 95 % limits of agreement	-8,2 % to 5,3 %
Passing Bablok slope	0,96
Passing Bablok intercept (mg/L)	2,36
Instrument Variation – Pentra 400 vs. AU400	
Bland Altman bias	-3,6 %
Bland Altman 95 % limits of agreement	-4,1 % to 11,2 %
Passing Bablok slope	1,01
Passing Bablok intercept (mg/L)	2,06

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 limitations
	Expiration date
	Consult instructions for use
	Manufacturer
REF	Catalogue number

Manufacturer

Gentian AS,
Bjornasveien 5
N-1509 Moss, Norway
TEL: +47 99 33 99 05
FAX: +47 69 24 09 62
http://www.gentian.no

Distributor

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 ABX Pentra 400

General Parameters

Characteristics:

Sample type:	Serum / Plasma
Number of reagents:	2

Reagent:

Reagent short name:	C-CRP*
Reagent number:	999*
On board stability:	---

☐ Cassette

Automatic Rerun:

☐ Post dilution:	---
☐ Post concentration:	---

Results:

Decimal positions:	1
☐ Manual patient validation	
☐ Pre-dilution	
Diluent Name:	
Factor:	---
Incubation time (cycles):	---

☑ **Linearity:** **Correlation:**

Low limit:	High limit:	Slope:	Intercept:
15.0	300.0	1.00	0.00

☐ **Delta Check:**

Delta check validity:	Absolute variation:	Relative variation:
---	---	---

**Defined by user.*

Reference Check (mg/L):

		Man / Default:	Women:	Child:
☐	Low:	---	---	---
☐	High:	---	---	---

Rerun Range (mg/L):

		Man / Default:	Women:	Child:
☐	Low:	---	---	---
☐	High:	---	---	---

Calibration Parameters

☐ **Pre-dilution:**

Type:	Calibrator diluent:
---	---

Factor 1	**Factor 2**	**Factor 3**	**Factor 4**
---	---	---	---
Factor 5	**Factor 6**	**Factor 7**	**Factor 8**
---	---	---	---

Checks:

☐ Reagent limit absorbance check

Reagent range low: ---

Reagent range high: ---

☐ Reagent blank limit absorbance check

Blank range low limit: ---

Blank range high limit: ---

☐ **Control Required**

☐ Control used 1: ---

☐ Control used 2: ---

☐ Control used 3: ---

Validity Backup:

☐ Backup time frame without calibration required

Interval: 30 Time unit: Day

Calibration:

Calibration mode:	Logit / Log5
Level:	6
Calibration factor:	---
Run(s):	2
☑ Dev_rep. (%):	10.0
☐ Dev_c. (%):	---
Calibrator used:	C-CRP*

Validity:

☑ On request

☐ Time validity

Interval: ---

Time unit: ---

Factor Calibration:

☐ Low limit check: ---

☐ High limit check: ---

☐ Relative variation check: ---

Analysis Parameters

☐ **Cleaner:**

Cleaner solution:

☐ H_2O

☐ Before ☐ After

**Defined by user.*

Wavelength (nm):

Primary wavelength: 600

Secondary wavelength: ---

Blank:

☑ Reagent blank

Diluent:

H_2O

Analysis Sequence:

Cycle:	Reagent needle:	Volume (µL):	Sample needle:	Volume (µL):	H_2O volume (µL):
1	R1	270.0	SAMPLE	3.0	10.0
19	R2	75.0	---	---	---
---	---	---	---	---	---
---	---	---	---	---	---

Mixing Speed: 80

Test Name: C-CRP* **Channel:** 999* **Code:** C-CRP* **Local Code:** C-CRP*

☑ Enable Modified on: N/A

Calculation Parameters

Correlation Factor:

Slope: 1.000

Intercept: 0.000

☐ **Sample Limit Check:**

Sample limit (Δ OD): ---

Sample limit cycle: ---

Reaction Direction:

☑ Reaction direction check

Reaction direction: Increase

☐ **Antigen Excess Check:**

Antigen excess limit (%): ---

Antigen excess point: ---

☑ **Definition Step A:**

Calculation type: End point

First reading (cycle): 21

Last reading (cycle): 42

☐ **Reaction Limit Check:**

Reaction limit absorbance: ---

Cycle: ---

OD Deviation Check:

☐ Linear regression ☐ First point ☐ Last point

r^2 threshold SD First point threshold SD Factor

☐ **Definition Step B (N/A)**

Calculation type: End point

First reading (cycle): ---

Last reading (cycle): ---

☐ **Reaction Limit Check**

Reaction limit absorbance: ---

Cycle: ---

OD Deviation Check:

☐ Linear regression ☐ First point ☐ Last point

r^2 threshold SD First point threshold SD Factor

☐ **Definition Step C (N/A)**

Calculation type: End point

First reading (cycle): ---

Last reading (cycle): ---

☐ **Reaction Limit Check**

Reaction limit absorbance: ---

Cycle: ---

OD Deviation Check

☐ Linear regression ☐ First point ☐ Last point

r^2 threshold SD First point threshold SD Factor

☐ **Definition Step D (N/A)**

Calculation type: End point

First reading (cycle): ---

Last reading (cycle): ---

☐ **Reaction Limit Check**

Reaction limit absorbance: ---

Cycle: ---

OD Deviation Check:

☐ Linear regression ☐ First point ☐ Last point

r^2 threshold SD First point threshold SD Factor

Formula

Unit Parameters

Unit:	Conversion Factor:
mg/L	1.0000
---	---
---	---

Unit:	Conversion Factor:
mg/L	1.0000
---	---
---	---