IVD TECHNOLOGIES

Brucella canis Antibody Test Kit

FOR DETECTION OF IgG ANTIBODY IN CANINE SERUM OR PLASMA

Product Number CBRC-1000

CANINE
BRUCELLA CANIS

INTENDED USE

The Brucella canis Antibody Test Kit is used for the detection of Brucella canis IgG antibody in canine plasma and serum.

ANALYTICAL PRINCIPLE

Brucella canis IgG-specific antibody in diluted samples is allowed to bind to microwell-bound Brucella canis antigen. After washing unbound materials, HRP-conjugate is allowed to bind to the Brucella canis IgG antibody-antigen complex. Unbound HRP-conjugate is washed away and TMB is allowed to react with bound HRP-conjugate. The reaction is stopped and the microwell is read. The intensity of the color produced in the HRP-TMB reaction is proportional to the amount of IgG-specific antibody in the sample.

SPECIMEN REQUIREMENTS

Serum and plasma samples are acceptable. Avoid repetitive freezing and thawing of samples.

REAGENTS

Precautions & Safety Notes

- WEAR LATEX GLOVES, FACE SHIELDS AND A LAB COAT WHEN HANDLING SPECIMENS AND OTHER HAZARDOUS REAGENTS
- FOR *IN VITRO* USE, POTENTIAL BIOHAZARDOUS MATERIAL. HANDLE ASSAY REAGENTS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT.
- The Sample Diluent, Positive Control, Negative Control, Cut-Off Calibrator, Wash Buffer, and HRP-Conjugate contain 0.1% ProClin 150. Avoid contact of these reagents with skin or eyes.

Kit Components

ID	Reagent	Part Number	Quantity
	Brucella canis Antibody Test Kit	**CBRC-1000**	**96 Test**
SD	Sample Diluent [Contains: PBS, BSA, 0.1% ProClin 150]	**SDFEA-1001**	**35 ml**
A	Negative Control [Contains: Normal Canine Serum, 0.1% ProClin 150] See QC Certificate for value	**CBRC-1001**	**100 µl**
B	Positive Control [Contains: Canine Serum Positive for Brucella canis IgG, 0.1% ProClin 150] See QC Certificate for value	**CBRC-1002**	**100 µl**
C	Microwell Plate [Contains: Brucella canis Antigen]	**CBRC-1003**	**96 Well Plate**
D	HRP Conjugate [Contains: HRP Conjugate, 0.1% ProClin 150]	**CBRC-1004**	**10 ml**
WB	20X Wash Buffer Concentrate [Contains: Tris Buffer, 0.01% Tween-20, NaCl, 0.1% ProClin 150]	**WBFEA-1001**	**50 ml**
TS	TMB Solution [Contains TMB] Keep away from light	**TMBS-1001**	**10 ml**
SS	Stop Solution [Contains: 1N Sulfuric Acid]	**SSFEA-1001**	**10 ml**

MATERIALS

The following materials are needed but not supplied.

- Variable Pipettors and Tips
- Stir Bar & Stirrer
- 12x75 mm disposable borosilicate glass culture tubes
- Test Tube Rack, polypropylene
- Vortexer
- Microwell Plate Film Sealer
- ELISA Plate Washer
- ELISA Plate Reader
- Refrigerator (for kit storage)

PROCEDURE PRECAUTIONS

- Bring reagents to room temperature before use.
- Use clean instruments & equipments.
- Unused microwells should be sealed tightly in the foil pouch until further use.
- Handle microwells with care.
- Minimize air bubbles in microwells.
- Microwells should not contain liquid after the washing steps. To ensure that liquids are removed from microwells after washing steps, microwells can be tapped against clean paper towels.

REAGENT PREPARATION

1X Wash Buffer Preparation

1. Add 1 unit of volume of 20X Wash Buffer Concentrate (**WB**) to 19 units of volume of DI water. For example, add **50 ml** 20X Wash Buffer Concentrate (**WB**) to **950 ml** of DI water.
2. Mix the Wash Buffer Preparation well.

PROCEDURE

1. Dilute samples, Negative Control (**A**), and Positive Control (**B**) by **1:25** by adding **10 µl** of samples, Negative Control and Positive Control to **240 µl** of Sample Diluent (**SD**) in glass tubes.
2. Vortex the tubes.
3. Dispense **100 µl** of the diluted samples, Negative Control, and Positive Control into designated wells of the Microwell Plate (**C**).
4. Cover the wells.
5. Incubate the wells for **30 minutes** at **room temperature**.
6. Wash each well **3 times** with **300 µl** of **1X Wash Buffer Preparation**. Tap microwells against clean paper towels to ensure no liquid remains.
7. Dispense **100 µl** of HRP Conjugate (**D**) to each well.
8. Cover the wells.
9. Incubate the wells for **30 minutes** at **room temperature**.
10. Wash each well **3 times** with **300 µl** of **1X Wash Buffer Preparation**. Tap microwells against clean paper towels to ensure no liquid remains.
11. Dispense **100 µl** of TMB Solution (**TS**) to each well.
12. Cover the wells.
13. Incubate the wells for **15 minutes** at **room temperature** and **protect from light**.
14. Dispense **100 µl** of Stop Solution (**SS**) to each well.

15. Read the wells in a microwell reader at **450 nm**.

PROCEDURAL NOTES

•Evaluation of samples should be based on the Sample to Negative Ratio.

EXPECTED VALUES

Cut off

Two hundred and fourteen (214) negative samples from normal dogs and 26 positive samples from dogs diagnosed with the disease were assayed with three lots of IVD Technologies Brucella canis Antibody Test Kit to determine the Cutoff OD value. ROC data analysis was used to determine Cutoff OD value. The Cutoff OD value was found to be 0.350.

Sample to Negative Ratio Calculation

Sample to Negative Ratio =
O.D. Sample / O.D. Negative Control

- The Sample to Negative Ratio is equal to the O.D. of the Sample over the O.D. of the Negative Control.

Sample to Negative Ratio Table

< 0.900	Negative
0.900 – 1.20	Borderline
> 1.20	Positive

CORRELATION ANALYSIS

Two hundred and eleven (211) dog serums were evaluated for Brucella canis antibody by a reference laboratory using the tube agglutination method and by IVD Technologies Brucella canis Antibody Test Kit. Thirty-five (35) samples, seven samples, and 168 samples were reported to be positive, borderline, and negative respectively by the reference laboratory. And 39 samples, four samples, and 168 samples were reported to be positive, borderline, and negative respectively by the IVD Technologies Brucella canis Test Kit.

Correlation Analysis Table

		Reference Laboratory			
		Positive	Borderline	Negative	**Total**
IVD Tech	Positive	35	1	3	**39**
	Borderline		4		**4**
	Negative		3	165	**168**
	Total	**35**	**7**	**168**	**211**

REPRODUCIBILITY

Intra-Assay

Five dog serum samples were assayed in quadruplicates in the same assay. The mean, standard deviation and CV are on the following table.

Inter-Assay

Five dog serum samples were assayed in five different assays. The mean, standard deviation and CV are on the following table.

	Intra-Assay			Inter-Assay		
ID	Mean	SD	CV	Mean	SD	CV
1	0.397	0.011	2.682	0.408	0.012	2.879
2	0.812	0.016	1.911	0.812	0.017	2.081
3	1.047	0.051	4.891	1.132	0.073	6.443
4	2.222	0.040	1.814	2.204	0.111	5.028
5	4.487	0.034	0.758	4.478	0.157	3.504

REFERENCES

- ADONE R., FRANCIA M., CIUCHINI F. (2008). *Brucella melitensis* B115-based complement fixation test to detect antibodies induced by *Brucella* rough strains.*J. Appl. Microbiol.* **105**, 567-574.
- Boeri E, Escobar G, Ayala S, Sosa-Estani S, Lucero N. Canine brucellosis in dogs in the city of Buenos Aires. Medicina (Buenos Aires) 2008; 68: 291-7.
- CARMICHAEL L.E., JOUBERT, J.C. (1987). A rapid slide agglutination test for the serodiagnosis of *Brucella canis*
- Carmichael L, Joubert J. Transmission of *Brucella canis* by contact exposure. Cornell Vet 1988; 78: 63-73.
- Carmichael L. *Brucella canis*. In: Nielsen K, Duncan JR, editors. Animal Brucellosis. Florida, USA., CRC Press Boca Raton, 1990, p. 336-50.
- Carmichael L, Shin S. Canine brucellosis: a diagnostician's dilemma. Seminars in Vet Med Surgery (Small Animal) 1996; 11: 161-5.
- DUNNE J., SEHGAL K., MCMILLAN A., PERRET L. (2002). Canine brucellosis in a dog imported into the UK. *Vet. Rec.* **15**, 247
- Corbel M. Brucellosis: an overview. Emerg Infect Dis 1997; 3: 213-21.
- Lucero N, Bolpe J. Buffered plate antigen test as a screening test for diagnosis of human brucellosis. J Clin Microbiol 1998; 36: 1425-7.
- Lucero N, Escobar G, Ayala S, Lopez G. Sensitivity and specificity of an indirect enzyme-linked immunoassay for the diagnosis of *Brucella canis* infection in dogs. J Med Microbiol 2002; 51 : 656-60.
- Mateau-de-Antonio E, Martín M, Soler M. Use of indirect enzyme-linked immunosorbent assay with hot saline solution extracts of a variant (M-) strain of *Brucella canis* for diagnosis of brucellosis in dogs. Am J Vet Res 1993; 54: 1043-6.

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