# VetLine

## Brucella ELISA

## (BRUVT0050)

Performance Characteristics

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# 1 Introduction

Brucella species are very small, gram-negative immobile bacteria (0.4 - 0.8 microns in diameter and 0.4 - 3.0 microns in length). They were named after the English military doctor Bruce Davis, who isolated the pathogen 1887 on Malta from the spleen of a deceased soldier with undulating fever.

Especially following four species are of importance:

| | |
|---|---|
| Brucella abortus | - causative agent of bovine brucellosis |
| Brucella melitensis | - causative agent of ovine and caprine brucellosis |
| Brucella suis | - causative agent of porcine brucellosis |
| Brucella canis | - causative agent of canine brucellosis |

The symptoms of the disease are similar in cattle, sheep, goats and pigs. Sheep seem to be less susceptible so less frequently abortion is observed in this species. After infection, brucellosis begins with a clinically normal phase which is often unnoticed. Later there are often joint inflammation, and sometimes mastitis. The main symptoms of brucellosis are abortions, premature births and birth of dead or weak offspring. Infected bulls can develop orchitis and epididymitis.
The pathogen is transmitted mainly by ingestion and mating. Infection by milk, urine and faeces is also possible. In livestock brucellosis can become epidemic.

Brucellosis is a transmitted disease to humans (zoonosis). Individuals in risk are especially shepherds, farmers, animal keepers, veterinarians and laboratory staff.

Infections may be diagnosed:

- By genus specific PCR
- Serology: Detection of antibodies by ELISA

# 2 Intended Use

The NovaTec VetLine Brucella ELISA is intended for the qualitative determination of antibodies against Brucella in veterinary serum, pooled serum samples and milk samples.

# 3 Principle of the Assay

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

# 4 Performance Characteristics

## *4.1 Reproducibility (Precision)*

**Test Description**

The reproducibility of the NovaTec Brucella ELISA kit was determined by comparing 12-24 replicates of 2 different samples in one assay (within-run) and by comparing 2 different samples assayed in 12 different runs (between-run).

In accordance with the procedures prescribed in the instruction, the mean ($\bar{x}$) and the standard deviation (s) of the absorbance values (E) were determined.

The CV value [%] was then calculated using the following formula:

$$CV = s/\bar{x} \times 100\ \%$$

Acceptance Criterion: CV < 15 %

**Results**

Table 1: Within-Run Precision

| Sample | n | Mean (E) | CV [%] |
|---|---|---|---|
| #1 | 24 | 0.577 | 4.14 |
| #2 | 24 | 1.276 | 3.34 |
| #3 | 24 | 1.200 | 2.75 |

Table 2: Between-Run Precision

| Sample | n | Mean (NTU) | CV [%] |
|---|---|---|---|
| #1 | 12 | 23.22 | 4.97 |
| #2 | 12 | 20.13 | 6.05 |
| #3 | 12 | 5.10 | 8.55 |

**Conclusion**

The acceptance criterion was met for all samples.

## 4.2 Analytical Specificity

### 4.2.1 Interference from Hemoglobin, Bilirubin and Triglycerides

#### Introduction

Increased concentrations of possible interference materials such as bilirubin, triglycerides and hemoglobin may interfere with immunoassays creating false-negative or false-positive results.
In order to investigate this topic a literature research in combination with investigation of nearly 100 parameters were performed.

#### Material and Test Condition

Different members of the NovaLisa® and VetLine ELISA line were used including assays for the detection of different antibody isotypes (IgA, IgM, IgM, IgM + IgM, total antibody with protein A/G) to bacteria, viruses, fungi, parasites and worms as well as an antigen ELISA for the detection of TSH, Dirofilaria and FeLV.

Defined positive resp. negative or equivocal samples were used.

A certain amount of the potentially interfering substance was added to each sample. The final concentration of each substance was in a pathological range as also described by competitors (10 mg/ml hemoglobin, 0.5 mg/ml bilirubin and 5 mg/ml triglycerides). The results obtained with the sample with added “interfering substance” should be 60-140 % of the result of the untreated sample in order to fulfil the specifications.

#### Conclusion

The internal specifications of 60-140 % were always fulfilled.
Interferences with hemolytic, lipemic or icteric samples were not observed up to a concentration of 10 mg/ml hemoglobin, 0.5 mg/ml bilirubin and 5 mg/ml triglycerides.
These results are also in agreement with literature data.

This conclusion is valid for the NovaLisa® as well as for the VetLine version of the assays.

*Dimeski, G. (2008) Interference Testing, Clin Biochem Rev 29, S43 – S48*
*Tate, J. and Ward, G. (2004) Interferences in Immunoassay, Clin Biochem Rev 25, 105 – 120*

## 4.3 Diagnostic Sensitivity and Specificity

### Introduction

To evaluate the diagnostic performance of the VetLine Brucella ELISA, external and internal studies were conducted in comparison to predetermined samples. Samples are of bovine and porcine origin.

### Materials

VetLine Brucella ELISA Lot: BRUVT-071-1

Production date: 2015-06 Expiry date: 2016-06-30

9 positive samples (5 porcine; 4 bovine)

31 negative samples (7 porcine; 24 bovine)

### Results

Total number of bovine and porcine samples: 40

| | Demand | | | |
|---|---|---|---|---|
| | | positive | negative | **Σ** |
| VetLine Brucella ELISA | positive | **9** | **0** | **9** |
| | negative | **0** | **31** | **31** |
| | **Σ** | **9** | **31** | **40** |

(Equivocal results were not included in the calculations)

Diagnostic Sensitivity: 100 % (95% confidence interval: 63.37 % - 98.62%)

Diagnostic Specificity: 100 % (95% confidence interval: 88.78 % - 98.62%)

Agreement: 100 % (40/40)

### Conclusion

For porcine and bovine samples the diagnostic sensitivity and the diagnostic specificity was 100 % (agreement: 100 %).