VetLine

Coxiella ELISA

(COXVT0600)

Performance Characteristics

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1 Introduction

Q-fever is caused by an infection with *Coxiella burnetii*, a small (0.3 - 0.7 microns), pleomorphic, Gram-negative bacterium.

Coxiella burnetii exists in 3 different forms:

SCV: small cell variant, small cells which are highly infectious
LCV: large cell variant, the cells are less infectious
SLP: spore-like particles with high environmental resistance. They can be infectious for years

Q-fever is a zoonotic disease that occurs worldwide with the exception of New Zealand and the Antarctic.
Reservoir animals are especially ruminants (sheep, goats, cattle) and ticks. Even pets like cats and dogs, as well as wildlife animals and ducks can be infected.
Transmission occurs primarily indirectly through inhalation of contaminated aerosols, but also directly by contact with infected organs or secretions (milk, feces, urine) of animals.
In ruminants, an infection often leads to epidemic abortions. During childbirth, large amounts of the agent are excreted.
Of particular importance in the transmission of *Coxiella burnetii* is the infestation of sheep with infected tick. The strong pathogen loaded, dried tick faeces in the fleece of the sheep is a high risk of infection.
The disease occurs in two variants, the acute and chronic phase. During the acute phase antibodies against the Phase 2-antigen are formed. High antibody titers against Phase 1-antigens typically occur within the chronic phase.
In humans, acute infection is connected with high fever, chills, muscle pain and headache. In the chronic phase organ manifestations such as endocarditis, osteomyelitis and hepatitis can occur.
Vulnerable persons are mainly veterinary staff, butchers, farmers and laboratory personnel.

Acute phase of Q-Fever

IgM specific to phase 2 after 2-3 weeks

IgG approximately 2 month after infection

Chronic phase of Q-Fever

From 6 weeks up to 4 month after infection phase 1 IgG- and IgA antibodies can be detected.

Infections may be diagnosed by:

- Complement binding reaction is still uses
- IFT (immuno fixation test)
- ELISA
- Cell culture
- PCR

2 Intended Use

The NovaTec VetLine Coxiella ELISA is intended for the qualitative determination of antibodies against Coxiella in veterinary serum.

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 VetLine Coxiella ELISA kit was determined by comparing 12-24 replicates of at least 2 different samples in one assay (within-run) and by comparing at least 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	0.303	6.75
2	24	0.601	7.03
3	24	1.380	11.19

Table 2: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
1	12	21.41	4.84
2	12	47.55	6.23
3	12	2.13	8.84

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 Novatec 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

4.2.2 Cross-Reactivity

Cross reactions cannot be excluded.

4.3 Diagnostic Sensitivity and Specificity

Introduction

The internal studies of the VetLine Coxiella ELISA with well defined samples from Biocheck (Germany), ID.vet (France) and The National Institute for Biological Standards and Control (UK) were conducted by NovaTec.

The external study was conducted at Biomedica (Austria), IDSA-Institutul de Diagnostic şi Sănătate Animală (Romania), and an accredited laboratory in Germany with well defined samples.

Materials

NovaTec VetLine Coxiella ELISA

79 positive bovine samples

199 negative bovine samples

Results

Total number of samples: 278

Table 3: Diagnostic Sensitivity and Specificity

		Demand		
		positive	negative	**Σ**
NovaTec VetLine Coxiella ELISA	positive	76	11	**87**
	negative	3	188	**191**
	Σ	**79**	**199**	**278**

Diagnostic Sensitivity bovine: 96,20 % (95% confidence interval: 89,3 % - 99,21 %)

Diagnostic Specificity bovine: 94,47 % (95% confidence interval: 90,32 % - 97,21 %)

Agreement 94,96 % (264/278)

Conclusion

The diagnostic sensitivity bovine is 96,20 % and the diagnostic specificity bovine is 94,47 % (agreement: 94,96 %).