Technical sheet

- ELISA monowell test
- Coating : culture lysate of *Besnoitia besnoiti*

- Sample : serum
- Dilution : 1/2

- Reading wavelength : 450nm
- Incubation time : 2h30
- Substrate : Single component TMB

Sensitivity

97,5% of animals tested positive in individual

99,5% of animals tested positive in pools of 10

Specificity

99,5% of animals tested negative in individual

100% of animals tested negative in pools of 10

This test can be used for diagnostic and screening purposes due to its exceptional performances

To place an order

Reference	Designation	Number of reactions
BIO K 466/2	Monoscreen AbELISA Besnoitia besnoiti / blocking, monowell	2 plates / 192 tests

Instructions for use and handling conditions: see instructions and MSDS (available at www.biox.com)

About AnalysiScreen

OBTAIN YOUR RESULTS IN A FEW CLICKS

AnalysiScreen™ is the new application to read and interpret all ELISA plate types Monoscreen™ and Multiscreen™.

AnalysiScreen™ is :

- Available on our website : https://www.biox.com
- Updated in real time
- Compatible with all plate formats of Bio-X Diagnostics
- Very easy to use

SCAN ME

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MONOSCREEN Ab ELISA

NEW

MonoScreen AbELISA - *Besnoitia Besnoiti*

Reference : BIO K 466/2

Validation in pools of 10

BIO-X Diagnostics

NEW

MONOSCREEN Ab ELISA - *Besnoitia besnoiti*

Update on the disease

Besnoitia besnoiti is an intracellular protozoan strictly responsible for bovine Besnoitiosis, often called «cattle anasarca». The disease mainly affects young cattle. Besnoitiosis is epizootic in the south of France, but it is now widespread in Africa, Asia and southwestern Europe. Given global warming and animal transfers, the disease is gradually moving north (first cases in Belgium in 2021, for example). The preferred route of transmission is transcutaneous, by biting insects (tabanids, stomoxes).

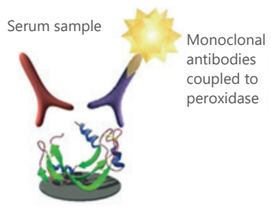
During the infection, an incubation phase of 3 to 6 days is followed by 3 successive clinical stages:

- A febrile stage of 3 to 7 days; the multiplication of tachyzoites in the endothelial cells of blood vessels causes hyperthermia in the animal.
- A second phase of 1 to 2 weeks; bradyzoite cysts generate subcutaneous edema.
- A chronic phase lasting several months, characterized by alopecia and scleroderma. The skin then becomes clearly thickened and wrinkled, and parasitic cysts are seen on the conjunctiva and sclera. This final phase generally leads to the death of the animal or to its euthanasia.

Bio-X was a pioneer in developing a PCR method which has the advantage of detecting infected animals in the very early febrile phase, by detecting tachyzoites in blood monocytes. A new diagnostic phase is now made possible for the detection of antibodies specific to Besnoitia besnoiti in the chronic phase by the ELISA test developed according to ANSES specifications..

Monoscreen AbELISA *Besnoitia besnoiti* *allows the detection of antibodies specific to Besnoitia besnoiti (IgG, IgM) in competition*

The test uses 96-well microtitration plates sensitised with a culture lysate of *Besnoitia besnoiti*. The operator transfers the previously diluted test serums into the microplate's wells. After 120 minutes of incubation and a rinse step, the operator adds the conjugate, which is a specific monoclonal antibody against *Besnoitia besnoiti* coupled to peroxidase. After incubating and washing the preparation, the operator adds the tetramethylbenzidine chromogen (TMB).



Monoscreen AbELISA *Besnoitia besnoiti* *has been validated according to ANSES specifications*

■ Interpretation methods and interpretation thresholds

Cut off at 40% based on the analysis of 150 serums

■ Analytical sensitivity- **100% positive**

LOD serum tested in 10 replicates per series, on two independent plates

■ Analytical specificity - **100% negative**

Serums from 14 *Neospora Caninum* seropositive cattle and 5 *Toxoplasma Gondii* seropositive cattle

■ Coherence of the dose-effect relationship - **Linearity**

2 series of at least 4 dilution levels of 2 to 2 covering the zone of linearity on 2 different plates

■ Intra-assay repeatability - **CV* = 6,34%**

*Coefficient of Variation ≤ 10% (3 plates tested entirely with a weak positive)

■ Intra-laboratory reproducibility - **CV* = 9,7%**

*Coefficient of Variation ≤ 15% (3 levels of dilution of the same sample located in the linear range analyzed in triplicate on 6 separate series by at least 2 operators on several different days)

■ Inter-laboratory reproducibility - **CV* < 8,93%**

*Coefficient of Variation ≤20%. The raw data from the labs (3 levels of dilution of 3 serums: strong, weak (close to LOD) and negative) are blindly analyzed 4 times by 5 different laboratories.

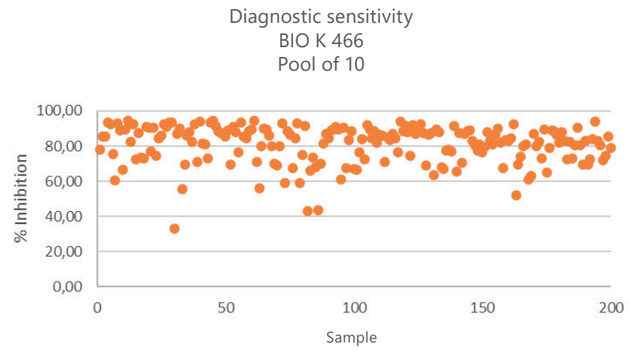
■ Control of robustness - **CV* = 6,6%**

Control under extreme conditions of temperature, time, washing (manual versus automatic) on 3 samples (strong, weak and negative) in duplicate.

French NRL approval for individual and pool

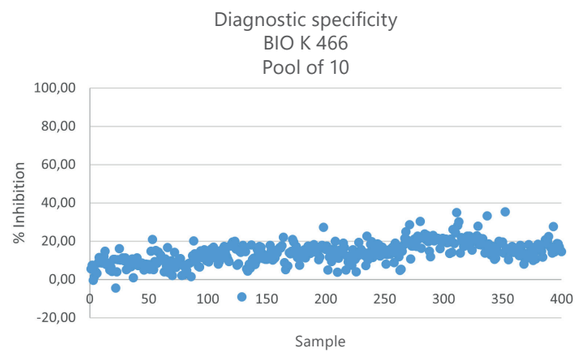
■ Diagnostic sensitivity : **99,5% of positive animals**
201 pools analytically and epidemiologically qualified from infested animals from 12 herds geographically representative of the national territory0

BIO K 466 - Diagnostic sensitivity



■ Diagnostic specificity: **100% of negative animals**
400 pools from several herds from European countries that have not recorded an outbreak of Besnoitiosis

BIO K 466 - Diagnostic specificity



A unique Cut-off of 40%

99,5% of diagnostic sensitivity in pools of 10

100% of diagnostic specificity in pools of 10

Additional validation tests

Validation on the LOD at 1/ 10th (92 repetitions)

	% Inh	Standard deviation	%CV
Whole	62,53	2,48	3,97
Edges	61,94	3,25	5,25
Center	62,85	1,91	3,04

Testing for pool of 10

Criteria	Result
Sensitivity	99,5% positive
Specificity	100% positive

Simplified use protocol of the BIO K 466 kit

1
In each well, add:
- 50 µL dilution buffer 1x
- 50 µL sample/controls

35°C 95°F · 120 min · 3X Wash

2
In each well, add :
- 100 µL diluted conjugate solution

35°C 95°F · 30 min · 3X Wash

3
In each well, add:
- 100 µL chromogen solution

18°C 64°F · 10 min

4
In each well, add:
- 50 µL stop solution

450 nm — *Recording of Optical Densities (OD)*