



To WOA
Biological Standards Commission

Institute for Bacterial Infections and
Zoonoses (IBIZ)
Head: Professor Dr. Heinrich Neubauer

Department: WOA RL Glanders
Responsible: Dr. Mandy Elschner
Phone: +49 3641-804-2428
Fax: +49 3641-804-2228
E-Mail: Mandy.elschner@fli.de

Datum: 20.03.2024

Report about inter-laboratory study to further validate serological diagnostic capacity
of ID Screen Glanders Double Antigen Multispecies ELISA (GLANDA ELISA) in
comparison with CFT

1. Background

The WOA-RL Glanders at the Friedrich Loeffler Institute (FLI) has been working for many years in an international network to improve serological glanders diagnostics. In 2011, the Western blot was established as a confirmatory test for positive CFT results (1) and attracted international interest and application in clarification tests. However, this test will always remain a sophisticated test. Later on, the results of international validation studies comparing alternative methods to CFT as a commercial test for glanders were published. In the first study funded by the WOA in 2019 (2), it was already shown that ELISA based on recombinant proteins produced comparable or better results than CFT in terms of sensitivity and specificity.

In a second study in 2021 (3), the commercial ID Screen Glanders Double Antigen Multispecies ELISA (IDvet, France) was validated and showed better sensitivity and specificity than CFT. In particular, the rapid and simple testing protocol qualifies the GLANDA-ELISA as a reliable method even for handling large number of samples in standard diagnostic laboratories.

In 2020, the FLI approved the test for glanders diagnostics in Germany, where it is subject to regular batch control by the FLI.

The WOA RL at FLI has suggested to WOA to accept this Double Antigen ELISA as test to identify infected animals with high confidence and to demonstrate the freedom from glanders in animals for movement. However, in addition to some samples from mules and donkeys, mainly samples from horses were tested, so that this test can only be considered sufficiently validated, for trade of horses.

As part of the revision of the glanders chapter of the manual, the WOA encouraged the FLI to organise a comparative laboratory study with interested countries. Depending on the results obtained, the test may then be incorporated into the manual.

(1) Elschner MC, Holger C, Scholz M, Peggy M, Rassbach A, Dietzsch M, Melzer F, Schmoock G, De Assis Santana VL, De Souza MM, Wernery R, WernerYU, Neubauer H (2011) Use of a Western blot technique for the serodiagnosis of glanders. *BMC Vet Res* 7, 4.

(2) Elschner MC, Laroucau K, Singha H, Tripathi BN, Saqib M, Gardner I, Saini S, Kumar S, El-Adawy H, Melzer F, Khan I, Malik P, Sauter-Louis C, Neubauer H (2019) Evaluation of the comparative accuracy of the complement fixation test, Western blot and five enzyme-linked immunosorbent assays for serodiagnosis of glanders. *PLoS One* 14(4), e0214963. doi: 10.1371/journal.pone.0214963.

(3) Elschner MC, Melzer F, Singha H, Muhammad S, Gardner I, Neubauer H (2021) Validation of a commercial Glanders ELISA as an alternative to the CFT in international trade of equidae. *Front Vet Sci* 8, 62.

2. Participants

Australia	Diagnosics and Avian Infectious Diseases Research Australian Centre for Disease Preparedness (formally AAHL) CSIRO Private Bag 24, Geelong, VIC 3220
Brazil	Pan American Center for Foot-and-Mouth Disease and Veterinary Public Health, PAHO/WHO; Av. Gov. Leonel de Moura Brizola 7778 Duque de Caxias, Rio de Janeiro, CEP 25045-002
China	Laboratory of Equine Infectious Anemia, Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences 678 Haping Road, Xiangfang District, Harbin 150069
France	Anses, 14 rue Pierre et Marie Curie, 94701 Maisons-Alfort Cedex FRANCE Maisons-Alfort
Germany	FLI, Institute for Bacterial Infections and Zoonoses, Naumburger Str. 96a, 07743 Jena
UAE	Central Veterinary Research Laboratory, Dubai
UK	Animal and Plant Health Agency New Haw, Addlestone, Weybridge, Surrey KT15 3NB

For the anonymised report, the laboratories were randomly assigned laboratory codes. From Lab 1 - Lab 7. All laboratories receive notification of their laboratory code separately.

3. Samples

In total, 40 lyophilized serum samples (37 horses, 3 mules) were provided.

#RR	Status of sample	Origin
1	Horse Glanders Negative Serum	Brazil
2	Horse Glanders Negative Serum	Brazil
3	Mule Glanders Positive Serum	India
4	Horse Glanders Negative Serum	Germany
5	Mule Glanders Positive Serum	India
6	Horse Glanders Positive Serum	Brazil
7	Horse Glanders Positive Serum	Brazil
8	Horse Glanders Positive Serum	Pakistan
9	Horse Glanders Negative Serum	Brazil
10	Horse Glanders Positive Serum	Brazil
11	Horse Glanders Negative Serum	Brazil
12	Horse Glanders Negative Serum	Germany
13	Horse Glanders Negative Serum	Germany
14	Horse Glanders Positive Serum	Brazil
15	Horse Glanders Positive Serum	Brazil
16	Horse Glanders Positive Serum	Brazil
17	Mule Glanders Positive Serum	India
18	Horse Glanders Positive Serum	India
19	Horse Glanders Positive Serum	India
20	Horse Glanders Negative Serum	Brazil
21	Horse Glanders Negative Serum	Germany
22	Horse Glanders Negative Serum	Germany
23	Horse Glanders Negative Serum	Brazil
24	Horse Glanders Positive Serum	Brazil
25	Horse Glanders Negative Serum	Germany
26	Horse Glanders Negative Serum	Brazil
27	Horse Glanders Negative Serum	Germany
28	Horse Glanders Negative Serum	Germany
29	Horse Glanders Positive Serum	Brazil
30	Horse Glanders Positive Serum	India
31	Horse Glanders Positive Serum	India
32	Horse Glanders Positive Serum	India
33	Horse Glanders Negative Serum	Brazil
34	Horse Glanders Positive Serum	India
35	Horse Glanders Negative Serum	Brazil
36	Horse Glanders Negative Serum	Brazil
37	Horse Glanders Negative Serum	Germany
38	Horse Glanders Positive Serum	Brazil
39	Horse Glanders Positive Serum	Brazil
40	Horse Glanders Negative Serum	Germany

The status of positive samples was confirmed by

- Positive PCR in samples of lesions found after the death of the animals or in purulent secretions of abscesses
or
- Confirmation of clinical signs and CFT
or
- Confirmation of clinical signs and isolation of *B. mallei* or PCR

in outbreak scenarios.

4. Preparation of samples

The samples were all steril filtrated and lyophilized at 0,2ml volume each. All participants received instruction for use.

5. Shipment of samples

Shipment was done as Biological sample, Category B; UN3373 by DHL Express Together with the respective import permits of the consignee laboratory.

Duration of shipment:

Country	Pick-up at FLI	Samples delivered
France	16.11.2023	21.11.2023
UK	20.11.2023	24.11.2023
UAE	20.11.2023	25.11.2023
Australia	20.11.2023	01.12.2023
Brazil	20.11.2023	06.12.2023
China	28.12.2023	03.03.2024

7. Results CFT:

#RR sample	intended	Lab 1	Lab 2	Lab 3	Lab 5	Lab 6	Lab 7
1	negative	negative	negative	negative	negative	negative	negative
2	negative	negative	negative	negative	negative	negative	negative
3	positive	positive	positive	suspicious	positive	positive	positive
4	negative	negative	negative	negative	negative	negative	negative
5	positive	positive	positive	positive	positive	positive	positive
6	positive	suspicious	positive	suspicious	positive	positive	positive
7	positive	negative	positive	negative	negative	positive	negative
8	positive	suspicious	positive	positive	positive	positive	positive
9	negative	negative	positive	negative	negative	negative	negative
10	positive	negative	negative	negative	negative	negative	negative
11	negative	negative	positive	negative	negative	positive	negative
12	negative	negative	negative	negative	negative	negative	negative
13	negative	negative	negative	negative	negative	negative	negative
14	positive	suspicious	positive	positive	positive	positive	positive
15	positive	negative	negative	negative	negative	negative	negative
16	positive	negative	negative	negative	negative	positive	negative
17	positive	suspicious	positive	positive	positive	positive	positive
18	positive	suspicious	positive	suspicious	positive	positive	positive
19	positive	positive	positive	positive	positive	positive	positive
20	negative	negative	negative	negative	negative	negative	negative
21	negative	negative	negative	negative	negative	negative	negative
22	negative	negative	negative	negative	negative	negative	negative
23	negative	negative	negative	negative	negative	negative	negative
24	positive	negative	negative	negative	negative	negative	negative
25	negative	negative	negative	negative	negative	negative	negative
26	negative	negative	negative	negative	negative	negative	negative
27	negative	negative	negative	negative	negative	negative	negative
28	negative	negative	negative	negative	negative	negative	negative
29	positive	suspicious	positive	positive	negative	positive	positive
30	positive	positive	positive	positive	positive	positive	positive
31	positive	negative	positive	positive	positive	negative	positive
32	positive	suspicious	positive	positive	positive	positive	positive
33	negative	negative	negative	negative	negative	negative	negative
34	positive	negative	negative	positive	positive	negative	positive
35	negative	negative	negative	negative	negative	negative	negative
36	negative	negative	negative	negative	negative	negative	negative
37	negative	negative	negative	negative	negative	negative	negative
38	positive	negative	positive	suspicious	positive	positive	positive
39	positive	positive	positive	positive	positive	positive	positive
40	negative	negative	negative	negative	negative	negative	negative

8. Discussion and Conclusion

Suspicious results were calculated as "positive" results.

ELISA	Lab 1	Lab 2	Lab 3	Lab 4 *	Lab 5	Lab 6	Lab 7
False negative	2	2	2	2	2	2	2
False positive	0	0	0	1	0	0	0
CFT							
False negative	8	5	5		6	5	5
False positive	0	2	0		0	1	0

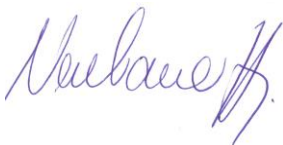
*The results of Lab 4 by CFT were excluded, because of anti-complementary reactions of most test samples

Samples #15 and #16 defined as "positive" were consistently detected as "false negative" by ELISA in all laboratories. Laboratory 4 was the only laboratory to recognise sample #4, defined as "negative", as a "false positive" by ELISA.

In the CFT, there was a higher deviation from the expected results. In total, between 5 and 8 "false negative" results were detected.

Overall, also in this international laboratory test the ELISA showed a higher concordance of results than the CFT, and confirms the suitability of the ELISA for serological glanders diagnostics. The higher number of "false negative" results in the CFT repeatedly proves that this method is difficult to standardize and should be replaced as soon as possible.

With best regards,



Prof. Dr. Heinrich Neubauer
Head of IBIZ and WOAHL RL Glanders



Dr. Mandy Elschner
Head of NRL Glanders