Eurasia Specialized Veterinary Publication

International Journal of Veterinary Research and Allied Science

ISSN:3062-357X

2023, Volume 3, Issue 2, Page No: 42-51

Available online at: www.esvpub.com/

Inter-Laboratory Harmonization of Complement Fixation Testing for *Trypanosoma equiperdum* Antibodies in Equids: A 7-Year European NRL Evaluation

Felipe Teixeira[1], Amanda Gomes[1*], André Pires[1]

[1]Department of Biochemistry, Health Sciences Institute, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

***E-mail** ✉ amanda.gomes2025@gmail.com

ABSTRACT

Dourine is a venereal parasitic disease of equids transmitted primarily during mating. The protozoan responsible, Trypanosoma equiperdum, is diagnosed according to official standards using the complement fixation test (CFT). Within the scope of our role as the European Reference Laboratory (EURL) for equine diseases (excluding African horse sickness), inter-laboratory proficiency tests (ILPTs) for dourine CFT were conducted in 2015, 2018, and 2022 to assess the analytical performance of National Reference Laboratories (NRLs) across the European Union.

Each ILPT set consisted of equine sera either positive or negative for antibodies to Trypanosoma spp., collected from healthy, immunised, or experimentally infected animals. In total, 22 NRLs took part in one or more rounds. The proportion of laboratories achieving perfect agreement (100%) with the reference results was 57%, 90%, and 80% in 2015, 2018, and 2022, respectively.

Findings from these ILPTs confirmed the importance of harmonising the detection limits of the CFT and demonstrated the ongoing necessity for regular performance evaluation of NRLs to maintain network reliability. The results also emphasised the value of establishing a well-characterised serum biobank, both to improve the representativeness of ILPT samples and to facilitate the validation of alternative serological techniques for global dourine monitoring.

Keywords: Dourine, Complement fixation test, Horse, Inter-laboratory proficiency testing

Received: 28 May 2023
Revised: 01 August 2023
Accepted: 03 September 2023

How to Cite This Article: Teixeira F, Gomes A, Pires A. Inter-Laboratory Harmonization of Complement Fixation Testing for *Trypanosoma equiperdum* Antibodies in Equids: A 7-Year European NRL Evaluation. Int J Vet Res Allied Sci. 2023;3(2):42-51. https://doi.org/10.51847/rKYgXN65WD

Introduction

Dourine is a sexually transmitted protozoan infection in horses and related species. Transmission generally occurs through mating, allowing the parasite to enter the host's tissues and bloodstream. During the initial infection stage, affected animals may develop fever, local swelling, emaciation, and anaemia [1]. In advanced phases, the parasite can penetrate the central nervous system [2, 3], causing neurological symptoms such as incoordination or paralysis, which frequently end in death.

Occurrences of dourine must be notified to the World Organisation for Animal Health (WOAH, formerly OIE) and the European Commission within Europe.

According to international diagnostic standards [1], the disease is attributed to Trypanosoma equiperdum, a member of the Trypanozoon subgenus. However, comparative genomic studies involving related species—T. brucei brucei (nagana) and T. brucei evansi (surra)—have revealed that the conventional classification into three subspecies does not reflect actual genetic relationships, since these lineages are polyphyletic [4]. To date, no

unified framework reconciles disease manifestation with phylogenetic groupings [5, 6], and therefore, biological and epidemiological features remain the principal criteria for strain identification [7].

Currently, equine trypanosomoses, including dourine, persist in parts of Africa [8, 9], Asia [10, 11], and Latin America [12–14]. Outside these regions, particularly in Europe and North America, only sporadic detections have been reported [15, 16], and both continents are generally regarded as disease-free [17]. Nevertheless, distinguishing dourine from surra and nagana continues to pose diagnostic challenges [17, 18] because:

1. The three diseases lack unique clinical markers;
2. There is a mismatch between the WOAH nomenclature and the numerous genetic lineages within the Trypanozoon complex [17];
3. Parasitaemia levels are often below the detection limits of microscopic and molecular assays [19].

Given these constraints, international trade surveillance for dourine relies on the CFT, as prescribed by the WOAH [1]. This assay employs crude antigen preparations of T. b. equiperdum, yet due to the high genomic similarity among members of Trypanozoon, the test cannot differentiate infections caused by other trypanosomes such as T. b. brucei or T. b. evansi [17]. Furthermore, the reliability of the CFT is influenced by the titration accuracy and quality of essential reagents, including antigen, complement, and red blood cells.

In Europe, dourine diagnosis and surveillance are performed within a coordinated framework of NRLs. In 2008, the European Commission appointed the French Agency for Food, Environmental, and Occupational Health & Safety (ANSES) as the EURL responsible for standardising and enhancing the diagnostic capacity of European laboratories for equine diseases (excluding African horse sickness). Following earlier ILPTs organised in 2009 and 2012 [20], this investigation aimed to assess the diagnostic accuracy of EU NRLs for dourine using the CFT method [1].

Materials and Methods

Composition of the test panels

The CFT ILPT panels were constructed from equine serum samples specifically selected to either contain or lack anti-Trypanosoma antibodies **(Table 1)**. Each panel was randomly coded before being distributed to participants at a controlled temperature of +2–8 °C. Samples were not heat-treated and had to be maintained under refrigerated conditions to prevent freeze–thaw degradation.

The stability of the lyophilised sera was verified through accelerated ageing trials, showing no change in CFT reactivity after three weeks at 30 °C. For quality assurance, in each ILPT session, a reference panel stored continuously at 4 °C was re-analysed at the end of the testing period to confirm storage stability.

Table 1. Characteristics of the dourine CFT ILPT panels for 2015, 2018, and 2022.

Sample Source	Serum Code	Dilution	Sample Form	Anticipated Dourine CFT Outcome	Dourine CFT Titres (EURL Reference Tests)	2015	2018	2022
Pool of sera from uninfected horses	Neg 1	–	Native	Negative	0	2		
Horse inoculated with *T. b. equiperdum* OVI antigen	HT	–	Native	Positive	60	1		
Horse inoculated with *T. b. equiperdum* OVI antigen	MT	–	Native	Positive	8	1		
	LT	3/5 dilution of MT serum	Native	Positive	5	2		
Horse immunised with *T. b. equiperdum* Teva antigen	Teva N	–	Native	Positive	80	1		
Horse immunised with *T. b. evansi* Rotat 1.2 antigen	Rotat 1.2 N	–	Native	Positive	40	1		

Horse experimentally infected with *T. b. equiperdum* Ethiopian strain	Ethiopian	–	Native	Positive	20	2		
Pool of sera from non-infected horses	Neg 2	–	Freeze-dried	Negative	0		7	3
Horse immunised with *T. b. equiperdum* Teva antigen	Teva	–	Freeze-dried	Positive	80		3	2
	Teva (1/2)	Half dilution of Teva serum	Freeze-dried	Positive	40		2	
	Teva (1/4)	Quarter dilution of Teva serum	Freeze-dried	Positive	20		3	2
Horse immunised with *T. b. evansi* Rotat 1.2 antigen	Rotat 1.2	–	Freeze-dried	Positive	60		1	1
	Rotat 1.2 (1/2)	Half dilution of Rotat 1.2 serum	Freeze-dried	Positive	30		2	
	Rotat 1.2 (1/4)	Quarter dilution of Rotat 1.2 serum	Freeze-dried	Positive	15		2	
Horse experimentally infected with *T. b. equiperdum* OVI strain	Trypeq	–	Freeze-dried	Positive	80			2
				Total samples per ILPT session:		**10**	**20**	**10**

CFT, complement fixation test; EURL, European Union Reference Laboratory; ID, identifier; ILPT, inter-laboratory proficiency test.

Participating laboratories

A total of 22 National Reference Laboratories (NRLs) for dourine across the European Union participated in at least one of the three CFT inter-laboratory proficiency tests (ILPTs) conducted in 2015, 2018, and 2022 **(Figure 1 and Table 2)**.

During each ILPT session, participants were assigned random identification codes to maintain the blind status of analyses. In this study, laboratories are designated numerically (1 to 22) to enable tracking of performance trends throughout the three ILPT rounds **(Table 2)**.

To eliminate any potential conflict of interest, ANSES—though acting as the EURL for equine diseases—coordinated the tests but did not take part in them as the French NRL.

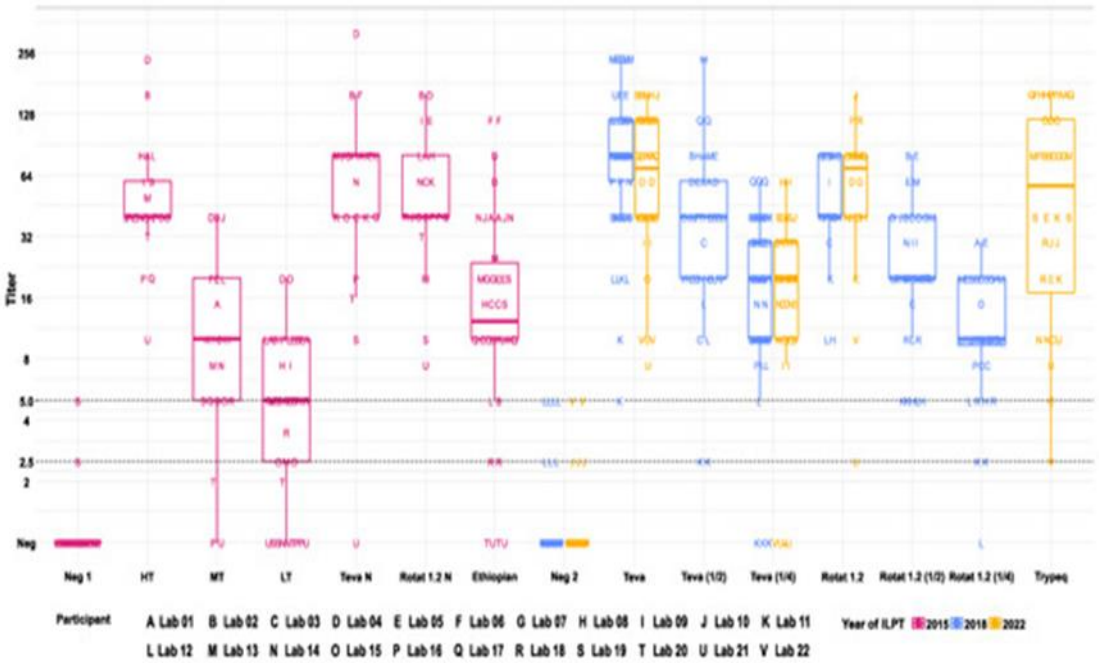

Figure 1. Box-and-whisker plots illustrating the distribution of CFT titres obtained by NRLs during the 2015, 2018, and 2022 dourine ILPTs, grouped by year and serum ID. Titres were derived according to the procedures outlined in the Materials and Methods. Horizontal dashed lines denote threshold intervals: sera were considered doubtful when 2.5 < titre < 5, and positive when titre > 5.

Table 2. Summary of the qualitative CFT results and antigens used by participating laboratories in the three ILPT sessions.

Participant	2015 – Unexpected Findings	2015 – Successful Identifications (%)	2015 – Antigen Type	2015 – Antigen Dilution	2018 – Unexpected Findings	2018 – Successful Identifications (%)	2018 – Antigen Source	2018 – Antigen Dilution	2022 – Unexpected Findings	2022 – Successful Identifications (%)	2022 – Antigen Source	2022 – Antigen Dilution
1	0	100	A-1	60	0	100	F-1	20	0	100	F-2	16
2	0	100	A-2	200	0	100	A-11	100	0	100	F-3	30
3	0	100	A-3	100	0	100	F-1	50	0	100	F-2	40
4	0	100	B-1	40	0	100	B-1	30	0	100	B-3	10
5	0	100	A-4	64	0	100	A-4	64	0	100	F-3	48
6	0	100	C-1	80	0	100	C-2	70	0	100	C-3	20
7	0	100	A-1	100	0	100	A-12	128	0	100	A-15	200
8	0	100	D-1	100	0	100	D-2	180	0	100	D-3	100
9	0	100	A-5	100	0	100	F-1	50	1	90	A-16	100
10	0	100	B-2	32	0	100	B-2	32	3	70	F-2	64
11	0	100	A-6	128	7	65	A-13	160	0	100	F-3	10
12	0	100	A-3	50	8	60	F-1	1400	–	–	–	–
13	2	80	A-7	50	0	100	F-1	30	0	100	F-2	30
14	2	80	A-3	10	0	100	F-1	40	0	100	F-2	256
15	2	80	A-8	100	0	100	F-1	30	0	100	F-2	30

16	3	70	A-3	15	0	100	F-1	60	0	100	F-2	75
17	3	70	A-9	40	0	100	A-14	50	0	100	F-2	20
18	3	70	A-10	200	0	100	F-1	30	0	100	F-3	30
19	5	50	E-1	128	0	100	F-1	30	0	100	A-17	20
20	5	50	E-1	16	–	–	–	–	–	–	–	–
21	6	40	E-1	32	0	100	A-12	100	3	70	A-18	9
22	–	–	–	–	–	–	–	–	6	40	F-3	10

Percentage of laboratories achieving 100% successful identification
2015 → **57%** | 2018 → **90%** | 2022 → **80%**
a: Laboratory did not join that specific ILPT.
b: To preserve confidentiality, antigen identities were coded—letters denote suppliers, while numbers represent production batches. Antigens B, C, and D were prepared in-house, whereas A, E, and F were commercial sources. The intensity of red shading in the table corresponds to the number of unexpected outcomes per laboratory.

2015 serum panel

Each test panel consisted of 150 µL aliquots containing various serum samples **(Table 1)**. These sera, stored frozen for long-term preservation, were thawed, subdivided into multiple vials, and shipped at 4–8 °C to participants. Laboratories were allotted two weeks to submit their CFT results.

2018 and 2022 serum panels

For both the 2018 and 2022 sessions, the panels were composed of freeze-dried sera. Each sample was to be kept refrigerated (+2–8 °C) both before and after reconstitution.

To prepare the sera, participants added 0.5 mL of distilled water, mixed vigorously, and incubated for 30 minutes at room temperature with periodic agitation until fully dissolved.

Participants had four weeks to return their data.

Before distribution, panel homogeneity was verified according to ISO 17043 [21]: ten aliquots of each serum were tested in duplicate to confirm that all aliquots of the same serum yielded identical qualitative outcomes.

EURL CFT protocol

Each participant laboratory conducted the dourine complement fixation test (CFT) following the diagnostic procedure routinely used in-house. The interpretation of haemolysis inhibition (HI) followed the WOAH Terrestrial Manual, Chapter 3.6.3 [1]. HI was recorded at 1/5 dilution using the following scoring system:

0, trace, 1+, 2+, 3+, 4+, corresponding respectively to 0%, trace, 25%, 50%, 75%, and 100% unlysed red blood cells.

The qualitative assessment at 1/5 dilution was defined as:

HI of 0 or trace: Negative (Neg)

HI of 1+: Doubtful/Suspicious (Susp)

HI of 2+, 3+, or 4+: Positive (Pos)

Sera with anti-complementary activity: Inconclusive (IC)

Some laboratories reported nonstandard classifications; in such cases, their results were evaluated based on their own internal criteria.

Samples yielding positive results at 1/5 dilution could be serially diluted (1:2) for determining the positivity endpoint. This endpoint titre was calculated with the formula:

Titre=Last dilution with % of HI ≥ 50% × % of HI of the dilution × 2

For example, a 3+ result at 1/10 dilution equates to: 10 × 75% × 2 = 15. According to the WOAH standard, the positivity threshold is 5 (5 × 50% × 2 = 5).

Analytical methods

Participants were required to provide detailed information regarding the testing methodology, including the antigen source, batch identification, and expiration date, as well as the suppliers of positive and negative control sera. They also indicated whether each new antigen batch underwent titration. The outcomes reported by

participants were then compared with the reference results generated by the EURL during preliminary assessments for homogeneity and stability.

To assess the analytical performance of the assays across laboratories, the parameters of specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) were computed using the following formulas:

$$\text{Sensitivity} = \frac{\sum \text{True}\ (+)}{\sum \text{True}\ (+) + \sum \text{False}\ (-)} \quad (1)$$

$$\text{Specificity} = \frac{\sum \text{True}\ (-)}{\sum \text{True}\ (-) + \sum \text{False}\ (+)} \quad (2)$$

$$\text{PPV} = \frac{\sum \text{True}\ (+)}{\sum \text{True}\ (+) + \sum \text{False}\ (+)} \quad (3)$$

$$\text{NPV} = \frac{\sum \text{True}\ (-)}{\sum \text{True}\ (-) + \sum \text{False}\ (-)} \quad (4)$$

The box-and-whisker plot illustrating the distribution of dourine CFT titres was produced using the R statistical software [22].

Results and Discussion

Organisation of the dourine CFT ILPT and description of panels

The composition of the panels distributed to participants across the three dourine CFT ILPT rounds is outlined in **Table 1**. Because it was challenging to obtain sera from horses naturally or experimentally infected with the parasite, only two sera—Ethiopian and Trypeq—from experimentally infected animals were available for inclusion. To complete the sets, additional samples were produced using sera from horses immunised with whole-cell lysates of various Trypanozoon strains. To generate graded antibody levels, the sera from these immunised horses were diluted with negative serum samples, as indicated in **Table 1**.

Since the CFT relies on crude antigen preparations, cross-reactivity can occur in sera from animals that were infected or vaccinated with Trypanozoon organisms from different lineages. Consequently, positive CFT outcomes obtained with sera immunised using T. b. evansi type A antigens were anticipated and considered correct.

In 2015, the sera were provided in liquid form at 4 °C, while for 2018 and 2022, the panels were freeze-dried before shipment. This adaptation enabled the inclusion of four identical sera—Neg 2, Teva, Teva (1/4), and Rotat 1.2—across both the 2018 and 2022 ILPT rounds **(Table 1)**. All participating laboratories reported successful reconstitution of samples, with no technical difficulties encountered.

Assessment of ILPT participant performance

Across all three ILPTs, 22 European Union National Reference Laboratories (NRLs) took part: 21 in 2015, and 20 in both 2018 and 2022 **(Figure 1)**. Among them, 19 laboratories participated in every round, one joined two, and two joined only one.

As the WOAH Terrestrial Manual recognises the dourine CFT as a qualitative diagnostic assay, the evaluation focused solely on the positive, negative, or suspicious classifications of the test sera **(Table 2)**. Of the 19 laboratories present in all three sessions, 8 (42%) achieved 100% correct classifications in every round, 10 (53%) did so in two, and 1 (5%) did so in only one.

In 2015, 12 out of 21 (57%) labs matched all expected results, while 9 (43%) recorded between two and three discrepancies. Among these, Lab 19 exhibited specificity issues, and nine laboratories experienced sensitivity errors, particularly with LT (9/9) and MT (5/9) sera, which had low expected titres. Additionally, laboratories employing E-1 antigens generated the highest proportion of unexpected results.

In 2018, 18 of 20 (90%) laboratories reported entirely correct data, while 2 (10%) showed eight inconsistencies. Lab 12 demonstrated specificity problems, and Labs 11 and 12 had sensitivity issues. These two also used the most diluted antigen preparations (160 and 1400, respectively).

In 2022, 16 out of 20 (80%) achieved fully accurate results, while 4 (20%) presented between one and six incorrect classifications. Among these, Labs 10 and 22 showed specificity concerns, while Labs 9, 21, and 22 exhibited sensitivity deficiencies. No association was identified between the unexpected findings and antigen batch or dilution for that year.

Serum-by-serum quantitative evaluation

The sera composing the panels are detailed in **Table 1**, and the returned data are shown in **Figure 1**.

In 2015, 20 out of 21 (95%) laboratories correctly identified the Neg 1 samples as negative, with one laboratory classifying both replicates as suspicious. All participants recognised HT and Rotat 1.2 N sera as positive, whereas Lab 21 incorrectly labelled the Teva N serum as negative. The MT and LT samples—both with low titres—were classified as positive by 12 out of 21 (57%) laboratories. The Ethiopian serum, derived from an infected horse, was misclassified as negative by two labs and suspicious by one.

In 2018 and 2022, three laboratories did not identify Neg 2 samples as negative, resulting in 12 unexpected outcomes (0.6%) out of 200 total. As expected, the mean titres for sera obtained from horses immunised with T. b. equiperdum (Teva) or T. b. evansi (Rotat 1.2) decreased with increasing dilution.

False negatives occurred most often in 1/4 dilutions, with 7/100 (7%) for Teva and 3/40 (7.5%) for Rotat 1.2, compared to 0/100 (0%) and 2/40 (5%) for undiluted samples and 1/20 (2.5%) and 0/40 (0%) for 1/2 dilutions, respectively. For the Trypeq serum from experimentally infected horses, all laboratories correctly identified both replicates as positive, except Labs 9 and 22, which marked one and two replicates as suspicious, respectively.

Although equine trypanosomosis, particularly dourine, has disappeared from many geographic regions, it still poses a potential threat to global horse health and continues to justify international trade surveillance measures [19, 23]. Within the European Union (EU), the network of dourine National Reference Laboratories (NRLs) plays a crucial role in monitoring and early detection of possible outbreaks. One of the main responsibilities of the European Union Reference Laboratory (EURL) for equine diseases (excluding African Horse Sickness) is to assess both the sensitivity and specificity of diagnostic activities carried out within this network [24].

Because trypanosomes can appear intermittently in the bloodstream, serological approaches remain the most dependable method for detecting infection, while microscopic and molecular tools are considered secondary. Consequently, the complement fixation test (CFT) continues to serve as the officially recommended diagnostic assay for dourine within international trade regulations [1]. A 2014 evaluation of the EU NRL network highlighted that harmonisation of critical reagents and the creation of reference sera from T. equiperdum could enhance diagnostic reliability [20]. The present paper, therefore, reports on the outcomes of the dourine CFT inter-laboratory proficiency tests (ILPTs) carried out in 2015, 2018, and 2022 across the EU NRL network.

Specificity Assessment

To examine CFT specificity, each ILPT panel contained several replicates of negative sera. Across all 61 total participating entries from the three sessions, four laboratories (6.6%) incorrectly reported negative samples as positive or doubtful. These same participants repeatedly produced similar inconsistencies within a single session, and their recorded titres were typically close to the positivity limit (≤5). This pattern likely indicates improper titration of key reagents—such as red blood cells, complement, or haemolytic serum—which can lead to incomplete erythrocyte lysis even in the absence of antibodies.

It is therefore advised that laboratories verify complete haemolysis in the control well for each tested serum (a well lacking antigen) and, if necessary, include an additional control well without serum where the complement is diluted to 1/2 or 1/4 to confirm that the complement volume is adequate to ensure total red cell lysis.

Threshold Determination and Sensitivity Considerations

Serological assays aim to detect pathogen-specific antibodies, making the positivity cut-off value a critical factor influencing sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) [25]. During CFT reagent calibration, this threshold is typically established based on the complement fixation percentage from low-titre sera. However, as Europe has remained free of dourine for decades (except a 2011 outbreak in Italy [15]), obtaining authentic low-reactivity sera from infected animals remains highly challenging. To address this, most laboratories rely on sera from horses immunised with crude Trypanosoma antigens, which exhibit low but measurable reactivity in the CFT. Nonetheless, such sera can produce artificial threshold values that may not reflect natural infection profiles. Using sera from experimentally infected horses allows for a more accurate representation of biochemical and hematological changes that accompany real infections. For this reason,

the Ethiopian serum—from horses experimentally infected with the T. b. equiperdum Ethiopian strain [8]—was included in the 2015 ILPT.

Three laboratories (Labs 18, 20, and 21) failed to classify this serum as positive, indicating sensitivity shortcomings and reinforcing the need for threshold standardisation. In the 2022 ILPT, another experimentally derived serum, Trypeq (T. equiperdum OVI), was used. None of the 20 participating NRLs reported Trypeq as negative; however, two labs—Lab 9 and Lab 22—returned suspicious outcomes. Lab 9 recorded one positive and one suspicious replicate, consistent with lower positivity rates across all sera, while Lab 22 displayed extensive sensitivity (43%) and specificity (33%) issues. The wide titre range (2.5–160) for these sera suggests that further harmonisation of reagents and procedures across laboratories remains necessary.

Reagent Standardisation and Quantitative Evaluation

For the 2018 ILPT, the use of freeze-dried serum samples prepared in large homogeneous batches permitted reliable cross-session comparison of laboratory performance. During preparation, Teva and Rotat 1.2 sera were diluted to produce undiluted, half-diluted, and quarter-diluted variants. The analysis of returned data revealed a strong correlation between reported titres and serum dilution factors, confirming that the CFT response is proportional to the antibody concentration present in the tested sera.

During the 2015 dourine CFT ILPT, 9 of 21 laboratories (43%) demonstrated sensitivity deficiencies, particularly evident with the LT serum. To assist laboratories in correctly establishing positivity thresholds, two reference control sera—one high-titre and one low-titre—were subsequently produced and distributed across the EU diagnostic network, alongside an antigen derived from T. b. equiperdum OVI parasites propagated in rats. After these corrective measures were introduced, the number of laboratories experiencing sensitivity issues declined markedly in the later sessions: 2 of 20 (10%) in 2018, and 3 of 20 (15%) in 2022.

In the CFT system, diagnostic specificity depends largely on the antigen employed. Across the ILPT sessions, participating laboratories used six antigen sources—three prepared internally and three obtained commercially. Those employing non-commercial (in-house) antigen preparations achieved 100% of expected results, indicating that laboratories capable of producing their own dourine antigen possess the technical expertise required for reliable diagnostics. Conversely, the three laboratories using the commercial E-1 antigen reported unsatisfactory results for 50–60% of the tested samples. Based on these findings, participants were advised to discontinue the use of E-1 antigens, which were subsequently excluded from both the 2018 and 2022 ILPTs.

For the other two commercial antigen batches (A and F), no consistent relationship was found between supplier, batch number, or participant performance. Although certain laboratories reported inconsistent outcomes with these preparations, the limited number of users for each batch prevented any statistically meaningful correlation between antigen choice and test performance. Interestingly, even within a single batch, considerable variation in antigen dilution was observed—F-1 being used at dilutions from 1/20 to 1/1400 and F-2 between 1/16 and 1/256—yet final diagnostic outcomes appeared unaffected by dilution level.

Recent phylogenetic studies have revealed that dourine, surra, and nagana are caused by polyphyletic Trypanozoon lineages [26]. For example, parasite clades responsible for dourine may share closer genetic relationships with surra agents than with other dourine isolates [5, 6]. These findings challenge the traditional disease classifications and suggest that dourine may instead represent a clinical syndrome triggered by a host-specific immune response in equids following Trypanozoon infection [17].

Because CFT antigens consist of whole Trypanozoon cell lysates, the assay inherently lacks clade specificity, meaning it cannot reliably differentiate between dourine, surra, and nagana [17]. However, for pre-movement health checks and international trade certification, this cross-reactivity is not problematic, since detection of any of these regulated trypanosomoses ensures the health security of traded horses. Consequently, positive dourine CFT results obtained from sera immunised with T. b. evansi Rotat 1.2 antigens **(Table 1)** were interpreted as expected during the ILPT assessments.

Conclusion

Within the framework of the EURL's mandate, Inter-Laboratory Proficiency Tests (ILPTs) are periodically organised to evaluate the performance of EU National Reference Laboratories using the dourine CFT. Considering that, depending on national testing demands and horse movement frequency, individual NRLs may conduct anywhere from a handful to several hundred assays annually, such evaluations are essential to prevent erroneous results that could lead to serious economic or sanitary repercussions.

The three ILPT rounds conducted to date have enabled the implementation of corrective strategies, such as advising against certain antigen batches, and have collectively improved overall network performance through the distribution of standardised sera and antigens supplied by the EURL.

Future improvements in laboratory capability will depend on several initiatives: the development and dissemination of clear, user-oriented SOPs, continued ILPT sessions incorporating high-quality sera from experimentally infected horses, and targeted training programmes for underperforming or newly established laboratories intending to adopt the dourine CFT.

At present, the principal barrier to full standardisation remains the limited availability of representative field samples from diverse geographic regions, encompassing multiple Trypanozoon clades and infection stages. Establishing field serum banks would make it possible to define precise detection thresholds based on authentic infection-derived biological material, rather than immunised animal sera. This approach would enhance sensitivity while maintaining acceptable specificity.

Furthermore, such a biobank would support comprehensive field validation of novel diagnostic tools—following WOAH-endorsed validation frameworks—for promising alternative assays like ELISA [27, 28], Luminex [29], and ICT [30]. More broadly, this study underscores the necessity for all laboratories performing—or intending to perform—CFT-based diagnostics, whether for dourine or any other infectious disease, to participate in external quality assurance programmes such as ILPTs to ensure the sustained reliability and comparability of their diagnostic results.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. World Organisation for Animal Health. Dourine in horses (Trypanosoma equiperdum infection). In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Paris: OIE; 2022. p. 1–13.
2. Hébert L, Guitton E, Madeline A, Géraud T, Carnicer D, Lakhdar L, et al. Validation of a new experimental model for assessing drug efficacy against infection with Trypanosoma equiperdum in horses. Vet Parasitol. 2018;263(1):27–33.
3. Hébert L, Guitton E, Madeline A, Géraud T, Zientara S, Laugier C, et al. Melarsomine hydrochloride (Cymelarsan®) fails to cure horses with Trypanosoma equiperdum OVI parasites in their cerebrospinal fluid. Vet Parasitol. 2018;264(1):47–51.
4. Carnes J, Anupama A, Balmer O, Jackson A, Lewis M, Brown R, et al. Genome and phylogenetic analyses of Trypanosoma evansi reveal extensive similarity to T. brucei and multiple independent origins for dyskinetoplasty. PLoS Negl Trop Dis. 2015;9(1):e3404.
5. Oldrieve G, Verney M, Jaron KS, Hébert L, Matthews KR. Monomorphic Trypanozoon: Towards reconciling phylogeny and pathologies. Microb Genom. 2021;7(8):000632.
6. Lukeš J, Kachale A, Votýpka J, Butenko A, Field MC. African trypanosome strategies for conquering new hosts and territories: The end of monophyly? Trends Parasitol. 2022;38(8):724–36.
7. Suganuma K, Narantsatsral S, Battur B, Yamasaki S, Otgonsuren D, Musinguzi SP, et al. Isolation, cultivation and molecular characterization of a new Trypanosoma equiperdum strain in Mongolia. Parasit Vectors. 2016;9(1):481.
8. Hagos A, Abebe G, Büscher P, Goddeeris BM, Claes F. Serological and parasitological survey of dourine in the Arsi–Bale highlands of Ethiopia. Trop Anim Health Prod. 2010;42(4):769–76.
9. Yasine A, Ashenafi H, Geldhof P, Van Brantegem L, Vercauteren G, Bekana M, et al. Histopathological lesions in reproductive organs, distal spinal cord and peripheral nerves of horses naturally infected with Trypanosoma equiperdum. BMC Vet Res. 2019;15(1):175.

10. Mizushima D, Amgalanbaatar T, Davaasuren B, Kayano M, Naransatsral S, Myagmarsuren P, et al. Nationwide serological surveillance of non-tsetse-transmitted horse trypanosomoses in Mongolia. Parasite Epidemiol Control. 2020;10(1):e00158.
11. Mungun-Ochir B, Horiuchi N, Altanchimeg A, Koyama K, Suganuma K, Nyamdolgor U, et al. Polyradiculoneuropathy in dourine-affected horses. Neuromuscul Disord. 2019;29(6):437–43.
12. Sánchez E, Perrone T, Recchimuzzi G, Cardozo I, Biteau N, Aso PM, et al. Molecular characterization and classification of Trypanosoma spp. Venezuelan isolates based on microsatellite markers and kinetoplast maxicircle genes. Parasit Vectors. 2015;8(1):536.
13. Yamazaki A, Suganuma K, Kayano M, Acosta TJ, Saitoh T, Valinotti MFR, et al. Risk factors for equine trypanosomosis and hematological analysis of horses in Paraguay. Acta Trop. 2022;233(1):106543.
14. Hébert L, Polledo G, Lecouturier F, Giorgi M, Beck C, Lowenski S, et al. Serological evidence of equine infectious anaemia, West Nile fever, surra and equine piroplasmosis in a herd of horses in northern Argentina. Vet Parasitol Reg Stud Rep. 2021;24(1):100566.
15. Pascucci I, Di Provvido A, Camma C, Di Francesco G, Calistri P, Tittarelli M, et al. Diagnosis of dourine in outbreaks in Italy. Vet Parasitol. 2013;193(1–3):30–8.
16. Caporale VP, Battelli G, Semproni G. Epidemiology of dourine in the equine population of the Abruzzi Region. Zentralbl Veterinarmed B. 1980;27(7):489–98.
17. Büscher P, Gonzatti MI, Hébert L, Inoue N, Pascucci I, Schnaufer A, et al. Equine trypanosomosis: Enigmas and diagnostic challenges. Parasit Vectors. 2019;12(1):234.
18. Desquesnes M, Sazmand A, Gonzatti M, Boulangé A, Bossard G, Thévenon S, et al. Diagnosis of animal trypanosomoses: Proper use of current tools and future prospects. Parasit Vectors. 2022;15(1):235.
19. Desquesnes M, Gonzatti M, Sazmand A, Thevenon S, Bossard G, Boulange A, et al. A review on the diagnosis of animal trypanosomoses. Parasit Vectors. 2022;15(1):64.
20. Cauchard J, Soldan A, Madeline A, Johnson P, Büscher P, Petry S. Inter-laboratory ring trials to evaluate serological methods for dourine diagnosis. Vet Parasitol. 2014;205(1–2):70–6.
21. International Organization for Standardization. ISO/IEC 17043: Conformity assessment—General requirements for proficiency testing. Geneva: ISO; 2010.
22. R Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2022.
23. Beck C, Lowenski S, Durand B, Bahuon C, Zientara S, Lecollinet S. Improved reliability of serological tools for the diagnosis of West Nile fever in horses within Europe. PLoS Negl Trop Dis. 2017;11(3):e0005936.
24. Laroucau K, Colaneri C, Jay M, Corde Y, Drapeau A, Durand B, et al. Interlaboratory ring trial to evaluate CFT proficiency of European laboratories for diagnosis of glanders in equids. Vet Rec. 2016;178(24):632.
25. Álvarez-Rodríguez A, Jin BK, Radwanska M, Magez S. Recent progress in diagnosis and treatment of human African trypanosomiasis has made the elimination of this disease a realistic target by 2030. Front Med. 2022;9(1):1037094.
26. Cuypers B, Van den Broeck F, Van Reet N, Meehan CJ, Cauchard J, Wilkes JM, et al. Genome-wide SNP analysis reveals distinct origins of Trypanosoma evansi and Trypanosoma equiperdum. Genome Biol Evol. 2017;9(7):1990–7.
27. Bonfini B, Tittarelli M, Luciani M, Di Pancrazio C, Rodomonti D, Iannetti L, et al. Development of an indirect ELISA for the serological diagnosis of dourine. Vet Parasitol. 2018;261(1):86–90.
28. Davaasuren B, Amgalanbaatar T, Musinguzi SP, Suganuma K, Otgonsuren D, Mossaad E, et al. The evaluation of GM6-based ELISA and ICT as diagnostic methods on a Mongolian farm with an outbreak of non-tsetse transmitted horse trypanosomosis. Vet Parasitol. 2017;244(1):123–8.
29. Verney M, Gautron M, Lemans C, Rincé A, Hans A, Hébert L. Development of a microsphere-based immunoassay for the serological diagnosis of equine trypanosomosis. Sci Rep. 2022;12(1):1308.
30. Mizushima D, Amgalanbaatar T, Davaasuren B, Molefe NI, Battur B, Battsetseg B, et al. The utility of an rTeGM6-4r-based immunochromatographic test for the serological diagnosis of non-tsetse-transmitted equine trypanosomosis in rural areas of Mongolia. Parasitol Res. 2018;117(9):2913–9.