European Union Reference Laboratory for Parasites Istituto Superiore di Sanità

Proficiency Testing for the detection of anti-*Toxoplasma* IgG in ovine serum samples





Seventeenth Annual EURLP Workshop, Rome 15-16 September 2022



The aims of the proficiency testing (PT) were :

To evaluate:

- the laboratories competence in detection of anti-Toxoplasma IgG in ovine serum samples
- the performance of the commercial kits, utilized for the detection of anti-Toxoplasma IgG in ovine.





Participating laboratories



Netherland	
Norway	
Poland	-
Portugal	
Romania	
Slovack	
Slovenia	-
Spain	<u>s</u>

Seventeenth Annual EURLP Workshop, Rome 15-16 September 2022 Alessandra Ludovisi

Methods

Any serological test based on the detection of anti-Toxoplasma IgG could

be used. Each laboratory should choose the test/s routinely used.





Materials and Methods 1

The test material forwarded to each laboratory consisted of a panel of 4 serum samples collected from *T. gondii* free or *T.gondii* experimentally infected animals.

Serum sample Code	Expected Result	S/P%*
s.1	Positive	> 100%
s.2	Positive	82.8%
s.3	Positive	59.1%
s.4	Negative	4.2%

Serum sample panel

*These serum samples were:

individually tested for anti-*Toxoplasma* IgG by the: "ID Screen Toxoplasmosis Indirect Multi-species





Seventeenth Annual EURLP Workshop, Rome 15-16 September 2022

Materials and Methods 2.

Serum samples were:

 \succ distributed in 100 µL aliquots,

> preserved with 1% merthiolate solution,

Iabelled with a code





Materials and Methods 3.

Each laboratory received a link available to NRLs on the EURLP website to the online survey platform Microsoft Forms, through which PT results and other details could be submitted





Results1

The methods or kits used by the participants were:

- **IDVet ID Screen**, **15 labs:** (A,C,D,E,G,H,L,M,N,O,P,Q,R,S,T)
- Indirect Immunofluoresence antibody test (IFAT) / OIE, 1 lab: F
- Toxoreagent Mast Kit, 2 labs: B and I
- VetLine Toxoplasma ELISA (NovaTec, Immundiagnostica GmbH), 1 lab: J

Two labs performed an additional test:

- Immunoblot full Antigen and P30 Antigen, 1 lab : E
- Pigtype Toxoplasma Ab, Indical Bioscience, 1 lab : A



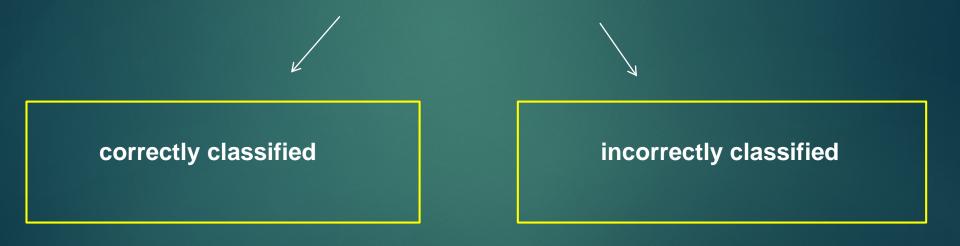




Criteria for the results evaluation:

The participating laboratory had to indicate the positivity or negativity of each tested item.

The result of the analysis of each serum sample was reported as







Seventeenth Annual EURLP Workshop, Rome 15-16 September 2022

RESULTS 2.

11/19 NRLs correctly classified (as positive or negative) all serum samples

Sample code	Expec. results	Lab. code																		
Code	TCSUITS	А	E	F	Н	I	Μ	Ν	0	Р	Q	S	В	С	D	G	J	L	R	Т
1	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+/-	-	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	-	+/-	+/-	-	-	+/-	-	+/-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Seventeenth Annual EURLP Workshop, Rome 15-16 September 2022



Test interpretation

 $S/P \le 40\%$ Negative40% < S/P < 50%Doubtful $S/P \ge 50\%$ Positive



Serum sample Code	Expected Result	S/P
s.1	positive	152%
s.2	positive	82.8%
s.3	positive	59.1%
s.4	negative	4.2%







Taking into consideration:

- Serum sample n°3 is clearly positive sample
- Aliquots were homogeneous because they were prepared from the same batch

Possible causes of disagreement:

- Not uniform distribution of the antigen in the plate
- Not appropriate resuspension of all Kit components before use
- Human error







Criteria for the final PT evaluation

The result evaluation is qualitative

IgG titers will be considered as additional information to compare the performance of tests used by participants

Final evaluation is considered as

"positive" if all samples are correctly classified "negative" in all the other cases.





Seventeenth Annual EURLP Workshop, Rome 15-16 September 2022

PT evaluation

Laboratory code	N° of samples correctly classified	N° of samples NOT correctly classified	Final evaluation
Α	4/4	0/4	Positive
E	4/4	0/4	Positive
F	4/4	0/4	Positive
H	4/4	0/4	Positive
Ι	4/4	0/4	Positive
Μ	4/4	0/4	Positive
Ν	4/4	0/4	Positive
0	4/4	0/4	Positive
Р	4/4	0/4	Positive
Q	4/4	0/4	Positive
S	4/4	0/4	Positive
В	1/4	3/4	Negative
С	3/4	1/4	Negative
D	3/4	1/4	Negative
G	2/4	2/4	Negative
J	2/4	2/4	Negative
L	3/4	1/4	Negative
R	3/4	1/4	Negative
Т	3/4	1/4	Negative





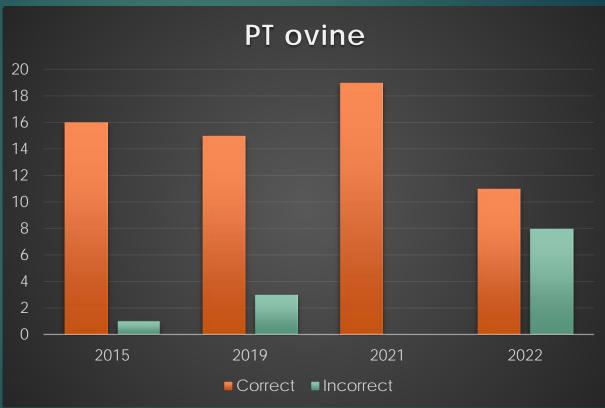
Seventeenth Annual EURLP Workshop, Rome 15-16 September 2022

Conclusion

More than a half (58%) of the laboratories correctly classified all serum samples

The more frequently used commercial kits are immunoenzimatic assays

Overtime comparison







Seventeenth Annual EURLP Workshop, Rome 15-16 September 2022



Thanks for your attention!





Seventeenth Annual EURLP Workshop, Rome 15-16 September 2022