Report of the 1st NRL proficiency testing on "Detection of anti-*Toxoplasma* IgG in ovine serum samples"

March - April, 2015

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1 Introduction

The obligate intracellular parasite *Toxoplasma gondii* is a protozoan of the Phylum Apicomplexa highly prevalent in humans and animals worldwide. Its clinical relevance is mainly due to potential congenital transmission to the foetus by seronegative women, causing intrauterine death or severe sequelae in the newborn and adult life. The major role in disease transmission is played by the oral ingestion of either bradyzoites encysted in the tissues of chronically infected warm blooded animals or sporozoites contained in the highly resistant environmental stage oocyst (Dubey, 2009). Sheep, along with goats and pigs, are the animal species mostly associated with human infections (Esteban-Redondo et al., 1999, Jones et al., 2009). Consequently, field studies on the seroprevalence of *T. gondii* are needed to estimate the risk for humans.

2 Scope

The proficiency testing (PT) aims to evaluate the competence of the appointed NRLs to detect anti-*Toxoplasma* IgG in ovine serum samples. The organization of PTs falls in the duties of the EURL for Parasites, as it is stated in the Regulation (EC) No 882/2004 of the European Parliament and of the Council (Commission Regulation No. 882/2004). The 1st PT on detection of anti-*Toxoplasma* IgG in ovine serum samples was organized following the request of NRLs during the 2014 EURLP annual workshop.

3 Time frame

The PT was first announced to NRLs by email on January 15th, 2015. A second email was sent to all NRLs on February 6th, 2015, including the participation forms (see Annex 1; MO/MAQPVI/01.PT Request Form) and the deadline to send it back. On March 16th, 2015, the samples were dispatched to participants by an international courier.

4 Test material

The test material forwarded to each laboratory consisted of a panel of 10 serum samples (Annex 2). Serum samples were collected from *T. gondii* naturally infected and uninfected sheep. All serum samples were individually tested for anti-*Toxoplasma* IgG by a commercial kit (ID Screen® Toxoplasmosis Indirect Multi-species Indirect ELISA TOXOS-MS). Serum samples were pooled according with their potency, optical density (OD) value, preserved with 1% merthiolate solution, and then tested again to confirm their positivity/negativity. Samples were further distributed in 250 µL aliquots, labelled with a code, and then put in a plastic bag sealed under vacuum, identified by an alphanumeric code stuck on the plastic bag and stored at +4 °C. Samples were forwarded to each participant laboratory by an international courier in a polystyrene box with cooler packages to maintain a temperature lower than +15 °C during shipping. Packages were forwarded according to the international forwarding regulations.

The stability of the samples was evaluated by testing twice, before and 10 days after the forwarding, the panel of serum samples. Differences between the two OD determinations were lower than 0.5 units (Annex 3).

To detect anti-*Toxoplasma* IgG in serum samples, any serological test based on the detection of anti-*Toxoplasma* IgG could be used. Each laboratory had to use the test/s routinely used.

5 Instructions to participants

From February 6th, 2015, general information on the PT aim, instructions and procedures were made available to NRLs on the EURLP web site (http://www.iss.it/crlp/index.php?lang=2&anno=2015& tipo=28).

Electronic copies of the forms, which had to be filled in and sent back to EURLP, were sent by e-mail on March 16th, 2015, (see Annex 1; MO/POPVI/00 from 01.06 to 04.06):

1) "Check package", to inform about the content and the condition of the PT material at the arrival in the lab;
2) "Instruments and Materials List" to be reported if instruments and/or materials different from those present in the original kit were used;
3) "Procedure", to inform about the kit used and its expiration date. Any variation from the used method had be reported;
4) "Result", to report the positivity or negativity of each sample.

The code assigned to the laboratory was sent individually to each participant laboratory by a separate e-mail on March 16th 2015.

6 Criteria for result evaluation and statistical analysis

The participating laboratory had to indicate the positivity or negativity of the tested samples. Evaluation of the results is qualitative for each serum sample, and it is reported as "correctly classified" or "incorrectly classified", irrespective of the IgG level found in the sample/s. Final evaluation is considered as "positive" if all samples are correctly classified and "negative" in all the other cases.

Due to the low number of samples in the panel, no statistical analysis of the results was performed. In April 2015, the EURLP sent an Individual PT Report (see Annex 4) to each participant laboratory as a pdf file by email.

7 Participating laboratories

Sixteen NRLs, coded from B to Q, participated in the PT (Annex 5).

8 Results

8.1 Package delivery to NRLs

All packages but two were delivered to participant laboratories within 24-72 hours (24 hours for 11 labs, 48 hours for 2 labs, 72 hours for one lab), one package was delivered after 7 days and one delivery date was not reported. At delivery, the internal temperature of the packages was ≤ 13 °C in 15 (94%) parcels; no information was provided from one NRL. The time elapsed from package delivery at the NRL and its control was ≤ 1 h for 14 laboratories, approximately 13 h for 1 laboratory, and it was not report by one laboratory.

8.2 Test methods

Fourteen laboratories tested the serum samples by one method, and two laboratories used two methods (see Annex 6). The methods used by the participants were:

- Indirect Immufluorescent assay
  - "in house" by laboratories B, K and O
  - Toxo-spot IF from Biomerieux by laboratories C and N;
- Enzyme Linked Immunosorbent Assay
  - LSIVET™ ruminant toxoplasmosis serum ELISA kit by laboratory O;
  - ID SCREEN® toxoplasmosis indirect multi-specie by laboratories F, G, J, L, P, Q;
  - IDEXX TOXOTEST Ab by laboratories D and H;
- Latex agglutination
  - "in house" by laboratory I;
  - Toxo-screen from Biomerieux by laboratories C, E and M.

8.3 Evaluation

All participant NRLs sent the results. All NRLs but one, correctly classified (as positive or negative) all serum samples (see Annex 7).

9 Conclusions

All laboratories but one (laboratory 'I') correctly classified all serum samples. All methods used but the "in house agglutination test" used by laboratory 'I', showed a 100% agreement with the expected results. The more frequently used commercial kits were immune-enzymatic assays, and among them, the ID SCREEN® toxoplasmosis indirect multi-species.

10 References

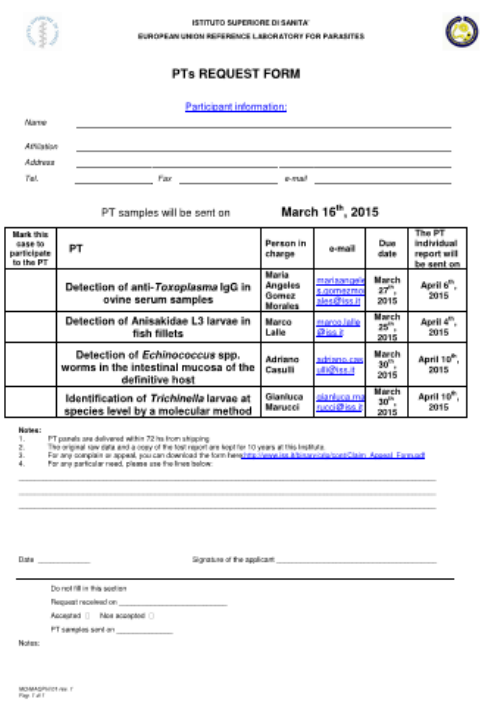
Dubey JP, 2009. Toxoplasmosis of animals and humans. CRC Press, 313 pp.

Esteban-Redondo I, Maley SW, Thomson K, Nicoll S, Wright S, Buxton D, Innes EA, 1999. Detection of *Toxoplasma gondii* in tissues of sheep and cattle following oral infection. Vet Parasitol 86, 155-171..

Jones JL, Dargelas V, Roberts J, Press C, Remington JS, Montoya JG, 2009. Risk factors for Toxoplasma gondii infection in the United States. Clin Infect Dis 49, 878-884.

Annex 1

Participation forms



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EUROPEAN UNION REFERENCE LABORATORY FOR PARASITES

PTs REQUEST FORM

Participant information:

Name _____

Affiliation _____

Address _____

Tel. _____ Fax _____ e-mail _____

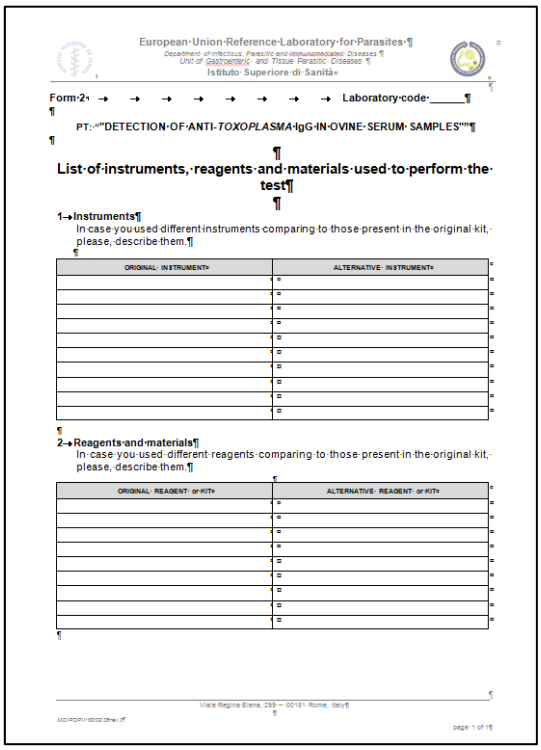
PT samples will be sent on **March 16th, 2015**

Mark this case to participate to the PT	PT	Person in charge	e-mail	Due date	The PT individual report will be sent on
	Detection of anti-*Toxoplasma* IgG in ovine serum samples	Maria Angeles Gomez Morales	maria angeles.gomezmorales@iss.it	March 27th, 2015	April 6th, 2015
	Detection of Anisakidae L3 larvae in fish fillets	Marco Lalle	marco.lalle@iss.it	March 25th, 2015	April 4th, 2015
	Detection of *Echinococcus* spp. worms in the intestinal mucosa of the definitive host	Adriano Casulli	adriano.casulli@iss.it	March 30th, 2015	April 10th, 2015
	Identification of *Trichinella* larvae at species level by a molecular method	Gianluca Marucci	gianluca.marucci@iss.it	March 30th, 2015	April 10th, 2015

Notes:
1. PT panels are delivered within 72 hs from shipping
2. The original raw data and a copy of the test report are kept for 10 years at this Institute.
3. For any complain or appeal, you can download the form Fees http://www.iss.it/binaries/cpue/Claim_Appeal_Form.pdf
4. For any particular need, please use the lines below:

Date _____ Signature of the applicant _____

Do not fill in this section
Request received on: _____
Accepted ☐ Not accepted ☐
PT samples sent on _____
Notes:



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Department of Infectious, Parasitic and Immunomediated Diseases
Unit of Gastroenteric and Tissue Parasitic Diseases
Istituto Superiore di Sanità

Form 2 → → → → → → → → Laboratory code: _____

PT: "DETECTION OF ANTI-*TOXOPLASMA* IgG IN OVINE SERUM SAMPLES"

List of instruments, reagents and materials used to perform the test

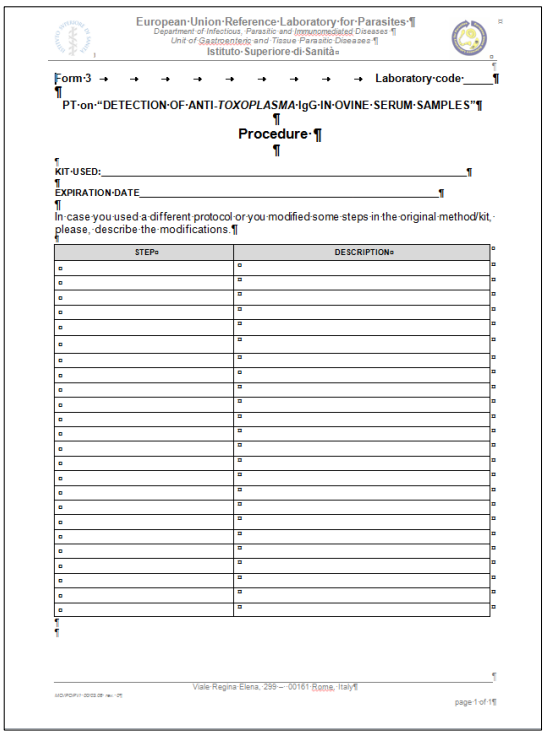
1 Instruments
In case you used different instruments comparing to those present in the original kit, please, describe them.

ORIGINAL INSTRUMENT	ALTERNATIVE INSTRUMENT

2 Reagents and materials
In case you used different reagents comparing to those present in the original kit, please, describe them.

ORIGINAL REAGENT or KIT	ALTERNATIVE REAGENT or KIT

Viale Regina Elena, 299 – 00161 Rome, Italy



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Form 3 → → → → → → → → → Laboratory code: _____

PT on "DETECTION OF ANTI-*TOXOPLASMA* IgG IN OVINE SERUM SAMPLES"

Procedure

KIT USED: _____

EXPIRATION DATE _____

In case you used a different protocol or you modified some steps in the original method/kit, please, describe the modifications.

STEP	DESCRIPTION

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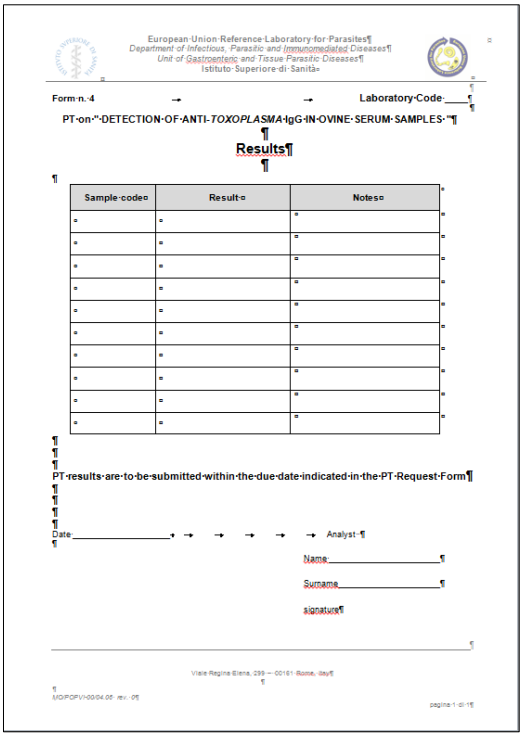

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Form n. 4 → → Laboratory Code: _____

PT on "DETECTION OF ANTI-*TOXOPLASMA* IgG IN OVINE SERUM SAMPLES"

Results

Sample code	Result	Notes

PT results are to be submitted within the due date indicated in the PT Request Form

Date _____ → → → → → Analyst

Name: _____

Surname _____

signature

Viale Regina Elena, 299 – 00161 Rome, Italy

Annex 2

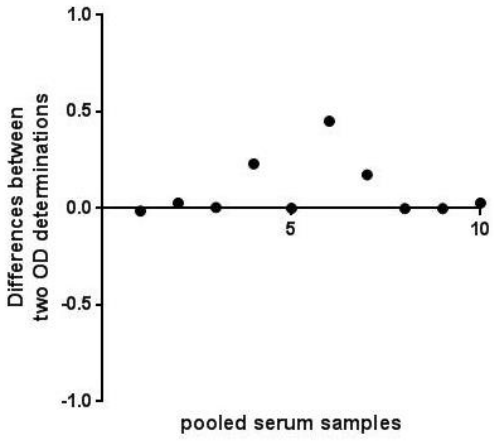
Optical Density values at 450 nm and expected results of the serum sample panel

Serum sample	Mean OD of duplicates	Expected results
1	2.490	positive
2	2.622	positive
3	0.086	negative
4	2.040	positive
5	0.079	negative
6	1.545	positive
7	1.805	positive
8	0.085	negative
9	0.086	negative
10	2.197	positive

Annex 3

Serum samples stability

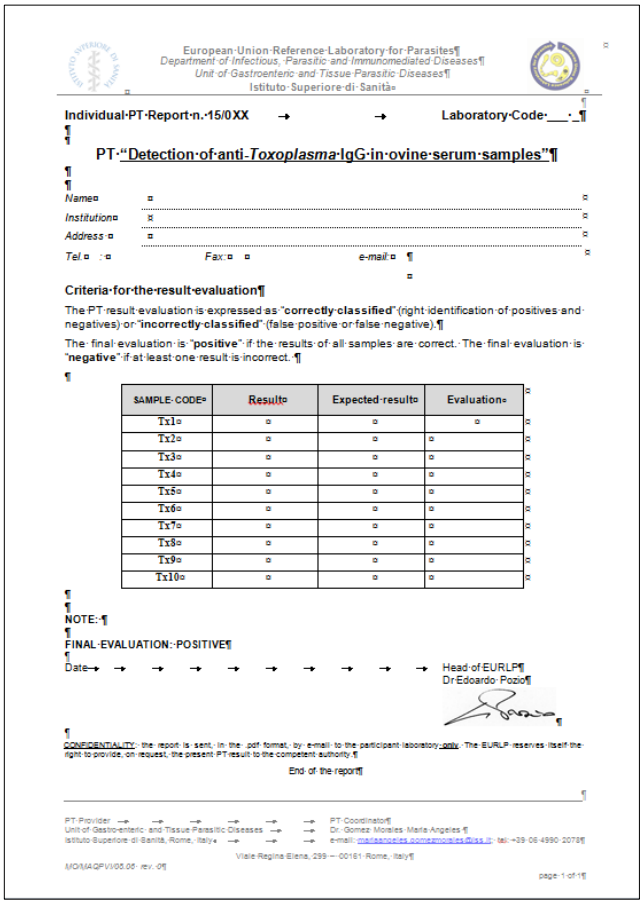
Sample	First test (Mean OD of duplicates)	Second test (Mean OD of duplicates)
1	2.475	2.490
2	2.650	2.622
3	0.090	0.086
4	2.271	2.040
5	0.080	0.079
6	1.997	1.545
7	1.979	1.805
8	0.085	0.085
9	0.086	0.086
10	2.225	2.197



Scatter plot of the differences between the optical density (OD) of two determinations of each pooled of serum samples. OD was recorded at 450 nm.

Annex 4

Report form



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Unit of Gastroenteric and Tissue Parasitic Diseases
Istituto Superiore di Sanità

Individual PT Report n. 15/0XX → → Laboratory Code: ___

PT "Detection of anti-*Toxoplasma* IgG in ovine serum samples"

Name ....
Institution ....
Address ....
Tel. .... *Fax:* .... *e-mail:* ....

Criteria for the result evaluation

The PT result evaluation is expressed as "**correctly classified**" (right identification of positives and negatives) or "**incorrectly classified**" (false positive or false negative).

The final evaluation is "**positive**" if the results of all samples are correct. The final evaluation is "**negative**" if at least one result is incorrect.

SAMPLE CODE	Result	Expected result	Evaluation
Tx1			
Tx2			
Tx3			
Tx4			
Tx5			
Tx6			
Tx7			
Tx8			
Tx9			
Tx10			

NOTE:

FINAL EVALUATION: POSITIVE

Date → → → → → → → → → → Head of EURLP
Dr Edoardo Pozio

CONFIDENTIALITY: the report is sent, in the .pdf format, by e-mail to the participant laboratory only. The EURLP reserves itself the right to provide, on request, the present PT result to the competent authority.

End of the report

PT Provider → → → → → → PT Coordinator
Unit of Gastro-enteric and Tissue Parasitic Diseases → → Dr. Gomez Morales Maria Angeles
Istituto Superiore di Sanità, Rome, Italy → → → e-mail: mariaangeles.gomezmorales@iss.it; tel: +39 06 4990 2078
Viale Regina Elena, 299 – 00161 Rome, Italy

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Annex 5

Participant National Reference Laboratories

Participant institutions	Country
Scientific Institute of Public Health (WIV-ISP), Brussels	Belgium
Estonian Veterinary and Food Laboratory, Tartu	Estonia
Finnish Food Safety Authority, Evira, Oulu	Finland
Laboratory for Animal Health, ANSES, Maisons-Alfort	France
Laboratorio Central de Sanidad Animal, Santa Fe, Granada	Spain
Friedrich-Loffler Institute, Federal Research Institute for Animal Health, Wusterhausen	Germany
Center of Athens Veterinary Institutions, Department of Parasitology, Athens	Greece
Institute of food safety, animal health and environment, Riga	Latvia
National Food and Veterinary Risk Assessment Institute, Vilnius	Lithuania
Faculty of Veterinary Medicine, Skopje	Macedonia
National Institute of Public Health and the Environment, RIVM, Bilthoven	Netherlands
National Veterinary Research Institute, Pulawy	Poland
National Laboratory of Veterinary Research, Lisboa	Portugal
Institute for Diagnosis and Animal Health, Bucharest	Romania
Institute for Medical Research, University of Belgrade, Belgrade	Serbia
Veterinary Faculty, University of Ljubljana, Ljubljana	Slovenia
Institute of Parasitology, University of Bern, Bern	Switzerland

Annex 6

Serological methods used by the participant laboratories

Method	In house/commercial kit	NRL code
IFA	in house	B, K, O
	Toxo-spot, Biomerieux	C, N
ELISA	LSIVET™ ruminant toxoplasmosis	O
	ID SCREEN® toxoplasmosis	F, G, J, L, P, Q
	IDEXX TOXOTEST Ab	D, H
Latex agglutination	in house	I
	Toxo-screen IF, Biomerieux	C, E, M

Annex 7

Proficiency testing results

Sample code	Expected results	NRL codes															
		B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Tx1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tx2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tx3	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Tx4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tx5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tx6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tx7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tx8	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Tx9	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Tx10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+