



European Union Reference Laboratory for Parasites

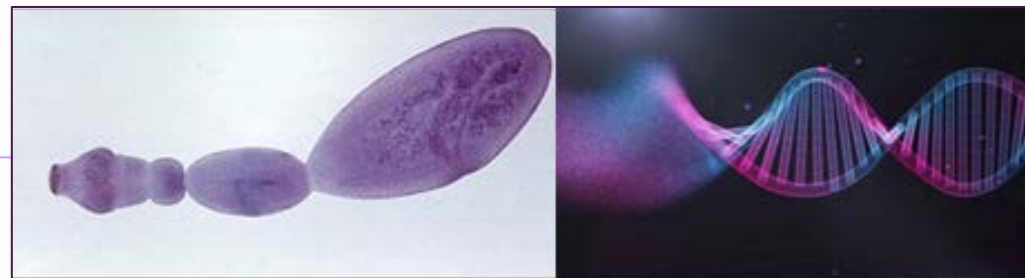
Unit of Foodborne and Neglected Parasitic Diseases

Department of Infectious Diseases

ISTITUTO SUPERIORE DI SANITÀ

7th PROFICIENCY TESTING

Molecular identification of *Echinococcus* spp. at the species level



XIX Workshop of National Reference Laboratories for Parasites

6th and 7th November 2024

Istituto Superiore di Sanità

Federica Santolamazza, Azzurra Santoro

European Union Reference Laboratory for Parasites (EURLP);

WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis;

ISTITUTO SUPERIORE DI SANITÀ (Rome, Italy)





PT-08 Molecular identification of Echinococcus at the species level-2024

Due date to submit results: April 12, 2024

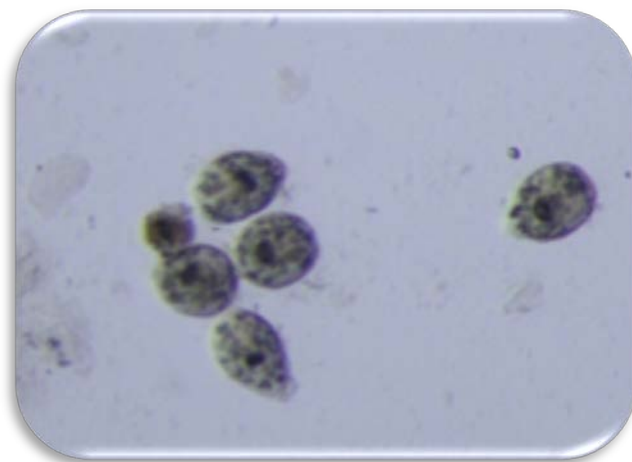
Individual report sent to participants within: April 30, 2024

Final report available on EURLP website from: May 31, 2024



Aim of the PT

Evaluation of laboratories competence in molecular identification of
Echinococcus granulosus sensu lato
and
Echinococcus multilocularis





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PT timing 2024



February 21st




 Istituto Superiore di Sanità
 Department of Infectious Diseases
 Unit of Foodborne and Neglected Parasitic Diseases
 European Union Reference Laboratory for Parasites

PTs REQUEST FORM 2024

<https://www.iss.it/en/web/iss-en/eurlp-proficiency-testing>

March 11th



April 12th

**DEADLINE
RESULT
REPORTING**

April 30th

Individual Report PT-08


 Istituto Superiore di Sanità
 Department of Infectious Diseases
 Unit of Foodborne and Neglected Parasitic Diseases
 European Union Reference Laboratory for Parasites

Individual PT Report n. _____ Laboratory Code _____

PT "Detection of Anisakidae L3 larvae in fish fillets"

Name _____
 Institution _____
 Address _____
 Tel. _____ Fax _____ e-mail _____

Criteria for the result evaluation
 The PT result evaluation is expressed as "correct" (right) for positives and negatives or "incorrect" (false positive or false negative).
 The final evaluation is "positive" if the number of correct results is greater than the number of incorrect results.
 "negative" if at least one result is incorrect.

SAMPLE CODE	Result (N° of detected larvae)	Evaluation
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

EVALUATION:
 Recommendations: _____
 Date _____ Head of EURLP Dr S.M. Cassi

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

End of the report

PT Provider: Unit of Foodborne and Neglected Parasitic Diseases, Istituto Superiore di Sanità, Viale Regina Elena, 280 - 00161 Rome, Italy
 PT Coordinator: Dr. Maria Lilla, e-mail: maria.lilla@iss.it, tel. +39 06 4980 2070
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May 31st


 Istituto Superiore di Sanità
 Department of Infectious Diseases
 Unit of Foodborne and Neglected Parasitic Diseases
 European Union Reference Laboratory for Parasites

Final report PT-04: An 1/2021
PT-04: "Detection of Anisakidae L3 larvae in fish fillets"

Design

Purpose	Evaluation of laboratories in charge of official control on fish
Scheme type	Single, simultaneous
Participants	Public and private, European laboratories
N° of participants	Depending on request
Method	not regulated
Test method	chosen by the participant
Matrix	3 fish fillet sandwiches
Item	Anisakidae live larvae
N° of samples	3 for each participant
Subcontracted activity	Immediate on-site preparation
Results evaluation	Positive

Information

PT Provider	30	fish fillet sandwiches	93
PT Coordinator	2	PT panel composition	3 fish fillet sandwiches: one spiked with 1 larva, two spiked with 3 larvae
NRL	28	Shipping	CHL
Shipping date	15/03/2021		

PT Provider: Unit of Foodborne and Neglected Parasitic Diseases, Istituto Superiore di Sanità, Viale Regina Elena, 280 - 00161 Rome, Italy
 PT Coordinator: Dr. Maria Lilla, e-mail: maria.lilla@iss.it, tel. +39 06 4980 2070

ACCREDITED
 PT-04: An 1/2021
 Member: Dr. Maria Lilla, e-mail: maria.lilla@iss.it, tel. +39 06 4980 2070
 Signature of Dr. Lilla and Dr. Lilla
 Italian Recognition Agreement





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Preparation of samples PT08-2024

The PT has been organized following the NRL request (2023)

The panel consists of 3 tubes:

each tube containing DNA extracted from canine faecal stool **spiked** with

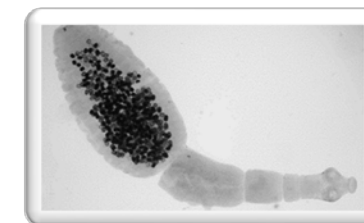
- *E. granulosus* s.s. cyst
- *E. multilocularis* worms
- DNA extracted from *Trichinella* spp. larvae



E. granulosus cysts collected from human hosts



E. multilocularis worms collected from foxes



The negative control was DNA extracted from *Trichinella* spp. larvae





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Detection method PT08-2024

The samples were identified at species level through multiplex PCR

E.g. = *E. granulosus* (117 bp)

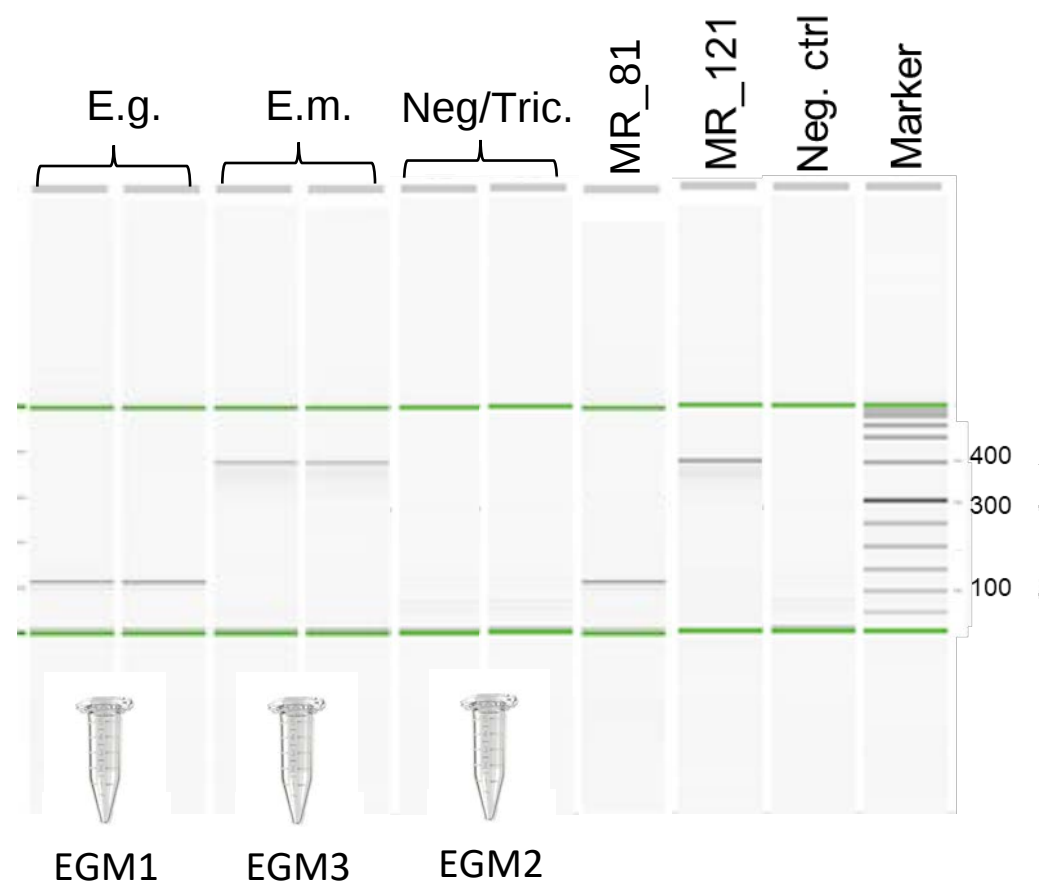
E.m. = *E. multilocularis* (395 bp)

Negative = *Trichinella*

EURLP-Reference Material

MR_81 = *E. granulosus* (G3)

MR_121 = *E. multilocularis*



Multiplex PCR (Trachsel *et al.* 2007)

In 30 μ l total reaction with 0.2 mM of each primer (Cest1, Cest2, Cest3, Cest4, Cest5)





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Detection method PT08-2024

Homogeneity was ensured by providing participants with aliquots of the same DNA preparation



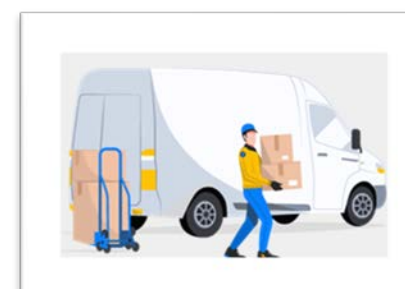
The tubes were plugged and sealed using plastic parafilm, individually coded



Each PT panel was inserted in polystyrene box with ice pack



All panels were delivered within 24-36 hours





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Evaluation criteria

The PT evaluation is qualitative and no statistical analysis of the results are applied

Lab code	Expected	Observed	Result (correct/incorrect)	Evaluation (positive/negative)
EGM X	<i>E. granulosus</i> negative <i>E. multilocularis</i>	<i>E. granulosus</i> negative <i>E. multilocularis</i>	correct correct correct	Positive
EGM X	<i>E. granulosus</i> negative <i>E. multilocularis</i>	<i>E. granulosus</i> <i>E. granulosus</i> <i>E. multilocularis</i>	correct incorrect correct	Negative
EGM X	<i>E. granulosus</i> negative <i>E. multilocularis</i>	<i>E. granulosus</i> negative <i>E. granulosus</i>	correct correct incorrect	Negative

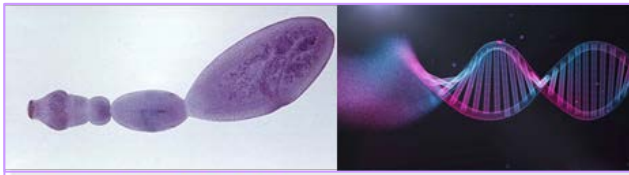
The result is “correct” if the PT items are correctly identified

The result is “incorrect” if the PT items are incorrectly identified

The PT is considered “POSITIVE” if the results of all samples are “correct”

The PT is considered “NEGATIVE” if at least one result is “incorrect”



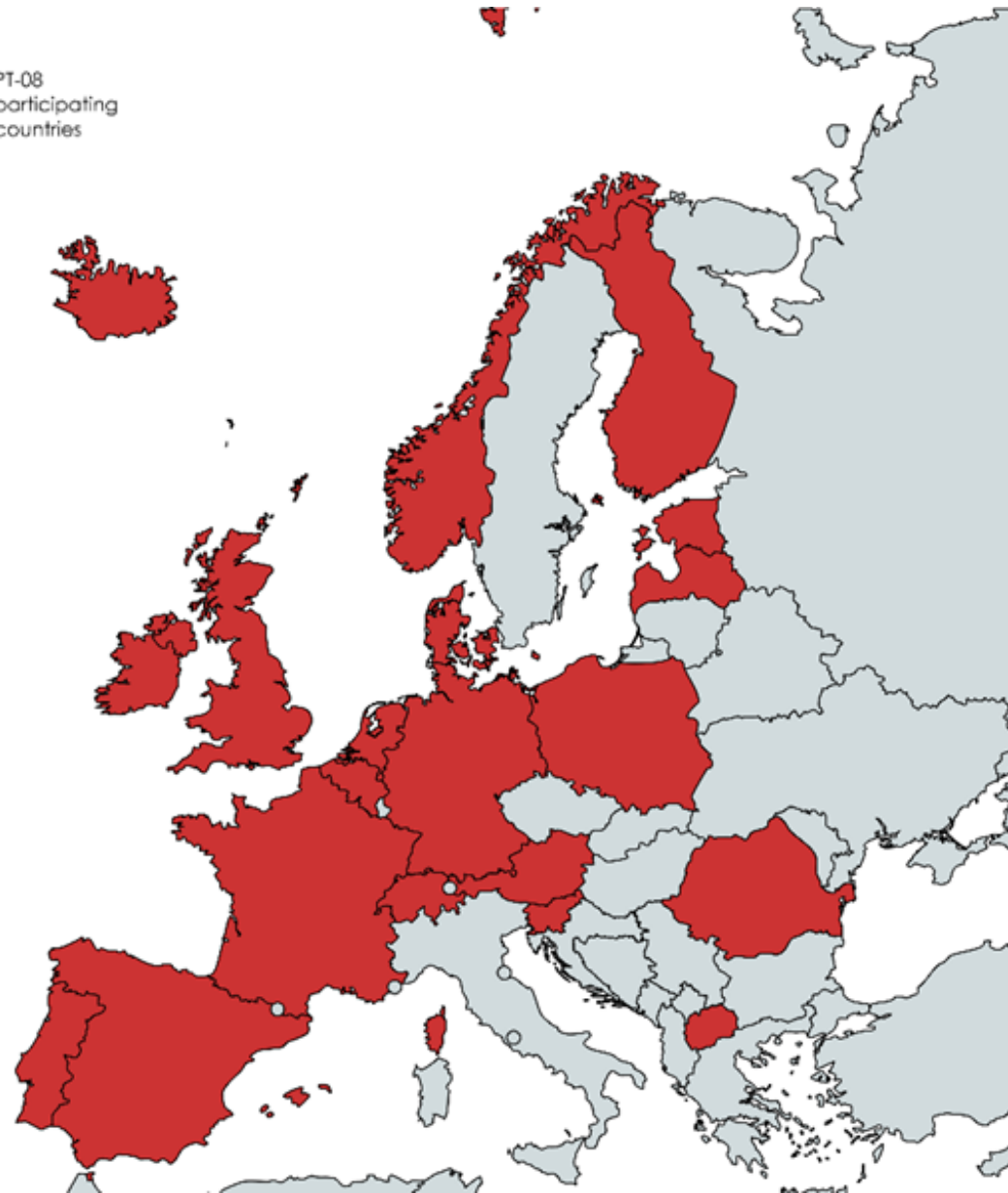


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Participants

PT-08 participating countries



Austria	Austria NRLP, Austrian agency for health and food safety
Belgium	Belgium NRLP, Institute of Tropical Medicine
Denmark	Denmark NRLP, Statens Serum Institut, laboratory of parasitology, SSI
Estonia	Estonian NRLP, Animal Health, Veterinary and Food Laboratory
Finland	Finland NRLP, Oulu, Finnish Food Authority, Ruokavirasto (ex Evira)
France	France NRL Echinococcus, ANSES, LRFS Nancy
Germany	Germany NRL Echinococcus, Friedrich-Loeffler-Institut fur Epidemiologie
Iceland	Iceland, Institute for Experimental Pathology at Keldur
Ireland	Ireland NRLP, Parasit section, Bact/Paras Division, Backweston Campus, Celbridge Kildare
Latvia	Latvia NRLP, Institute of food safety, animal health and environment, BIOR
Norway	Norway NRLP, Norwegian Veterinary Institute
Poland	Poland NRLP, National Veterinary Research Institute , Department of Parasitology and Invasive Diseases
Portugal	Portugal NRLP, Instituto nacional de investigacao agraria e veterinaria
Republic of North Macedonia	Republic of North Macedonia, Faculty of Veterinary Medicine, Skopje
Romania	Romania NRLP, Institute for Diagnosis and Animal Health GMO and Molecular Biology Unit
Slovenia	Slovenian NRLP, University of Ljubljana, Veterinary Faculty
Spain	Spain NRLP, Laboratorio Central de Sanidad Animal
Switzerland	Switzerland, Institute of Parasitology University of Bern
The Netherlands	The Netherlands NRLP, National Institute for Public Health and the Environment (RIVM)
United Kingdom	UK NRL for Trichinella and Echinococcus, Animal and Plant Health Agency, York (APHA)





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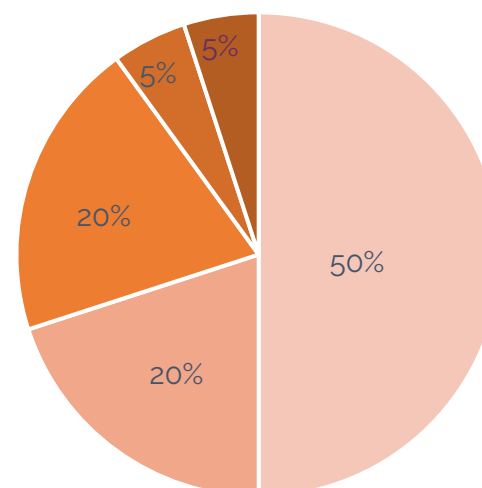
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Detection method applied

The EURLP recommends the use of the multiplex PCR by Trachsel *et al.* however, any other suitable molecular-based method is accepted

Method applied	N. labs
Trachsel <i>et al</i> , 2007 (multiplex PCR)	10
Trachsel <i>et al</i> 2007 + other methods	4
EURLP method (MI-15) + sequencing or + Real Time PCR (Øines <i>et al</i> , 2014)	4
Geysen <i>et al</i> , 2007 (PCR RFLP) + sequencing	1
In house method (PCR RFLP)	1





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Results

Participants N=20

Participants passed N=17

Participants failed N=3



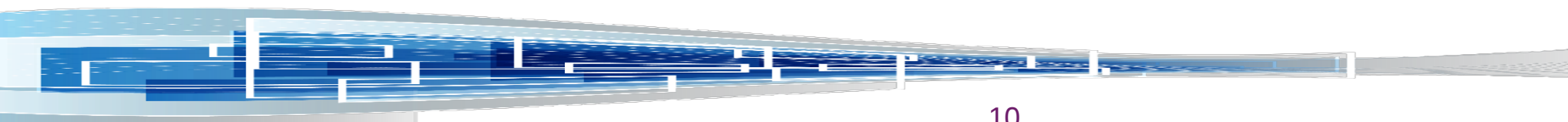
EGM1 Sample 1 (*E. granulosus* s. l.): 19 labs (95%) out of 20 obtained a positive evaluation



EGM2 Sample 2 (negative): 19 labs (95%) out of 20 obtained a positive evaluation



EGM3 Sample 3 (*E. multilocularis*): 18 labs (90%) out of 20 obtained a positive evaluation





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Results



Lab Code	Expected	Observed	Result (correct/incorrect)	Evaluation (positive/negative)	Lab Code	Expected	Observed	Result (correct/incorrect)	Evaluation (positive/negative)
EGM1	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive	EGM31	<i>E. granulosus</i>	negative	incorrect	negative
EGM2	negative	negative	correct		EGM32	negative	negative	correct	
EGM3	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct		EGM33	<i>E. multilocularis</i>	-	incorrect	
EGM4	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive	EGM34	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive
EGM5	negative	negative	correct		EGM35	negative	negative	correct	
EGM6	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct		EGM36	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct	
EGM7	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive	EGM37	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	negative
EGM8	negative	negative	correct		EGM38	negative	negative	correct	
EGM9	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct		EGM39	<i>E. multilocularis</i>	<i>E. granulosus</i>	incorrect	
EGM10	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive	EGM40	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive
EGM11	negative	negative	correct		EGM41	negative	negative	correct	
EGM12	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct		EGM42	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct	
EGM13	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive	EGM43	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive
EGM14	negative	negative	correct		EGM44	negative	negative	correct	
EGM15	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct		EGM45	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct	
EGM16	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive	EGM46	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive
EGM17	negative	negative	correct		EGM47	negative	negative	correct	
EGM18	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct		EGM48	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct	
EGM19	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	negative	EGM49	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive
EGM20	negative	<i>E. granulosus</i>	incorrect		EGM50	negative	negative	correct	
EGM21	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct		EGM51	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct	
EGM22	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive	EGM52	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive
EGM23	negative	negative	correct		EGM53	negative	negative	correct	
EGM24	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct		EGM54	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct	
EGM25	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive	EGM55	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive
EGM26	negative	negative	correct		EGM56	negative	negative	correct	
EGM27	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct		EGM57	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct	
EGM28	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive	EGM58	<i>E. granulosus</i>	<i>E. granulosus</i>	correct	positive
EGM29	negative	negative	correct		EGM59	negative	negative	correct	
EGM30	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct		EGM60	<i>E. multilocularis</i>	<i>E. multilocularis</i>	correct	





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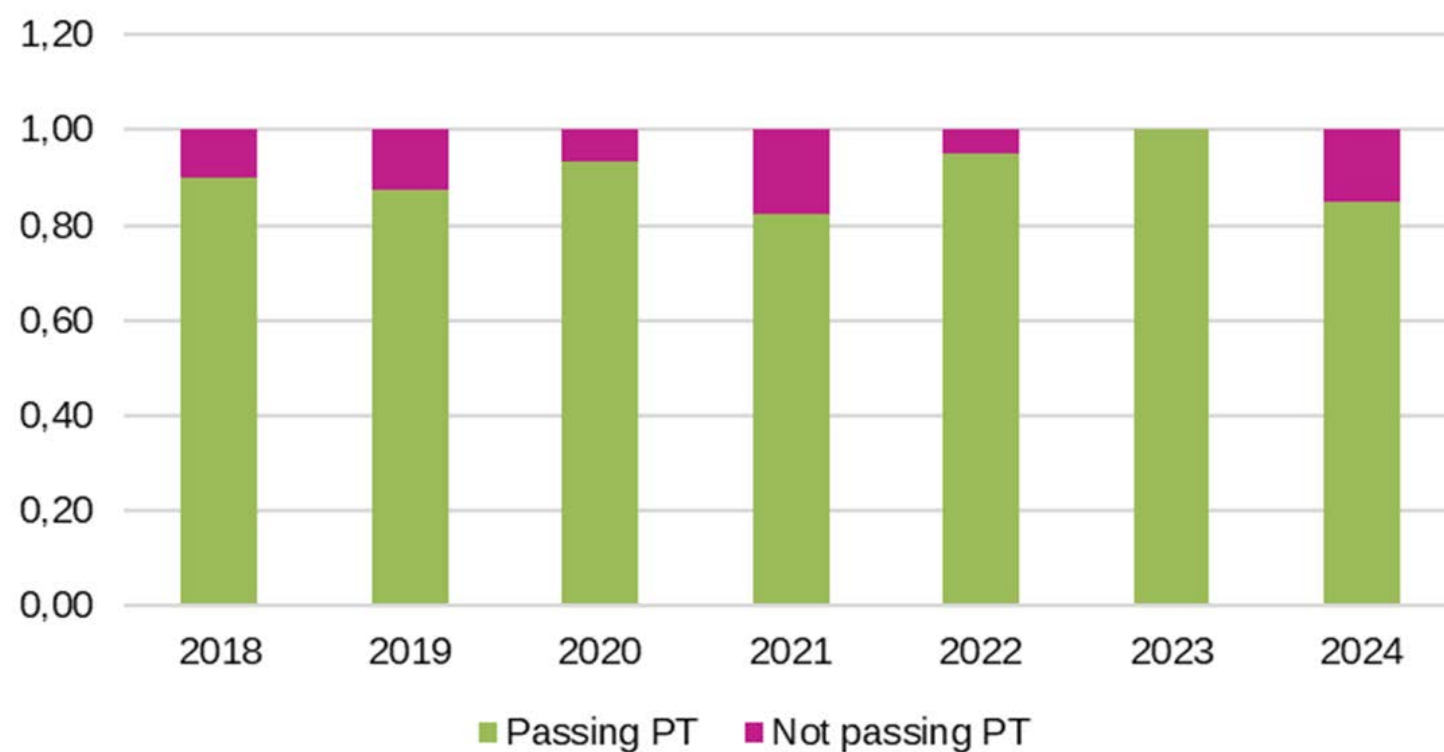
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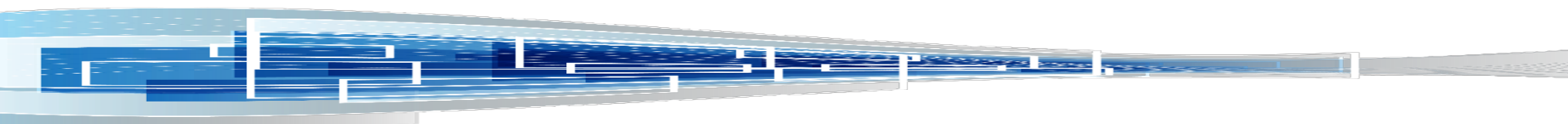
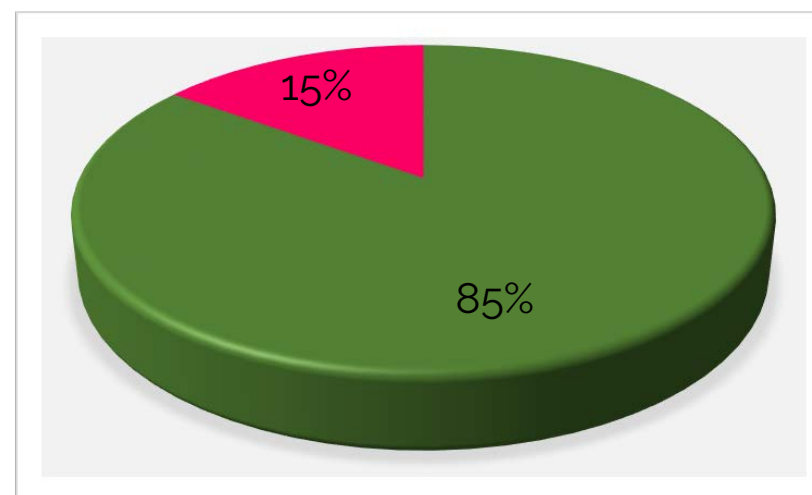
Results



PT-08 trend overs years



2024





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Conclusions

85% laboratories passed the Proficiency Testing 2024

95% of participants correctly identified the sample with DNA of *E. granulosus* s.l.

90% succeeded in the identification of the sample with DNA of *E. multilocularis*

95% detected the negative sample

Recommendations

- to optimize multiplex PCR settings to avoid unspecific amplifications and to sequence amplicons to confirm the identification
- to select the most suitable protocol for the identification of the two species covered by the Proficiency Testing



Thanks for your attention



ACKNOWLEDGMENTS

Adriano Casulli
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Azzurra Santoro
Irene Tartarelli



EURLP