

XVIII Workshop of National Reference Laboratories for Parasites
16th and 17th November 2023
Istituto Superiore di Sanità

5th PROFICIENCY TESTING

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

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WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis;
ISTITUTO SUPERIORE DI SANITÀ (Rome, Italy)

Aim of the PT



PT-08 Molecular identification of *Echinococcus*
at the species level-2023

Due date to submit results: April 14, 2023

Individual report sent to participants within: May 1, 2023

Final report available on EURLP website from: May 31, 2023

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

PT timing 2023

January 10th



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



PTs REQUEST FORM 2023

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

March 13th



April 14th

DEADLINE
RESULT
REPORTING

May 1st

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 identification of positives (negatives) or "incorrect" (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	N° of samples	Result	Evaluation

NOTE: _____

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 the right to provide, on request, the present PT result to the competent authority.

End of the report

PT Provider: _____ PT Coordinator: _____
Unit of Foodborne and Neglected Parasitic Diseases Dr. Maria Lallo
Istituto Superiore di Sanità, Italy e-mail: eurp@iss.it Tel: +39 06 4980 0874
Via Regina Elena, 299 - 00158 Rome, Italy

May 31st

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 control of food
Scheme type	Single, simultaneous
Participants	Public and private, European countries
N. of participants	Depending on request
Method	not regulated
Test method	chosen by the participant
PT items	fish fillet sandwiches
N. of samples	3 fish fillet sandwiches
Distribution	one splited with 1 larva, two splited with 3 larvae
Shipping	immediate shipment after preparation
NA	

Implementation

N. of participants	30	fish fillet sandwiches	93
Public laboratories	0		
Private laboratories	2		
PT items		3 fish fillet sandwiches: one splited with 1 larva, two splited with 3 larvae	
NRL	28	Shipping	CH4
Shipping dates	15/03/2021		

PT Provider: _____
Unit of Foodborne and Neglected Parasitic Diseases
Istituto Superiore di Sanità
Via Regina Elena, 299 - 00158 Rome, Italy
PT Person in charge: Dr. Maria Lallo
e-mail: eurp@iss.it Tel: +39 06 4980 0874

ACQUA
PTP n° 0000 P
Revised 2021/2022
Signature of Dr. M.L. and Dr. L. Lallo
Revised 2021/2022

Preparation of samples PT08-2023



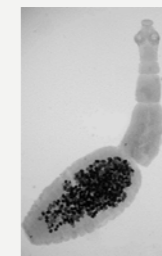
PT panel consists of 3 tubes, each containing 10 μ l DNA of *E. granulosus* s.s., *E. multilocularis*, and *Taenia* spp.

Echinococcus granulosus cysts were collected from human hosts



Echinococcus multilocularis worms were collected from foxes

Taenia crassiceps metacestode were collected from rodent



The samples were stored in 70% ethanol
A commercial kit was used for the DNA extraction

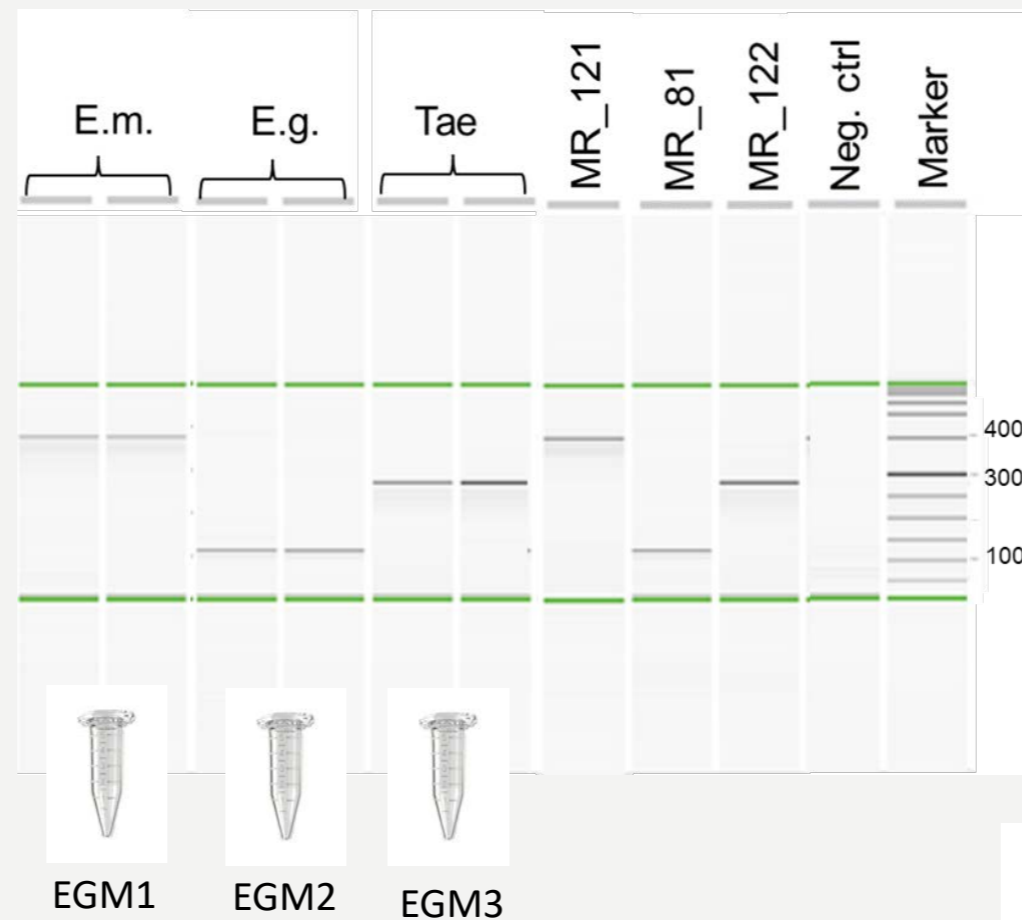




Detection method PT08-2023



The samples were individually identified at species level through multiplex PCR



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

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

Tae = *Taenia* spp (267 bp)

Reference Material EURLP

MR_121= *E. multilocularis*

MR_81 = *E. granulosus* (G3)

MR_122 = *Taenia crassiceps*

Multiplex PCR (Trachsel *et al.* 2007)

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



University of
Zurich

Zurich Open Repository and
Archive
University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2007

Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR
using targets in mitochondrial DNA

Trachsel, D ; Deplazes, P ; Mathis, A



Test material



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



Evaluation criteria

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

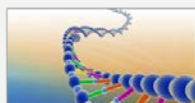
The result is “correct” if the PT items are correctly identified

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

Lab code	Expected	Observed	Result (correct/incorrect)	Evaluation (positive/negative)
Ed x	Eg	Eg	correct	Positive
	Em	Em	correct	
	neg	neg	correct	
Ed xx	Eg	Eg	correct	Negative
	Em	Em	correct	
	Eg	neg	incorrect	
Ed xxx	Eg	Em	incorrect	Negative
	Em	Em	correct	
	neg	neg	correct	

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

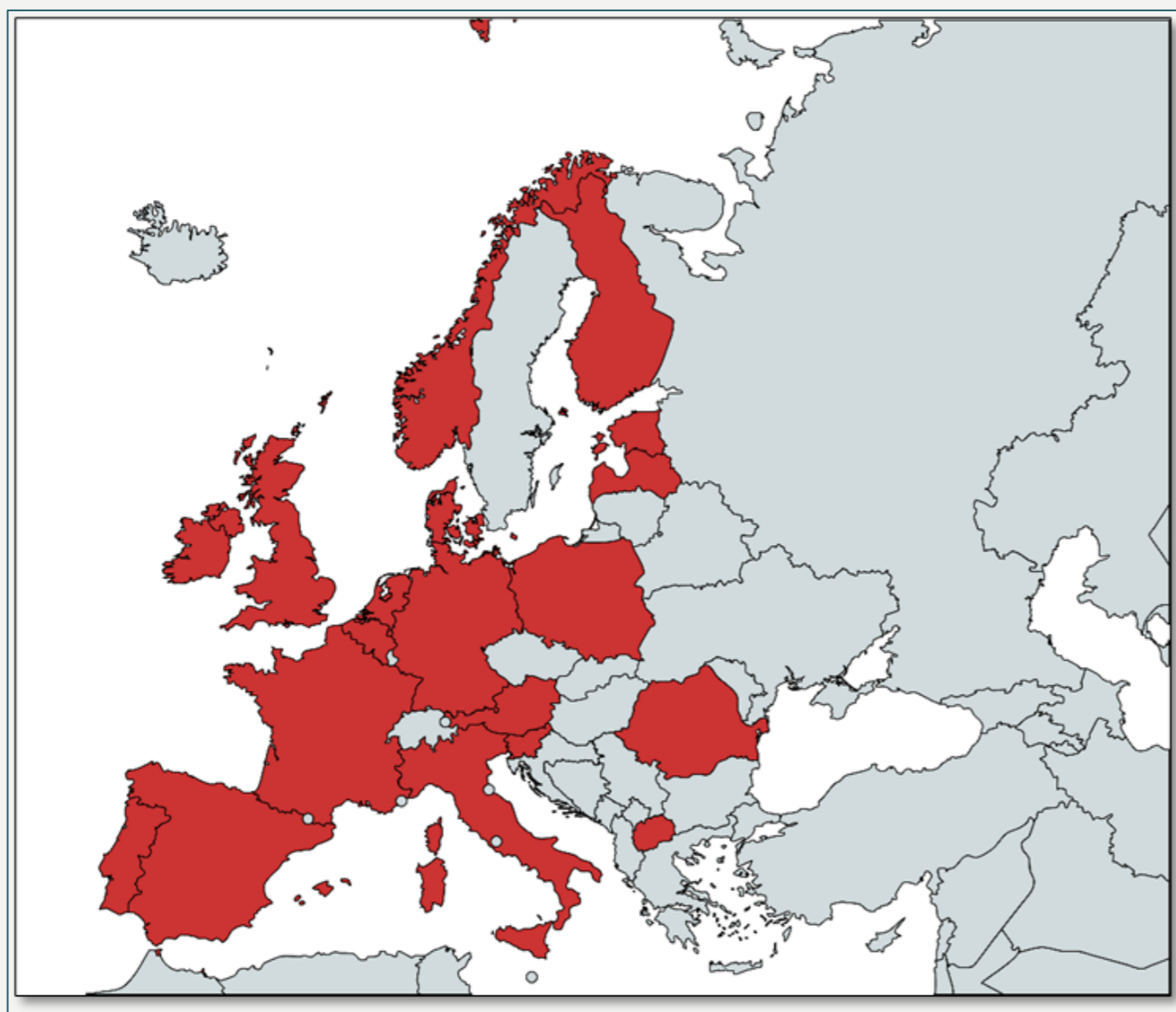
The PT is considered “NEGATIVE” if at least one result is “incorrect”



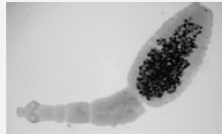
PT-08 Molecular identification of Echinococcus at the species level-2023

Due date to submit results: April 14, 2023
 Individual report sent to participants within: May 1, 2023
 Final report available on EURLP website from: May 31, 2023

Participants (N=19) 19 labs submitted results



Belgium NRLP , Institute of Tropical Medicine
Denmark NRLP , Statens Serum Institut, laboratory of parasitology
Estonian NRLP , Animal Health, Veterinary and Food Laboratory
Finland NRLP , Oulu, Finnish Food Authority, Ruokavirasto
France NRL Echinococcus , ANSES, LRFS Nancy
Germany NRL Echinococcus , Friedrich-Loeffler-Institut fur Epidemiologie
Ireland NRLP , Parasit section, Bact/Paras Division, Backweston Campus, Celbridge Kildare
Italy NRL Echinococcus , IZS Istituto Zooprofilattico Sperimentale della Sardegna
Latvia NRLP , Institute of food safety, animal health and environment, BIOR
Norway NRLP , Norwegian Veterinary Institute
Poland NRLP , National Veterinary Research Institute , Department of Parasitology and Invasive Diseases
Portugal NRLP , Instituto nacional de investigacao agraria e veterinaria
Republic of North Macedonia , Faculty of Veterinary Medicine, Skopje
Romania NRLP , Institute for diagnosis and animal health
Slovak Republic NRLP , Veterinary and Food Institute in Bratislava
Slovenian NRLP , University of Ljubljana, Veterinary Faculty
Spain NRLP , Laboratorio Central de Sanidad Animal
The Netherlands NRLP , National Institute for Public Health and the Environment (RIVM)
UK NRL for Trichinella and Echinococcus , Animal and Plant Health Agency, York

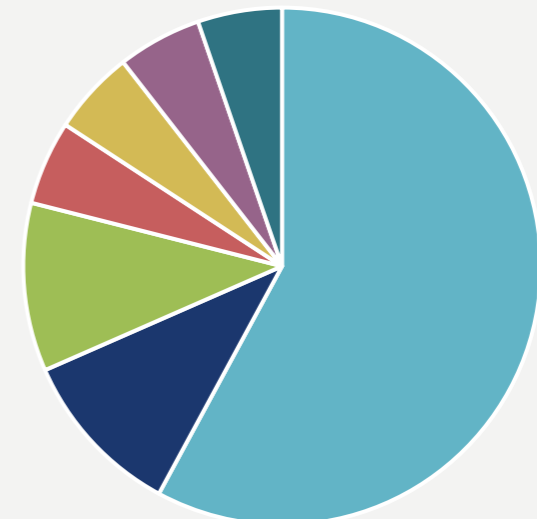


Detection method

The EURLP recommends the use of the multiplex PCR assay developed by Trachsel *et al.*



Method applied	N. labs
Multiplex PCR (Trachsel <i>et al.</i> , 2007)	11
EURLP method	2
EURLP method + in house PCR + sequencing	2
EURLP method + Real Time PCR (Øines <i>et al.</i> , 2014)	1
Semi-nested PCR+ sequencing (Geysen <i>et al.</i> , 2007)	1
PCR_RFLP (in house method)	1
PCR (in house method) + sequencing	1



RESULTS

Number of participant laboratories	19
Number of participants that passed the PT	19
Number of participants that failed the PT	0

Sample **1** (EGM1)- *E. multilocularis*: 19 labs (100%) out of 19 obtained a positive evaluation

Sample **2** (EGM2)- *E. granulosus s.l.*: 19 labs (100%) out of 19 obtained a positive evaluation

Sample **3** (EGM3)- *Taenia crassiceps* (negative): 19 labs (100%) out of 19 obtained a positive evaluation

RESULTS

Laboratory code	N° of samples correctly identified	N° of samples NOT correctly identified	Final evaluation
Ed1	3	0	Positive
Ed2	3	0	Positive
Ed3	3	0	Positive
Ed4	3	0	Positive
Ed5	3	0	Positive
Ed6	3	0	Positive
Ed7	3	0	Positive
Ed8	3	0	Positive
Ed9	3	0	Positive
Ed10	3	0	Positive
Ed11	3	0	Positive
Ed12	3	0	Positive
Ed13	3	0	Positive
Ed14	3	0	Positive
Ed15	3	0	Positive
Ed16	3	0	Positive
Ed17	3	0	Positive
Ed18	3	0	Positive
Ed19	3	0	Positive

Conclusions

The experience derived from the fifth PT carried out in 2023 on the molecular detection of *Echinococcus* spp. showed that the personnel of NRLs are skilled to detect this parasite in a qualitative test



100% of participants succeeded in the identification of *Echinococcus multilocularis*, *Echinococcus granulosus* s. l. and the negative sample

*THANKS FOR YOUR
ATTENTION*

