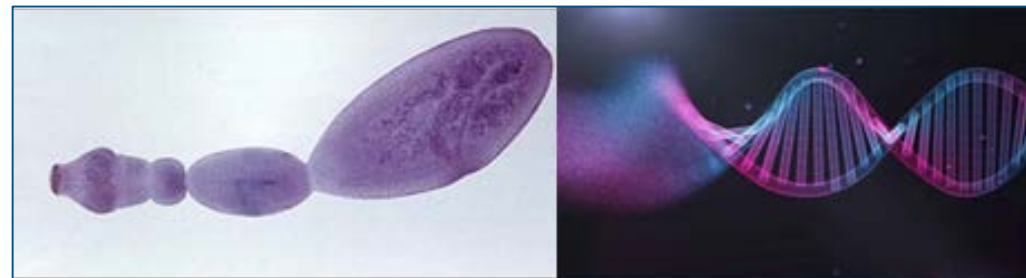


4th PROFICIENCY TESTING

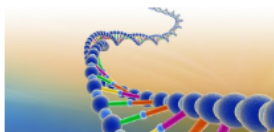
“Molecular identification of *Echinococcus* spp. at the species level”



XVII Workshop of National Reference
Laboratories for Parasites
15th and 16th september 2022
Istituto Superiore di Sanità

Federica Santolamazza, Azzurra Santoro, Adriano Casulli

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-2022

Due date to submit results: April 15, 2022

Individual report sent to participants within: May 2, 2022

Final report available on EURLP website from: May 31, 2022



Aim of the PT

Evaluation of laboratories competence in molecular identification of

Echinococcus granulosus sensu lato

and

Echinococcus multilocularis

PT timing 2022

January 25th



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

PTs REQUEST FORM 2022

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

March 14th



April 15th

**DEADLINE
RESULT
REPORTING**

April 29th

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 filets"

Name _____
Institution _____
Address _____
Tel _____ Fax _____ email _____

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

SAMPLE CODE	Result (N° of detected larvae)	Evaluation

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: Istituto Superiore di Sanità, Via Regina Elena, 286 - 00158 Roma, Italy
PT Coordinator: Dr. Maria Lupo, Via Regina Elena, 286 - 00158 Roma, Italy

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 filets"

Design

Field	Value
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	fish fillets, farmed fish fillets
Item	fish fillets, frozen live larvae
N. of samples	3 for each path
Subcontracted activities	Immediate shipment after preparation
Results evaluation	Blind
Accreditation	ISO 9001:2015
PT panel composition	3 fish fillet sandwiches, one spiked with 1 larva, two spiked with 3 larvae
Shipping	DHL
Shipping date	15/03/2021

PT Provider: Istituto Superiore di Sanità, Via Regina Elena, 286 - 00158 Roma, Italy
PT Coordinator: Dr. Maria Lupo, Via Regina Elena, 286 - 00158 Roma, Italy

ACCREDITED
PTP N° 2000-P
Istituto Superiore di Sanità
Via Regina Elena, 286 - 00158 Roma, Italy
PT Panel in charge: Dr. Maria Lupo, Via Regina Elena, 286 - 00158 Roma, Italy

**Final Report PT-08:
ECMOI 1/2022**



Preparation of samples PT08-2022



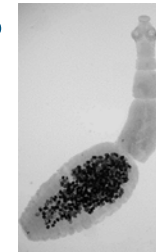
PT panel consists of 3 tubes, each containing 10 μ l DNA of *E. granulosus* s.s., *E. multilocularis*, and *Taenia* spp.

Echinococcus granulosus cysts were collected from human hosts



Echinococcus multilocularis worms were collected from foxes

Taenia spp. worms were collected from canids



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

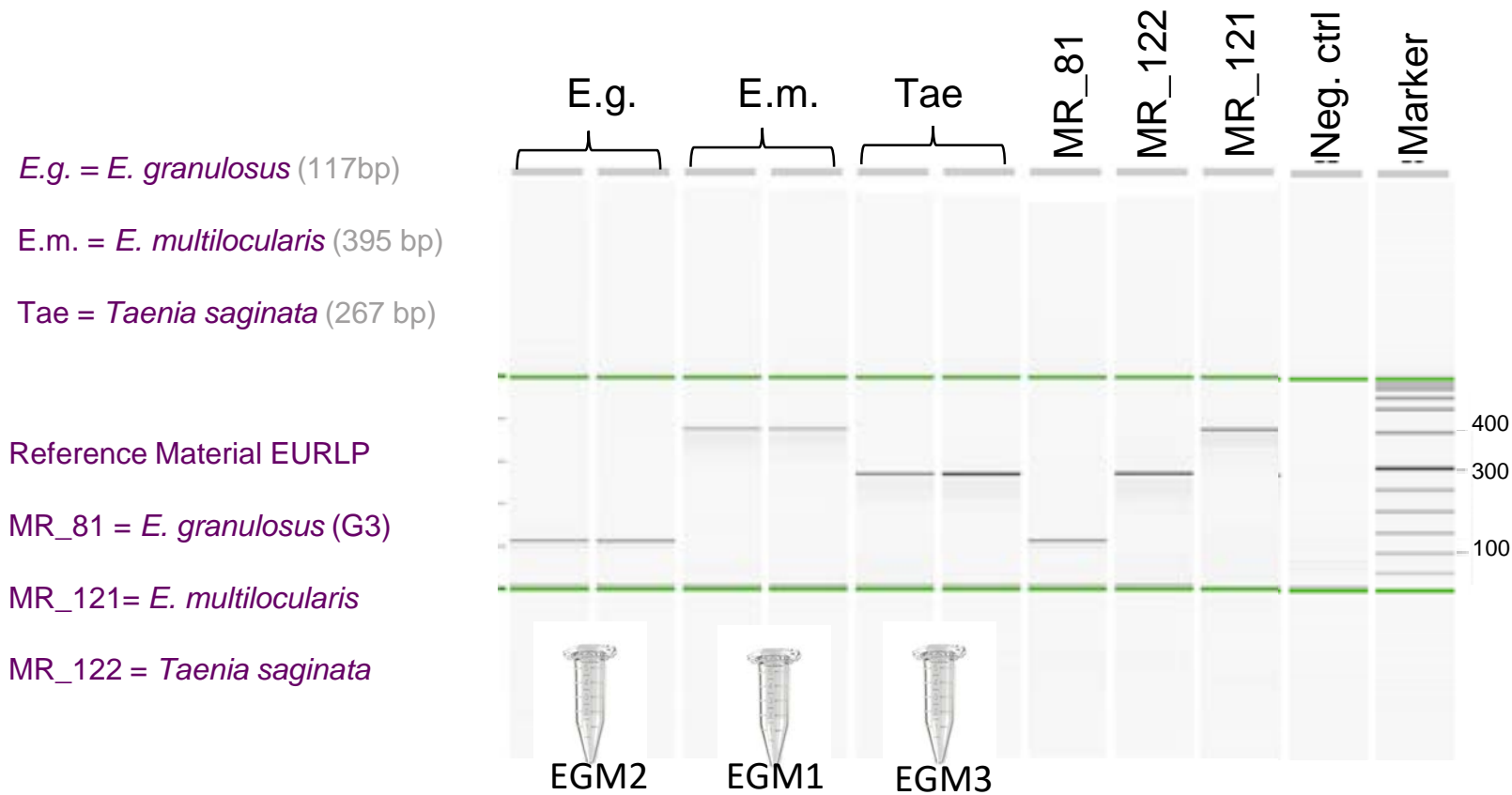




Detection method PT08-2022

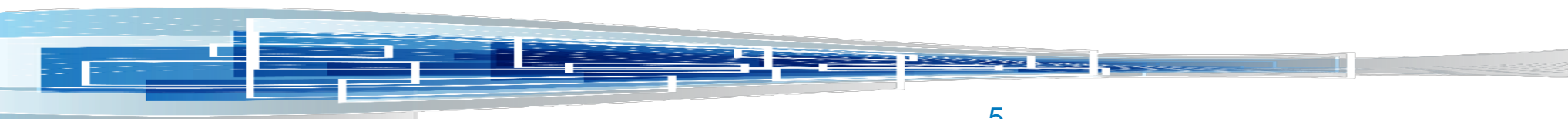


The samples were individually identified at species level through multiplex PCRs



Multiplex PCR (Trachsel *et al.* 2007)

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





Preparation of samples PT08-2022



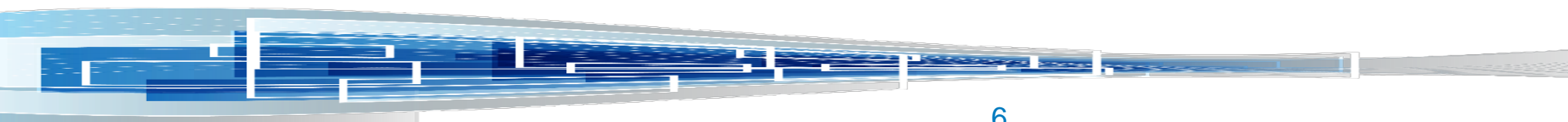
Homogeneity was ensured by providing participants with aliquots of the same DNA preparation



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

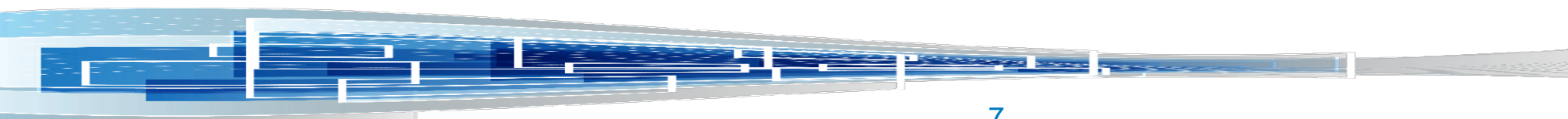
The result is “**correct**” if the PT items are correctly identified

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	
	neg	Eg	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”



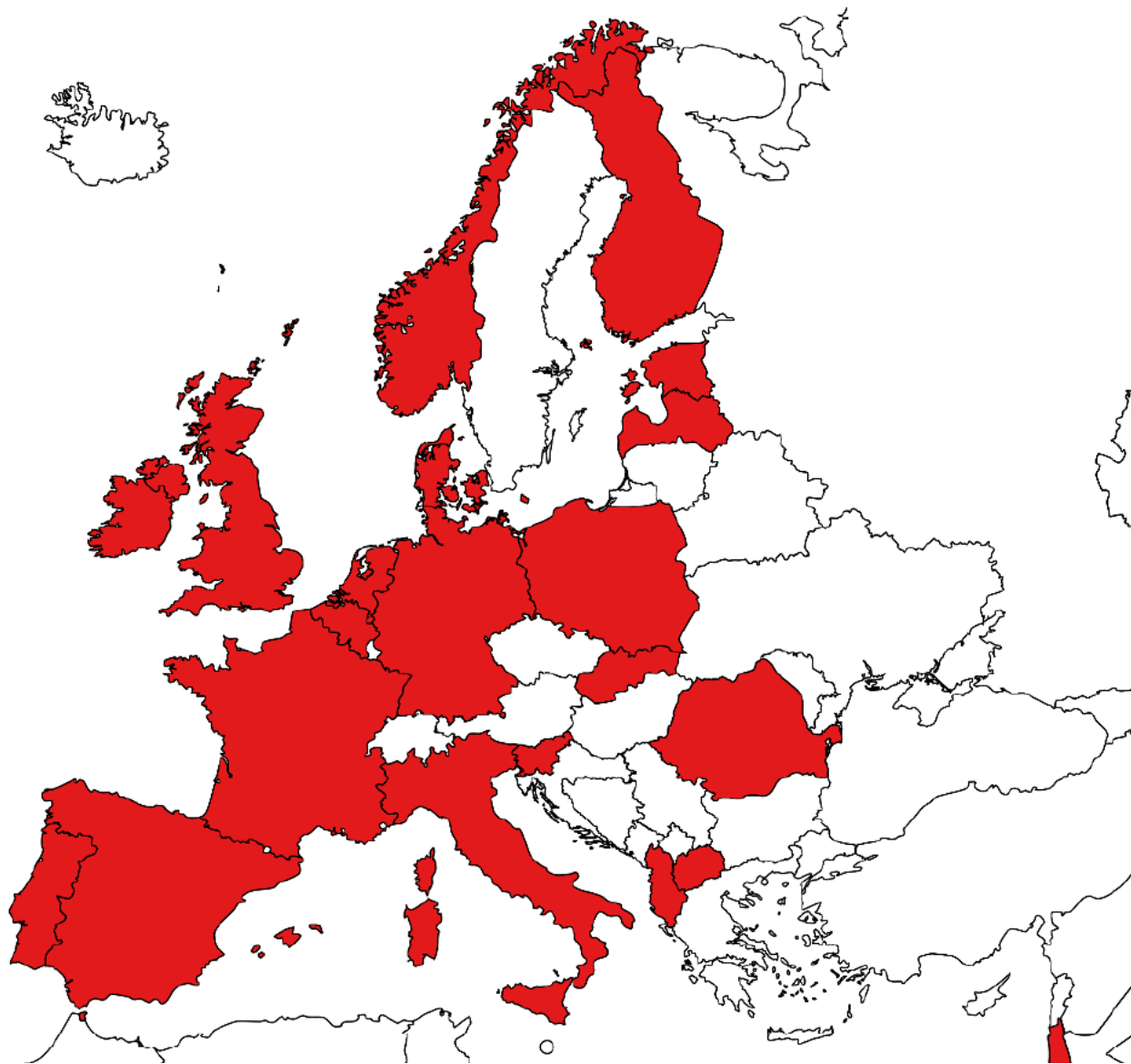


PT-08 Molecular identification of Echinococcus at the species level-2022

RESULTS



Participants (N=20)
 20 labs submitted results



Belgium NRLP, Institute of Tropical Medicine
Denmark NRLP, Statens Serum Institut, laboratory of parasitology, SSI
Estonian NRLP, Animal Health, Veterinary and Food Laboratory
Finland NRLP, Oulu, Finnish Food Authority, Ruokavirasto (ex Evira)
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
Israel, Division of Parasitology, Kimron Veterinary Institute, Israeli Veterinary Services
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

Created with mapchart.net



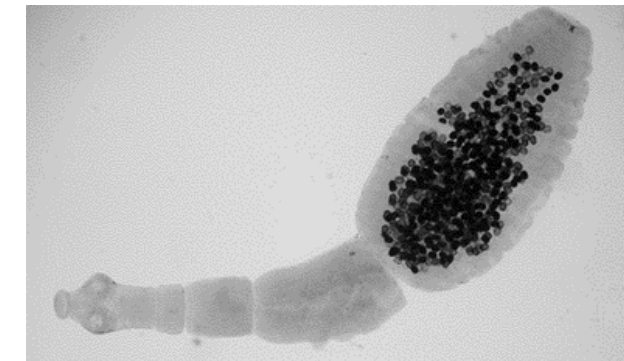
PT-08 Molecular identification of Echinococcus at the species level-2022

RESULTS

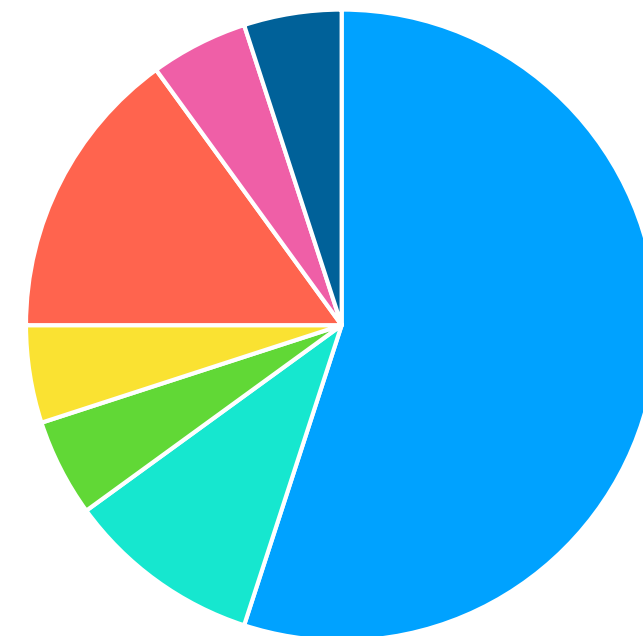


Detection method

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



Method applied	N. labs
Trachsel <i>et al.</i> , 2007	11
Bowles <i>et al.</i> , 1992	2
Geysen <i>et al.</i> , 2007	1
Laurimaa <i>et al.</i> , 2015	1
EURLP method+ sequencing	3
OIE Terrestrial Manual 2019	1
In house method: CO1 PCR and sequencing	1





PT-08 Molecular identification of Echinococcus at the species level-2022

RESULTS



Number of participant laboratories	20
Number of participants that passed the PT	19
Number of participants that failed the PT	1



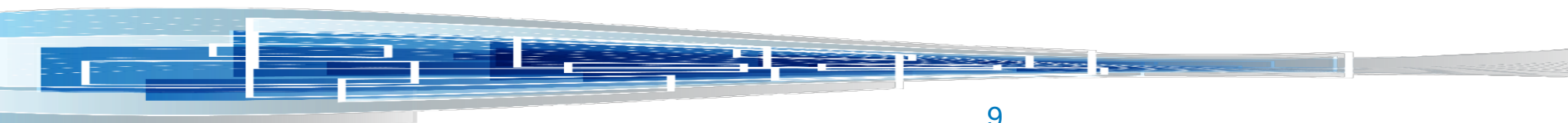
Sample 1 - *E. multilocularis*: 19 labs (95%) out of 20 obtained a positive evaluation

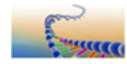


Sample 2 - *E. granulosus s.l.*: 20 labs (100%) out of 20 obtained a positive evaluation



Sample 3 - *Taenia saginata*: 20 labs (100%) out of 20 obtained a positive evaluation



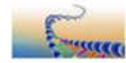


PT-08 Molecular identification of Echinococcus at the species level-2022

RESULTS



Lab code	Code	Expected	Observed	Results	Evaluation (Positive/Negative)	Lab code	Code	Expected	Observed	Results	Evaluation (Positive/Negative)
Ed1	EGM1	E. multilocularis	E. multilocularis	Correct	POSITIVE	Ed11	EGM31	E. multilocularis	E. multilocularis	Correct	POSITIVE
	EGM2	E. granulosus sl	E. granulosus sl	Correct			EGM32	E. granulosus sl	E. granulosus sl	Correct	
	EGM3	Negativo	Negativo	Correct			EGM33	Negativo	Negativo	Correct	
Ed2	EGM4	E. multilocularis	E. multilocularis	Correct	POSITIVE	Ed12	EGM34	E. multilocularis	E. multilocularis	Correct	POSITIVE
	EGM5	E. granulosus sl	E. granulosus sl	Correct			EGM35	E. granulosus sl	E. granulosus sl	Correct	
	EGM6	Negativo	Negativo	Correct			EGM36	Negativo	Negativo	Correct	
Ed3	EGM7	E. multilocularis	E. multilocularis	Correct	POSITIVE	Ed13	EGM37	E. multilocularis	E. multilocularis	Correct	POSITIVE
	EGM8	E. granulosus sl	E. granulosus sl	Correct			EGM38	E. granulosus sl	E. granulosus sl	Correct	
	EGM9	Negativo	Negativo	Correct			EGM39	Negativo	Negativo	Correct	
Ed4	EGM10	E. multilocularis	E. multilocularis	Correct	POSITIVE	Ed14	EGM40	E. multilocularis	E. multilocularis	Correct	POSITIVE
	EGM11	E. granulosus sl	E. granulosus sl	Correct			EGM41	E. granulosus sl	E. granulosus sl	Correct	
	EGM12	Negativo	Negativo	Correct			EGM42	Negativo	Negativo	Correct	
Ed5	EGM13	E. multilocularis	E. multilocularis	Correct	POSITIVE	Ed15	EGM43	E. multilocularis	E. multilocularis	Correct	POSITIVE
	EGM14	E. granulosus sl	E. granulosus sl	Correct			EGM44	E. granulosus sl	E. granulosus sl	Correct	
	EGM15	Negativo	Negativo	Correct			EGM45	Negativo	Negativo	Correct	
Ed6	EGM16	E. multilocularis	E. multilocularis	Correct	POSITIVE	Ed16	EGM46	E. multilocularis	E. multilocularis	Correct	POSITIVE
	EGM17	E. granulosus sl	E. granulosus sl	Correct			EGM47	E. granulosus sl	E. granulosus sl	Correct	
	EGM18	Negativo	Negativo	Correct			EGM48	Negativo	Negativo	Correct	
Ed7	EGM19	E. multilocularis	E. multilocularis	Correct	POSITIVE	Ed17	EGM49	E. multilocularis	E. multilocularis	Correct	POSITIVE
	EGM20	E. granulosus sl	E. granulosus sl	Correct			EGM50	E. granulosus sl	E. granulosus sl	Correct	
	EGM21	Negativo	Negativo	Correct			EGM51	Negativo	Negativo	Correct	
Ed8	EGM22	E. multilocularis	E. multilocularis	Correct	POSITIVE	Ed18	EGM52	E. multilocularis	E. multilocularis	Correct	POSITIVE
	EGM23	E. granulosus sl	E. granulosus sl	Correct			EGM53	E. granulosus sl	E. granulosus sl	Correct	
	EGM24	Negativo	Negativo	Correct			EGM54	Negativo	Negativo	Correct	
Ed9	EGM25	E. multilocularis	E. multilocularis and Taenia spp	Incorrect	NEGATIVE	Ed19	EGM55	E. multilocularis	E. multilocularis	Correct	POSITIVE
	EGM26	E. granulosus sl	E. granulosus sl	Correct			EGM56	E. granulosus sl	E. granulosus sl	Correct	
	EGM27	Negativo	Negativo	Correct			EGM57	Negativo	Negativo	Correct	
Ed10	EGM28	E. multilocularis	E. multilocularis	Correct	POSITIVE	Ed20	EGM58	E. multilocularis	E. multilocularis	Correct	POSITIVE
	EGM29	E. granulosus sl	E. granulosus sl	Correct			EGM59	E. granulosus sl	E. granulosus sl	Correct	
	EGM30	Negativo	Negativo	Correct			EGM60	Negativo	Negativo	Correct	

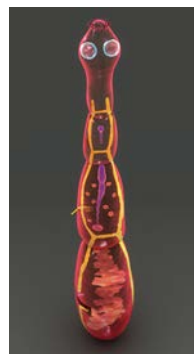


PT-08 Molecular identification of *Echinococcus* at the species level-2022

Conclusions



The experience derived from the fourth PT carried out in 2022 on the molecular detection of *Echinococcus* spp. showed that the personnel of NRLs are skilled to detect this parasite in a qualitative test



95% of participants succeeded in the identification of *Echinococcus multilocularis*, 100% of participants correctly identified *Echinococcus granulosus s. l.* and 100% detected the negative sample

Recommendations:

We suggest the laboratories to optimize multiplex PCR settings to avoid unspecific amplifications and to sequence amplicons to confirm the identification





PT-08 Molecular identification of Echinococcus
at the species level-2022

