9th Proficiency Test on "Molecular identification of Anisakid nematodes at the species level" – PT07

European Union Reference Laboratory for Parasites
Istituto Superiore di Sanità

XIX Workshop of National Reference Laboratories for Parasites
Rome, 28th-29th October 2025

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Aim of the Proficiency Test

Evaluation of laboratories competence in molecular identification of anisakidae nematodes species

The PT is accredited according to the ISO 17043

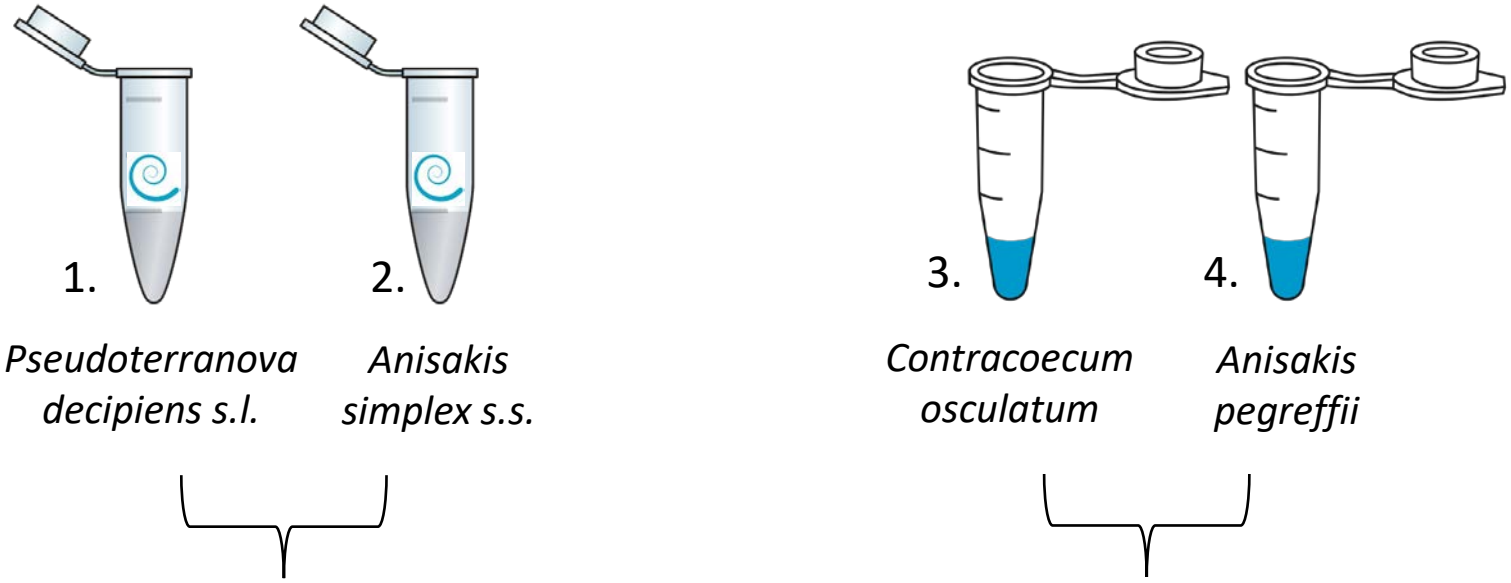
Timeline

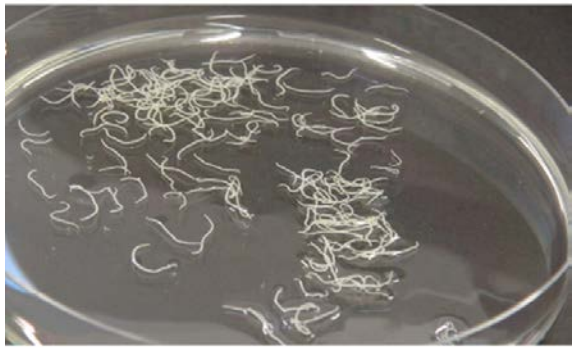
Call for participation	3rd February
Deadline for subscription	24th February
Pt sending	17th March
Deadline for submitting results	11th April
Individual report	30th April
Final report published on the EURLP webpage	31nd May

PT panel

Pseudoterranova decipiens

The single PT panel consisted of **4** items:





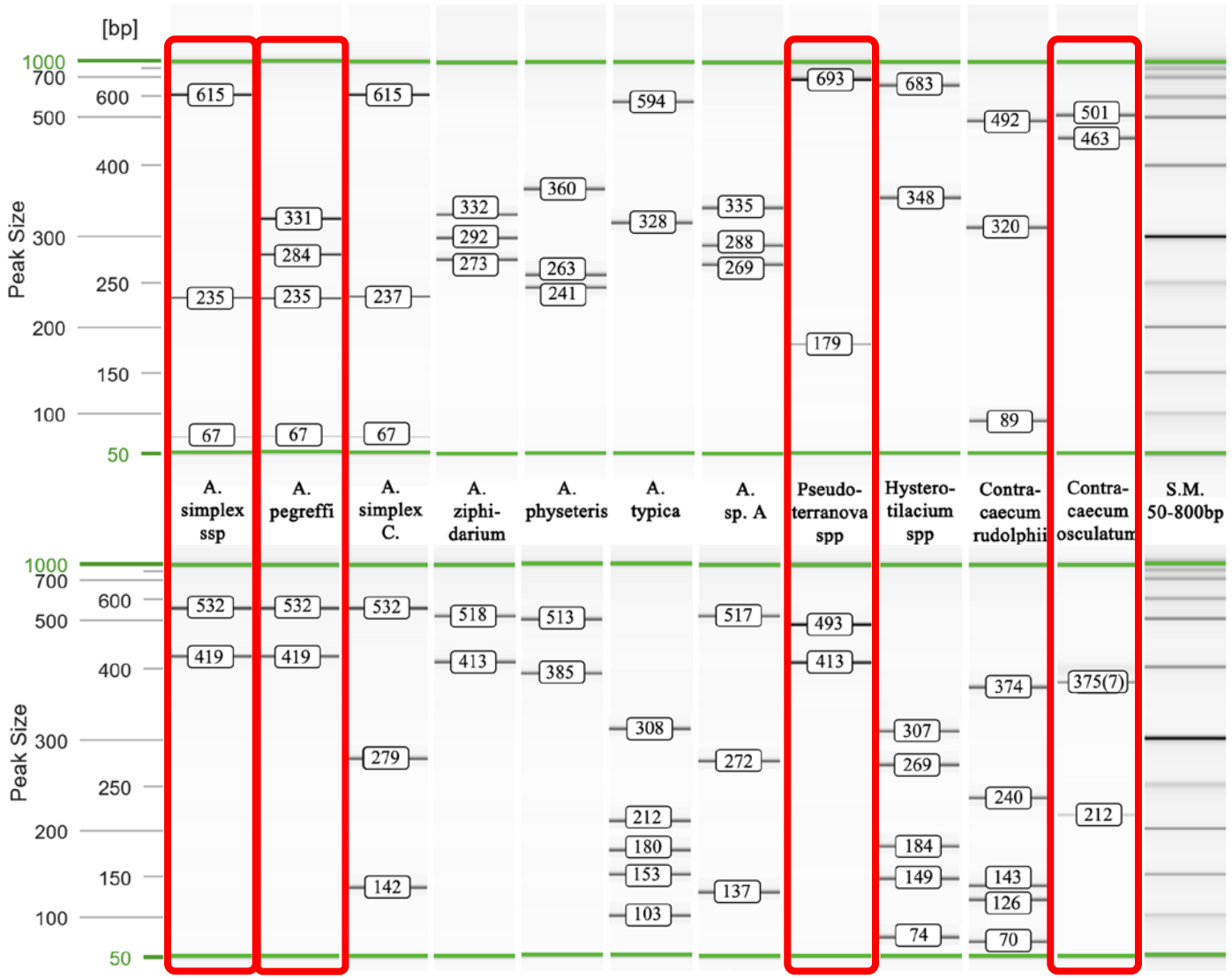
All larvae have been previously identified at species level by analysing one of their fragments by the EURLP method “Identification at species level of parasites of the family *Anisakidae* by PCR/RFLP”, MI04, same for DNA.

Identification at species level of parasites of the family Anisakidae by PCR/RFLP

1) PCR for ITS target (~900bp) on extracted DNA

2)

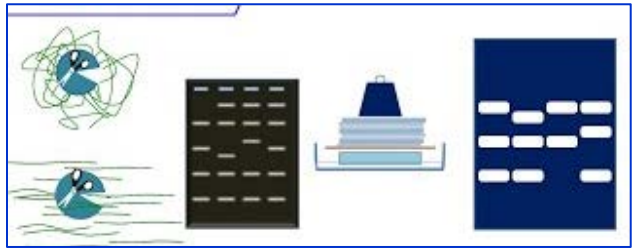
Restriction Enzyme	Target sequence
HinfI	5′...G▼ANTC...3′ 3′...CTNA▲G...5′
HhaI	5′...GCG▼C...3′ 3′...C▲GCG...5′



Molecular identification

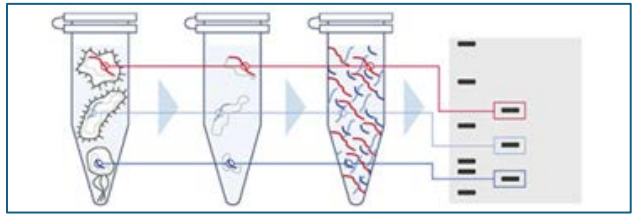
Recommended methods:

A) "Identification at species level of parasites of the family Anisakidae by PCR/RFLP"
MI04



A. simplex ss.
A. pegreffi
A. simplex/pegreffi hybrid
A. simplex C
A. ziphidarium
A. physeteris
A. typica
A. sp. A
Pseudoterranova spp
Hysterotilacium spp
Contracaecum rudolphii (A, B, C)   ***Contracaecum osculatum***

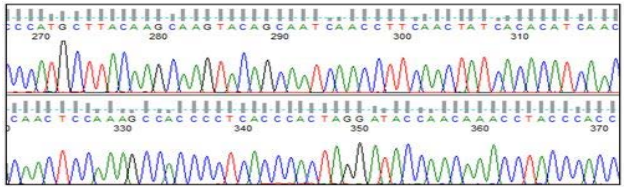
B) "Identification of Anisakidae Larvae at the species level by multiplex PCR"
MI10



A. pegreffii
A. simplex s.l.   *(incl. A. simplex/pegreffii hybrid)*
A. physeteris   *(incl. A. brevispiculata and A. paggiae)*
A. typica
Pseudoterranova spp
Hysterotilacium spp (H. aduncum)
Contracaecum rudolphii (A, B, C)   ***Contracaecum osculatum***

OR:

C) Any other suitable molecular method performed by the participant laboratory (i.e. PCR and sequencing)



Evaluation criteria

The PT evaluation is only **qualitative** and no statistical analysis of the results is applied.

For each PT item the result is **“correct”** or **“incorrect”** according to the correct or incorrect identification

- **The PT is considered “positive” if no “incorrect” results were obtained**
- **The PT is considered “negative” if at least one “incorrect” result was obtained**

PT participants

17 participants in 2025

- Belgium, NRLP ITPM
- Croatia, Veterinary Institute
- Denmark, NRL SSI
- Estonia, NRL LABRIS
- Finland, NRLP FINPAR
- France, NRL ANSES
- Germany, NRLP, Max Rubner-Institut
- Hungary, Food Chain Safety Laboratory Directorate
- Iceland, Institute for Experimental Pathology
- Italy, LNR IZS Sicilia
- Latvia, NRL BIOR
- Netherlands, NRL RIVM
- Norway, NRL Institute of Marine Research
- Poland, PIWet-PIB
- Portugal, INIAV I.P
- Institute of Veterinary Medicine of Serbia
- Slovenia, NRL University of Ljubljana, Veterinary Faculty

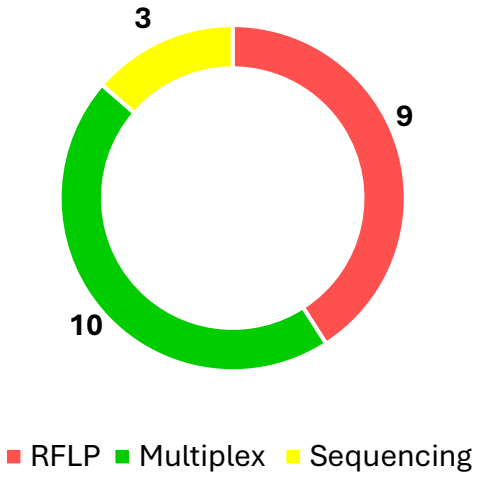
Results-Methods

Participation

- **17/17** labs sent the results

Methods

- **6** EURLP method 2 (Multiplex PCR)
- **5** EURLP method 1 (PCR-RFLP)
- **2** EURLP method 1; EURLP method 2 *
- **2** Published method (PCR and sequencing of ITS or COX2)
- **1** EURLP method 2; In house method (PCR-RFLP and multiplex PCR)
- **1** In house method (PCR-RFLP and sequencing of ITS)



*one lab sequenced PCR product COX1 for confirmation

Results- Detection

Laboratory code	N° of samples correctly identified		N° of samples NOT correctly identified		Method applied	Final evaluation
	Larvae	DNA	Larvae	DNA		
AMM01	1	2	1	0	In house method (PCR and sequencing)	NEGATIVE
AMM02	2	2	0	0	EURLP method 2 (multiplex PCR);	POSITIVE
AMM03	2	2	0	0	Published method (PCR and Sequencing)	POSITIVE
AMM04	2	2	0	0	EURLP method 2 (multiplex PCR);	POSITIVE
AMM05	2	1	0	1	EURLP method 1 (PCR-RFLP);	NEGATIVE
AMM06	1	2	1	0	EURLP method 1 (PCR-RFLP);	NEGATIVE
AMM07	2	2	0	0	EURLP method 2 (multiplex PCR);	POSITIVE
AMM08	2	2	0	0	EURLP method 1 (PCR-RFLP);	POSITIVE
AMM09	1	1	1	1	EURLP method 2 (multiplex PCR);	NEGATIVE
AMM10	2	2	0	0	EURLP method 2 (multiplex PCR);	POSITIVE
AMM11	2	2	0	0	EURLP method 2 (multiplex PCR); In house method (PCR-RFLP and multiplex PCR)	POSITIVE
AMM12	2	2	0	0	Published method (PCR and sequencing)	POSITIVE
AMM13	2	2	0	0	EURLP method 1 (PCR_RFLP); EURLP method 2 (multiplex PCR);	POSITIVE
AMM14	2	2	0	0	EURLP method 1 (PCR-RFLP); EURLP method 2 (multiplex PCR);In house method (PCR and sequencing)	POSITIVE
AMM15	2	2	0	0	EURLP method 2 (multiplex PCR);	POSITIVE
AMM16	2	2	0	0	EURLP method 1 (PCR_RFLP);	POSITIVE
AMM17	2	2	0	0	EURLP method 1 (PCR_RFLP);	POSITIVE

Missed identification

Wrong identification

13/17 (**77%**) of the reporting laboratories passed the PT

Results- Follow Up

Analysis of the cause of failure was provided by 2 the lab using the online follow-up form

AMM01 No feedback was received *

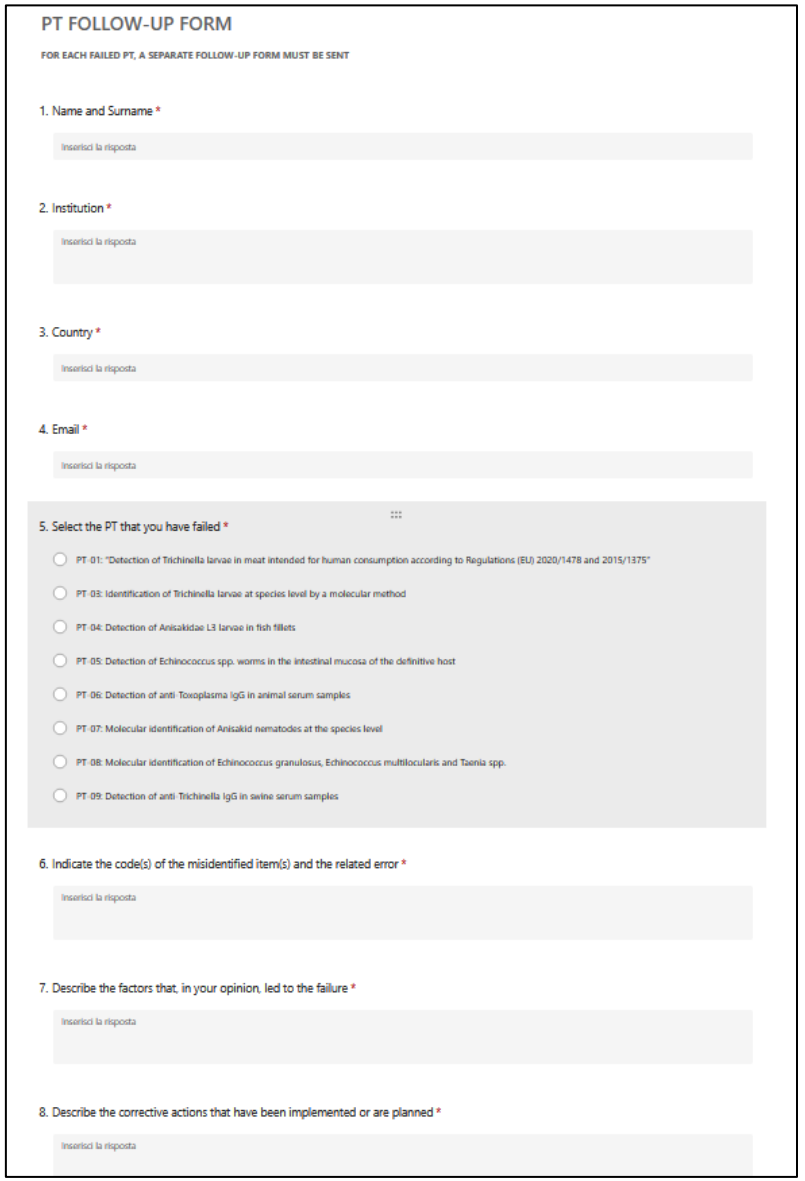
AMM05 No feedback was received *

AMM06 Reported a technical mistake during the PCR procedure

AMM09 Reported misreading of the method instructions and incorrect interpretation of the PCR fragments obtained

* PT failure could be due to:

- Incomplete removal of ethanol used to preserve the larvae.
- Errors or inappropriate changes made by the operator during the DNA extraction or PCR amplification steps.
- Use of unsuitable reagents during the DNA extraction or PCR amplification steps.



PT FOLLOW-UP FORM

FOR EACH FAILED PT, A SEPARATE FOLLOW-UP FORM MUST BE SENT

1. Name and Surname *

Inserisci la risposta

2. Institution *

Inserisci la risposta

3. Country *

Inserisci la risposta

4. Email *

Inserisci la risposta

5. Select the PT that you have failed *

- PT-01: "Detection of Trichinella larvae in meat intended for human consumption according to Regulations (EU) 2020/1478 and 2015/1375"
- PT-03: Identification of Trichinella larvae at species level by a molecular method
- PT-04: Detection of Anisakidae L3 larvae in fish fillets
- PT-05: Detection of Echinococcus spp. worms in the intestinal mucosa of the definitive host
- PT-06: Detection of anti-Toxoplasma IgG in animal serum samples
- PT-07: Molecular identification of Anisakid nematodes at the species level
- PT-08: Molecular identification of Echinococcus granulosus, Echinococcus multilocularis and Taenia spp.
- PT-09: Detection of anti-Trichinella IgG in swine serum samples

6. Indicate the code(s) of the misidentified item(s) and the related error *

Inserisci la risposta

7. Describe the factors that, in your opinion, led to the failure *

Inserisci la risposta

8. Describe the corrective actions that have been implemented or are planned *

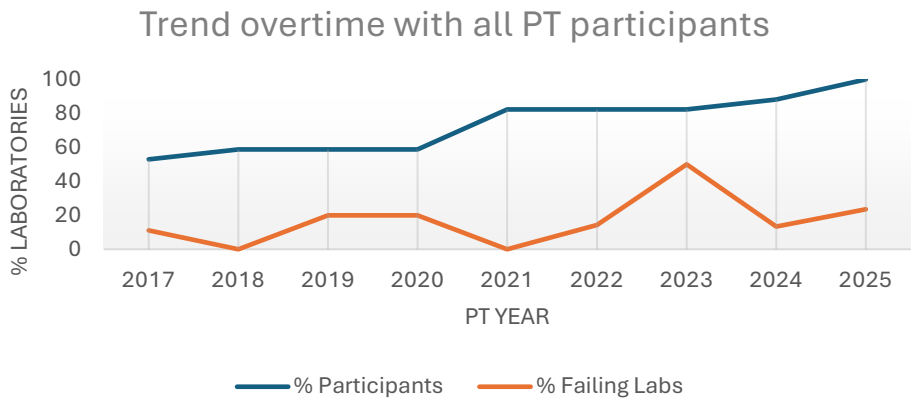
Inserisci la risposta

PT trend

Laboratory code 2025	PT year								
	2017	2018	2019	2020	2021	2022	2023	2024	2025
AMM01				P	P	P	N	P	N
AMM02	P	P	N	P	P	P	P	P	P
AMM03			P		P	P	P	P	P
AMM04				NA	P	P	P	P	P
AMM05	P	P	P	P	P	P		P	N
AMM06	P	P	P	N	P	P	N	P	N
AMM07	P	P	P	P	P	P	P	P	P
AMM08	P	NA			P	P	N	P	P
AMM09									N
AMM10									P
AMM11	P	P	P	P	P	P	P	N	P
AMM12									P
AMM13	P	P	P	P	P	P	P	P	P
AMM14		P		N	P	N	N	P	P
AMM15		P					N	P	P
AMM16			N		P	N	N	N	P
AMM17					NA	P	N	P	P

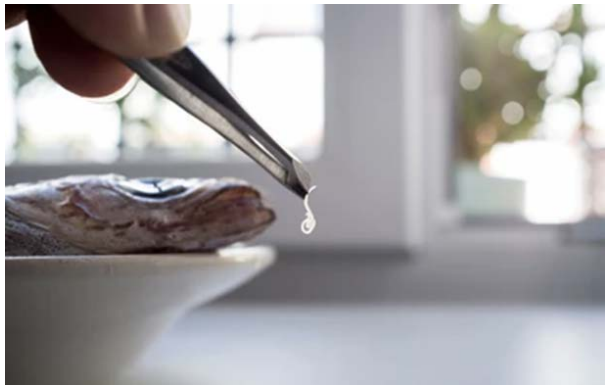
na=lab enrolled, no results submitted

New Entry



- 20 Laboratories enrolled in 2017-2025
- 5 Laboratories always partecipating
- 2 Laboratories always partecipating with a positive evaluation in every edition

Conclusions



- 13/17 (**77%**) reporting laboratories passed the PT.
- The PCR-RFLP and Multiplex PCR are the preferred methods, however sequencing, directly or in confirmation of other methods, is frequently performed.
- Method of choice and PT performance seems not correlated.
- Downward trend compared with the last year.

Thanks

See you in 2026!

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