8th Proficiency Test on "Molecular identification of Anisakid nematodes at the species level" – PT07

European Union Reference Laboratory for Parasites
Istituto Superiore di Sanità

XVIII Workshop of National Reference Laboratories for Parasites
Rome, 6th-7th November 2024

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Aim of the Proficiency Test

Evaluating the performance of the participant laboratory at correctly identifying larvae of anisakid nematodes at the species level applying molecular methods.

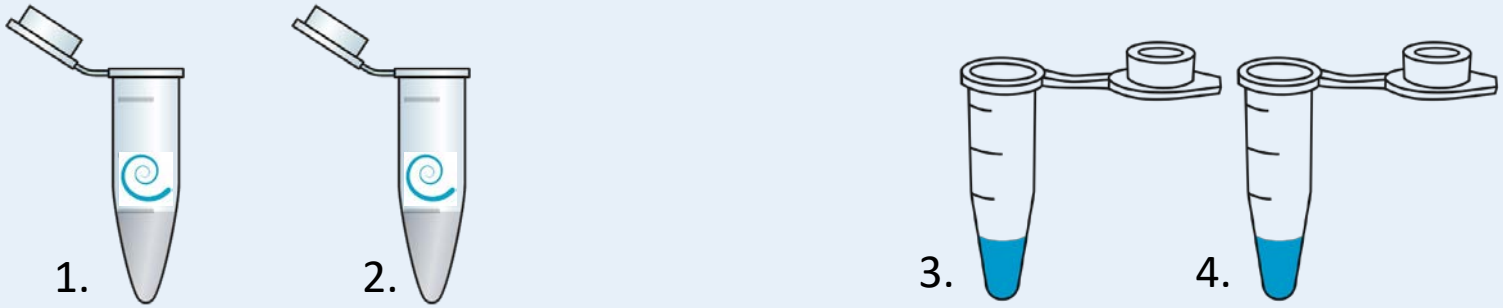
The PT is accredited according to the ISO 17043.

Timeline

Call for participing	23rd January
Deadline for subscription	28th February
Pt sending	11th March
Deadline for submitting results	12th April
Individual report	30th April
Final report published on the EURLP webpage	22nd May

PT panel

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

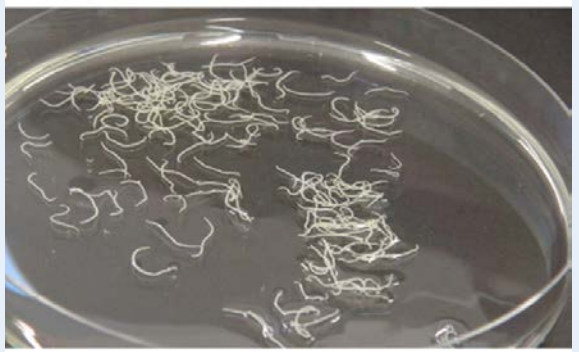


1. *Pseudoterranova decipiens s.l.*
2. *Anisakis pregreffii*

L3 larva fragments

3. *Contracoecum osculatum*
4. *Contracoecum osculatum*

Extracted DNA

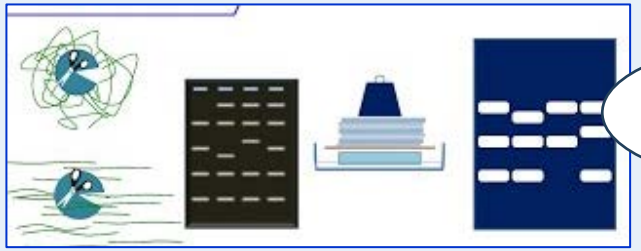


All larvae have been previously identified at species level by analyzing one of their fragments by the EURLP method “Identification at species level of parasites of the family *Anisakidae* by PCR/RFLP”, MI04, same for DNA.

Molecular identification

Recommended methods:

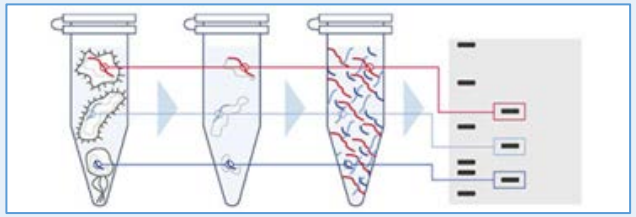
A) "Identification at species level of parasites of the family Anisakidae by **PCR/RFLP**" MI04



Updated!

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

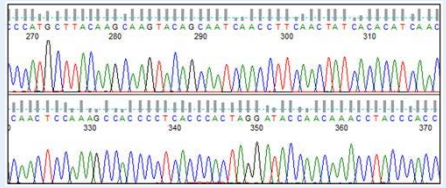
B) "Identification of Anisakidae Larvae at the species level by **multiplex** PCR" MI10



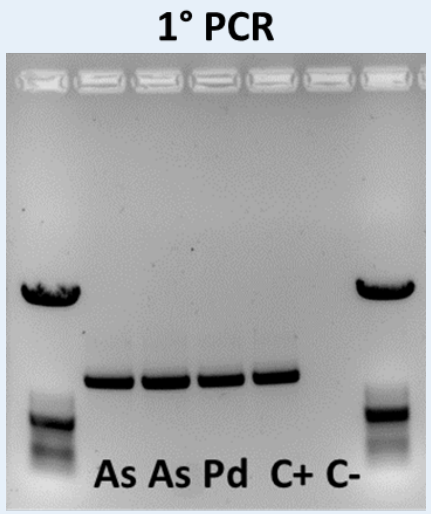
A. pegreffii
A. simplex s.l. *(incl. A. simplex/pegreffii hybrid)*
A. physeteris *(incl. A. brevispiculata and A. paggiae)*
A. typica
Pseudoterranova spp
Hysterotilacium spp (H. aduncum)
Contracaecum rudolphii (A, B, C)

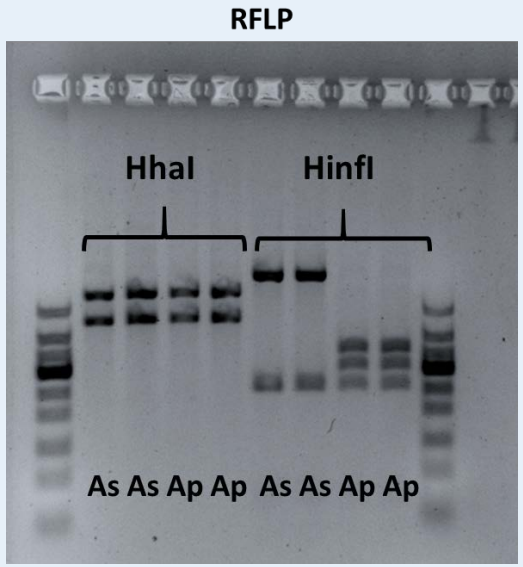
OR:

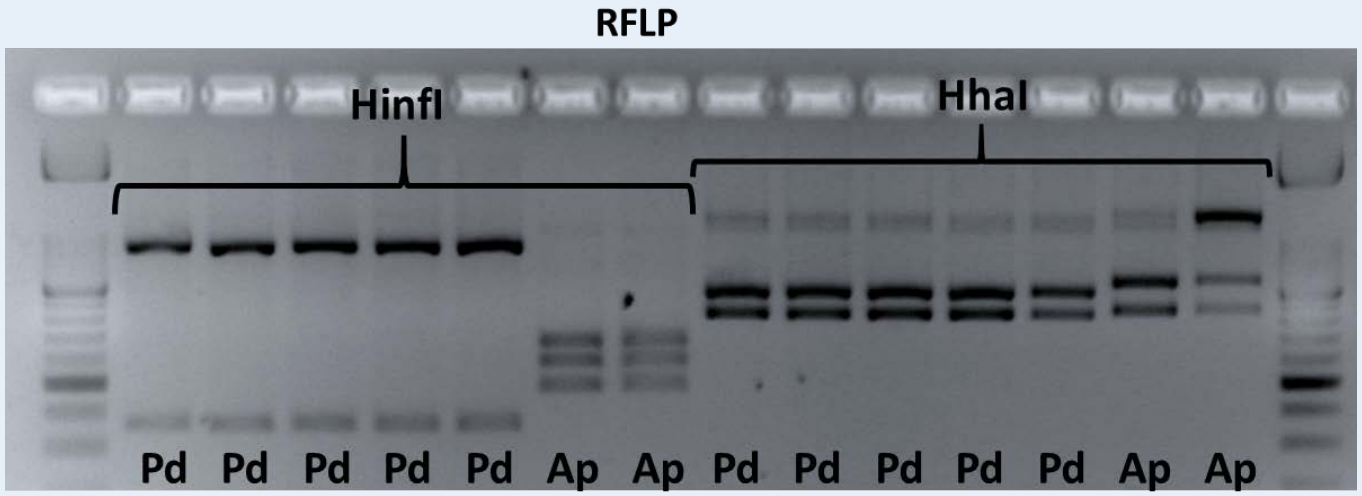
C) Any other suitable molecular method performed by the participant laboratory (i.e. PCR and sequencing)

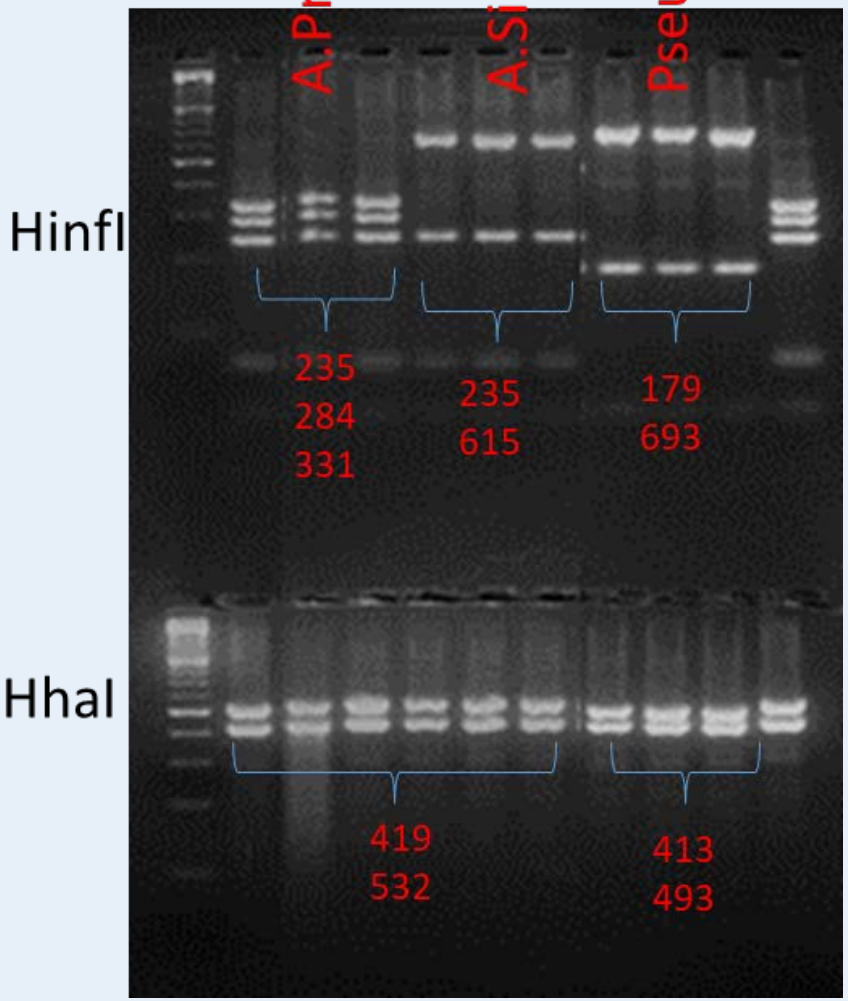


Identification at species level of parasites of the family *Anisakidae* by PCR/RFLP







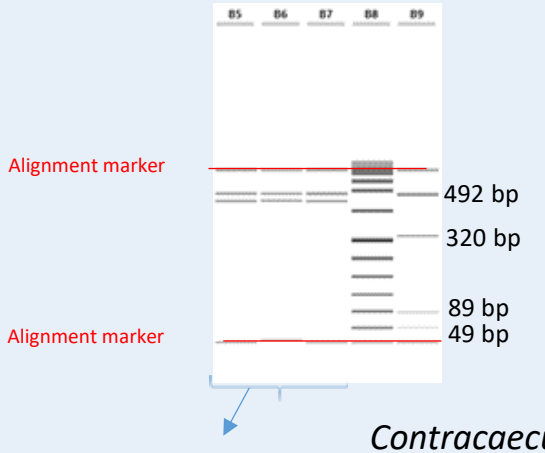


	PCR fragments of ITS amplicon	
Enzimi di restrizione	***HinfI***	***HhaI***
Contracaecum rudolphii (A, B, C)	**3, 49, 89, 320, 492**	**70, 126, 143, 240, 374**
Contracaecum osculatum	**463-501**	**212, 375, 377**

HhaI

Contracaecum osculatum *Contracaecum rudolphii*

HinfI



Contracaecum rudolphii

Contracaecum osculatum

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

Germany Max Rubner Institut

France ANSES

Italy IZS Sicily

Norway Institute of Marine Research

Denmark SSI

Poland PIWet-PIB

Netherlands RIVM

Belgium ITPM

Latvia BIOR

Romania IISPV

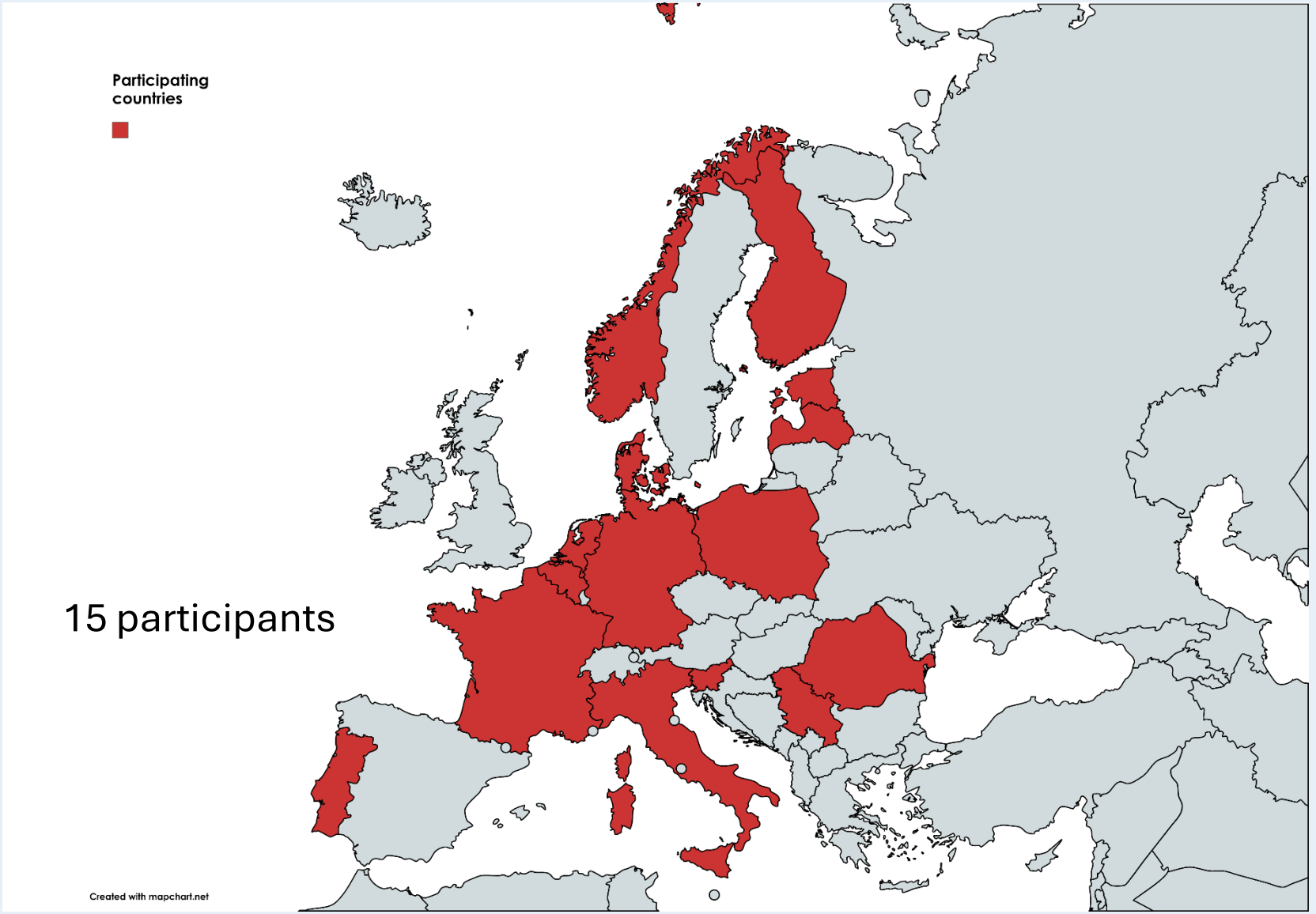
Finland FINPAR

Estonia LABRIS

University of Ljubljana

Institute of Veterinary Medicine of Serbia

Portugal INIAV,I.P.



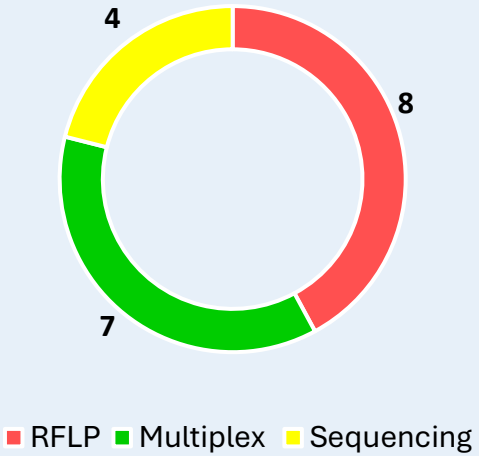
Results-Methods

Participation

- **15/15** labs sent the results

Methods

- **5** PCR-RFLP*
- **4** Multiplex PCR*
- **2** PCR & sequencing (ITS and/or cox2)
- **1** PCR-RFLP and Multiplex PCR
- **1** PCR RFLP, Multiplex and cox1 sequencing
- **1** PCR-RFLP and sequencing (not specified)
- **1** Multiplex PCR and in-house method (not specified)



*one lab each sequenced PCR products for confirmation

Results- Detection

13/15 (**87%**) of the reporting laboratories passed the PT

Laboratory code	N° of correctly identified		N° of uncorrectly identified		Method(s)	Final evaluation
	Larvae	DNA	Larvae	DNA		
AMM01	2	2	0	0	PCR RFLP + ITS sequencing	**positive**
AMM02	2	2	0	0	Multiplex PCR	**positive**
AMM03	2	0	0	2	Multiplex PCR + in house method (not specified)	**negative**
AMM04	2	2	0	0	cox2 sequencing	**positive**
AMM05	2	2	0	0	Multiplex PCR + ITS sequencing	**positive**
AMM06	2	2	0	0	PCR RFLP	**positive**
AMM07	2	2	0	0	PCR RFLP +Multiplex PCR + cox1 sequencing	**positive**
AMM08	2	2	0	0	PCR RFLP + ITS sequencing	**positive**
AMM09	1	2	1	0	ITS+cox2 sequencing	**negative**
AMM10	2	2	0	0	PCR RFLP	**positive**
AMM11	2	2	0	0	Multiplex PCR	**positive**
AMM12	2	2	0	0	PCR RFLP + Multiplex PCR	**positive**
AMM13	2	2	0	0	PCR RFLP	**positive**
AMM14	2	2	0	0	Multiplex PCR	**positive**
AMM15	2	2	0	0	PCR RFLP	**positive**

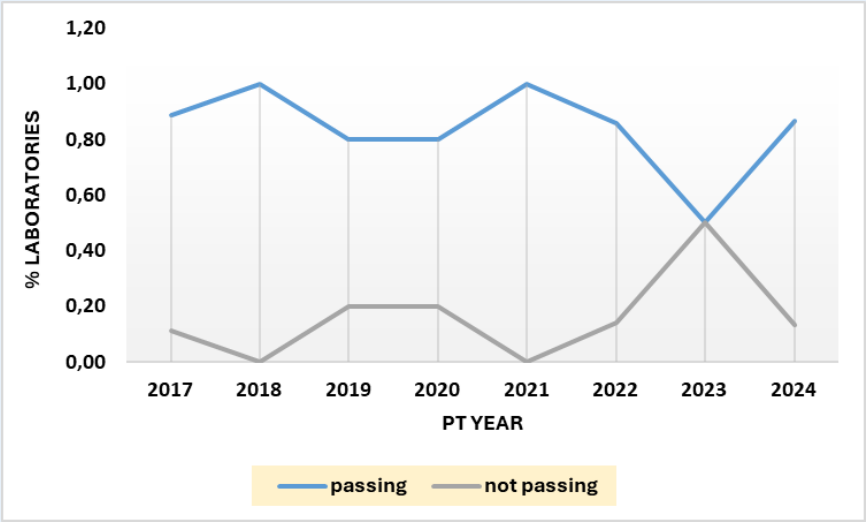
Mis-identification of the *C. osculatum* DNAs (*A. typica*)

Mis-identification of the *A. pregreffii* larva (*A. simplex*)

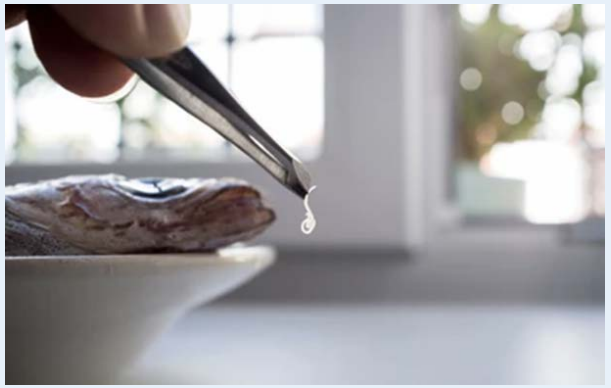
PT trend

Laboratory code	PT year							
	2017	2018	2019	2020	2021	2022	2023	2024
AMM01 (A15)								
AMM02 (A28)								
AMM03 (A6)								
AMM04 (A39)								
AMM05 (A40)				na				
AMM06 (A20)								
AMM07 (A8)								
AMM08 (A12)								
AMM09 (A3)								
AMM10 (A10)								
AMM11 (A16)								
AMM12 (A7)								
AMM13 (A26)					na			
AMM14 (A38)								
AMM15 (A31)		na						
(A11)		na						
(A17)								

na=lab enrolled, no results submitted



Conclusions



- 13/15 (**87%**) 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.
- Upward trend in 2024.

Thanks

See you in 2025!

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