# 6th Proficiency Test on molecular identification of Anisakid nematodes at the species level – PT07


European Union Reference Laboratory for Parasites
Istituto Superiore di Sanità


XVII Workshop of National Reference Laboratories for Parasites
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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.

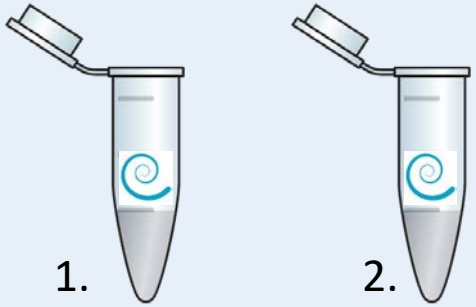

The PT is accredited according to the ISO 17043.

# Timeline

| | |
|---|---|
| Call for participing | 25° January |
| Deadline for subscription | 25° February |
| Pt sending | 14° March |
| Deadline for submitting results | 15° April |
| Individual report | 2° May |
| Final report published on the EURLP webpage | 31° May |

# PT panel

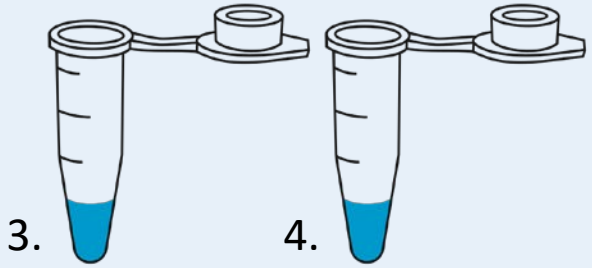

The single PT panel consisted of **4** items:




*Pseudoterranova decipiens*

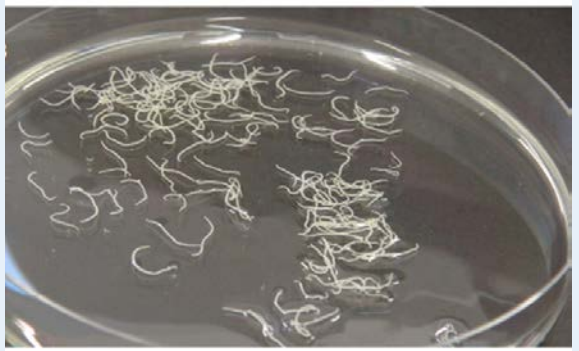
*Anisakis pregreffii*

L3 larva fragment




*Anisakis simplex sensu stricto*

Extracted DNA

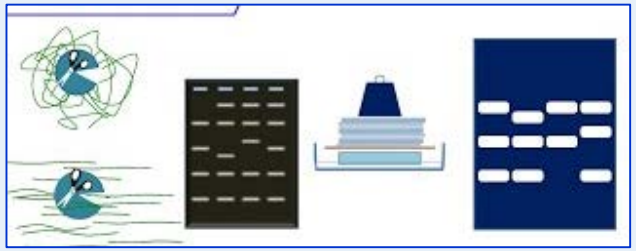


All larvae have been previously identified at species level by analyzing one of their fragments by the EURLP method “Identification at species level of parasites of the family *Anisakidae* by PCR/RFLP”, MI04, same for DNA.

# Molecular identification

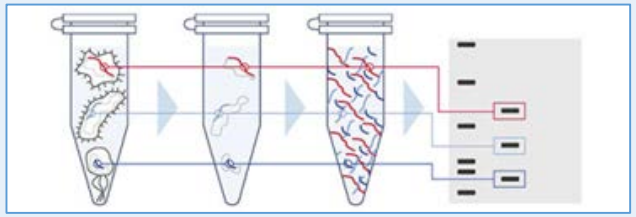
## Recommended methods:

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



| *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)* |

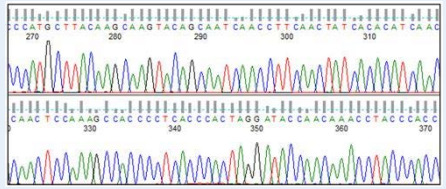
**B)** "Identification of Anisakidae Larvae at the species level by **multiplex** PCR" MI10



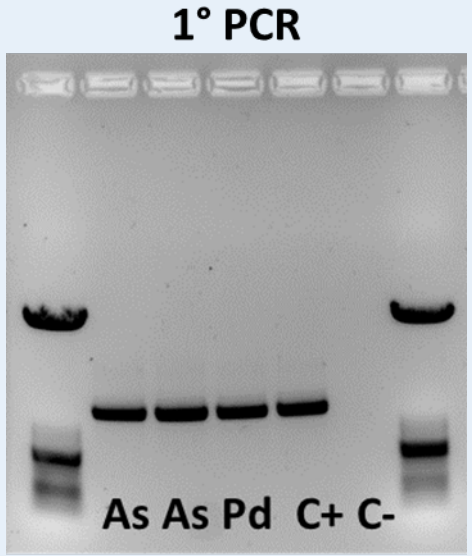
| *A. pegreffii* |
|---|
| *A. simplex s.l.* (incl. *A. simplex/pegreffii hybrid*) |
| *A. physeteris* (incl. *A. brevispiculata* and *A. paggiae*) |
| *A. typica* |
| *Pseudoterranova spp* |
| *Hysterotilacium spp (H. aduncum)* |
| *Contracaecum rudolphii (A, B, C)* |

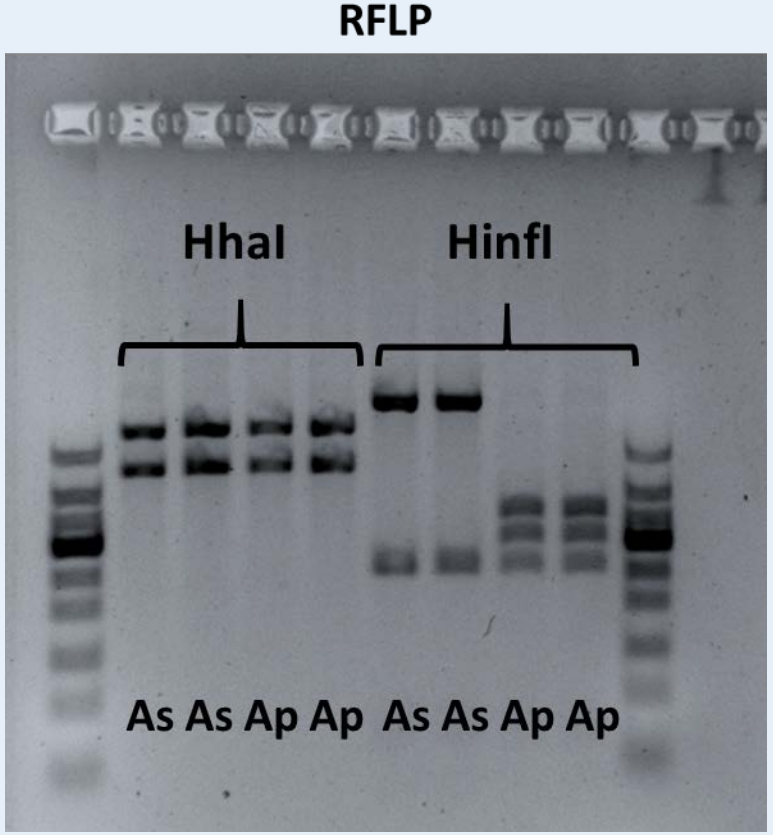
## OR:

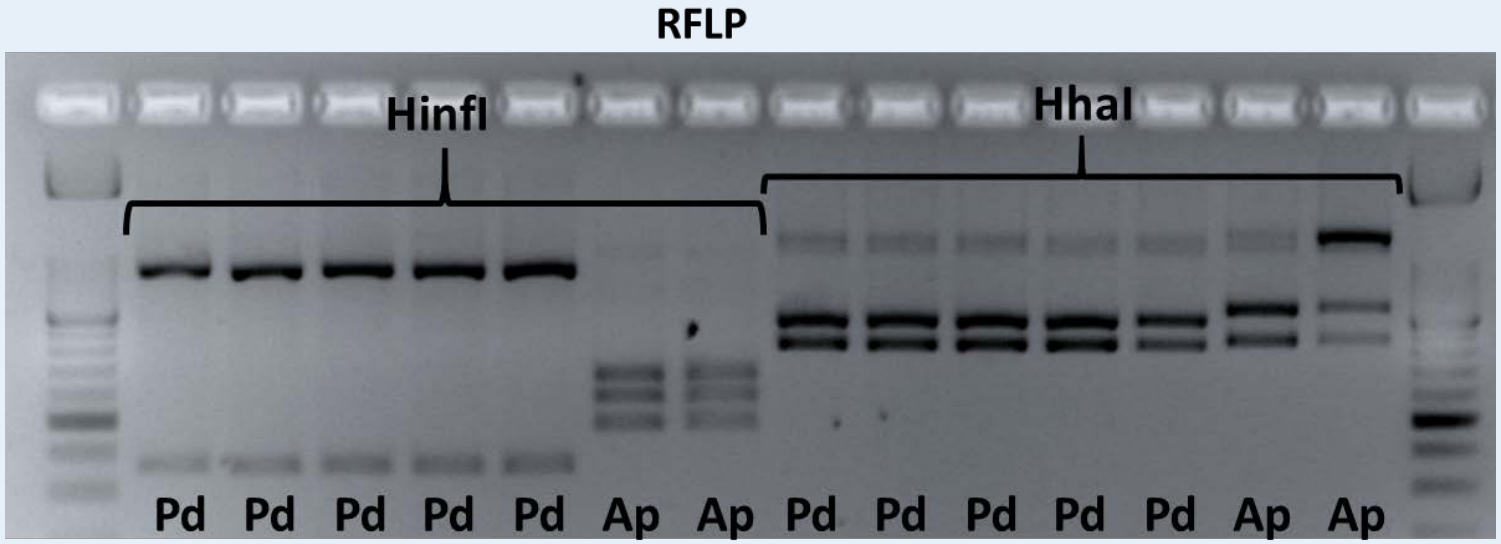
**C)** Any other suitable molecular method performed by the participant laboratory (i.e. PCR and sequencing)



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



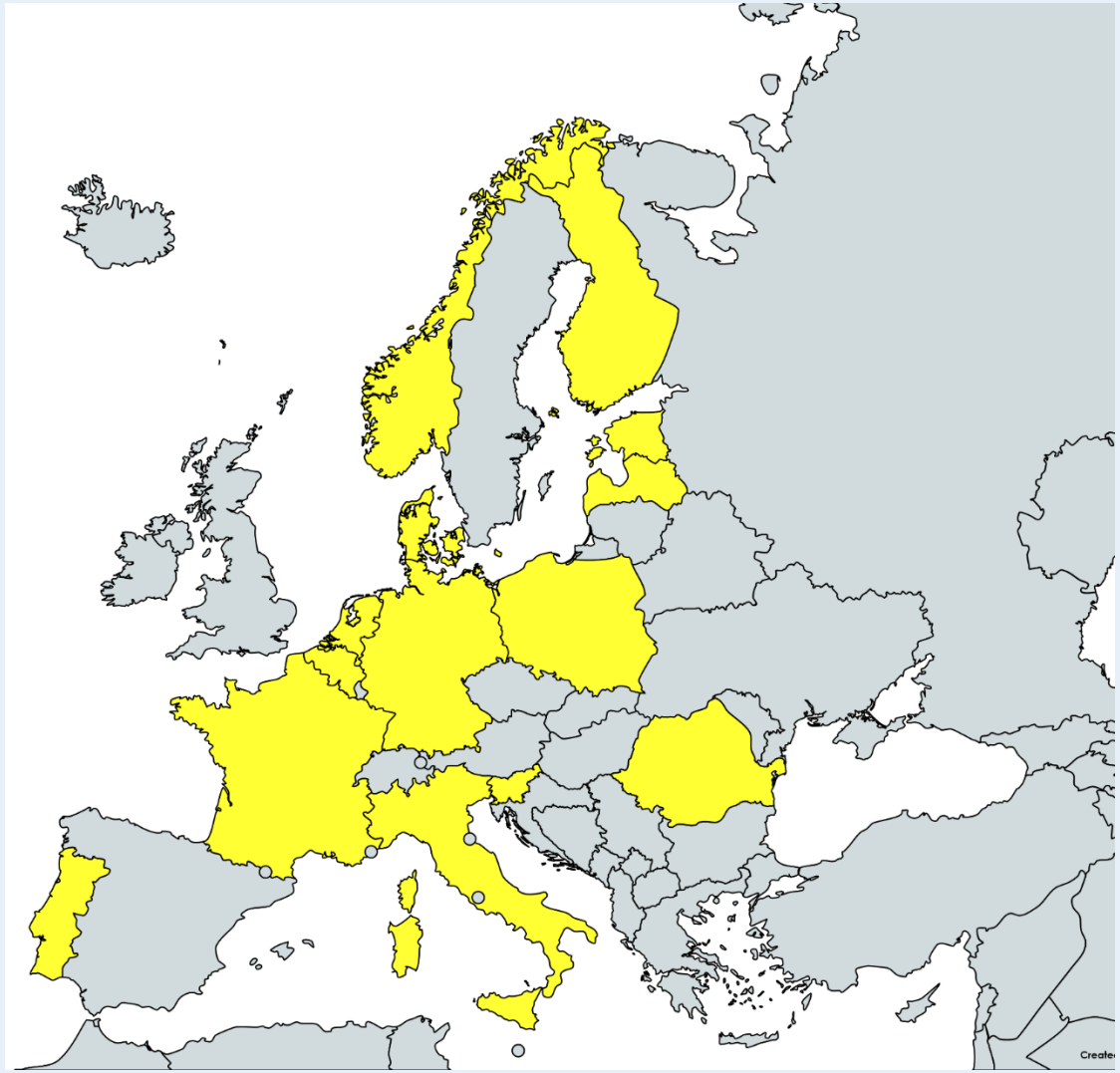
# Evaluation criteria

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



14 participants, 13 MS, 1 EEA

- Statens Serum Institut Copenhagen
- Estonian Veterinary and Food Laboratory Tartu
- Max Rubner-Institut Hamburg
- National Reference Laboratory for Anisakiasis Palermo
- Institute for diagnosis and animal health, Bucharest
- National Veterinary Research Institute Pulawy
- ANSES-Food safety Laboratory Boulogne Sur Mer
- Institute of food safety, animal health and environment Riga
- Institute of Tropical Medicine Antwerp
- Finnish Food Safety Authority Evira, Oulu
- University of Ljubljana Ljubljana
- Institute for Public Health and Environment Bilthoven
- Institute of Marine Research Bergen
- Instituto nacional de investigacao agraria e veterinaria Lisbon

# Results-1

**Participation**

- **14/14** labs sent the results

**Methods**

- **7** PCR-RFLP
- **1** PCR-RFLP and sequencing
- **1** PCR-RFLP and PCR Multiplex
- **3** PCR Multiplex
- **2** PCR + Sequencing (COX1 and COX2)

9 PCR-RFLP

4 PCR Multiplex

2 sequencing

**Detection**

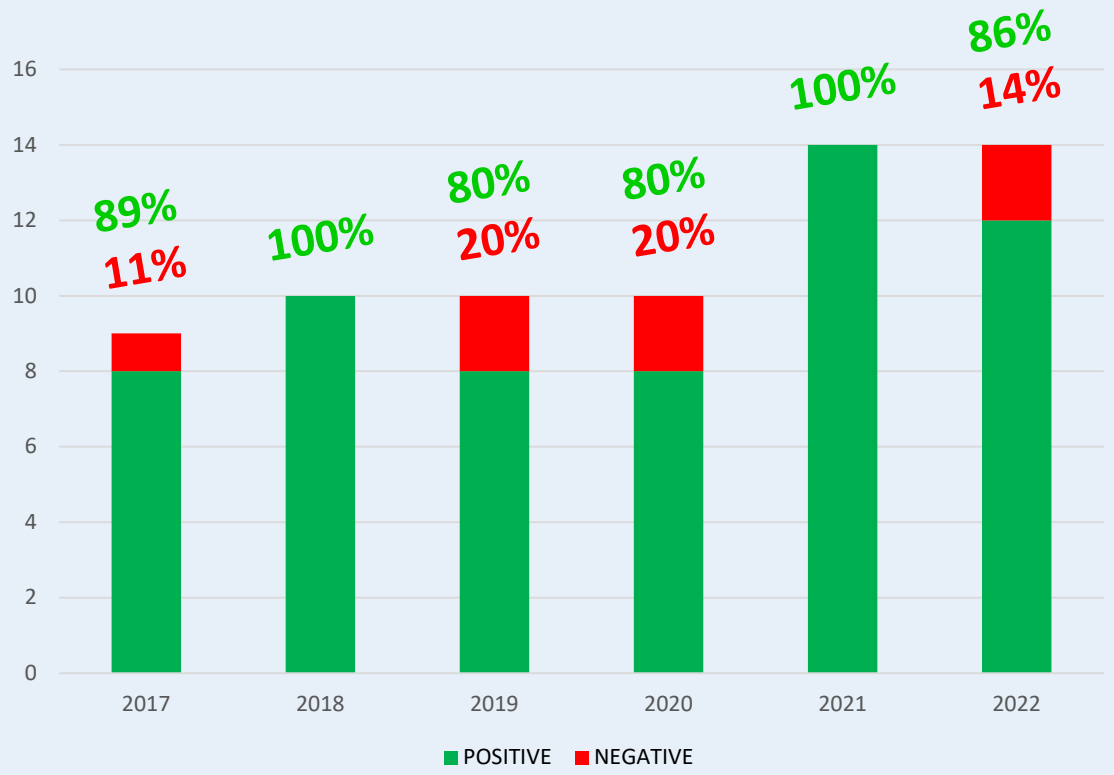
- 12/14 (86%) of the reporting laboratories passed the PT

# Results-2

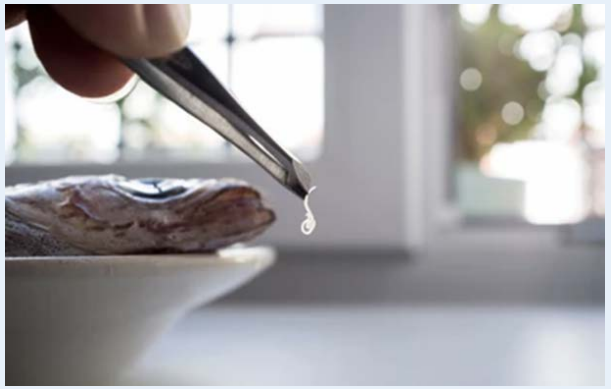
| Laboratory code | N° of samples correctly identified | | N° of samples NOT correctly identified | | Method(s) | Final evaluation |
|---|---|---|---|---|---|---|
| | Larva | DNA | Larva | DNA | | |
| **A3** | 1 | 2 | 1 | 0 | EURLP method 1 (**PCR_RFLP**) | **Negative** |
| **A6** | 2 | 2 | 0 | 0 | EURLP method 2 (**multiplex PCR**) | **Positive** |
| **A7** | 2 | 2 | 0 | 0 | EURLP method 1 (**PCR_RFLP**); EURLP method 2 (**multiplex PCR**) | **Positive** |
| **A8** | 1 | 2 | 1 | 0 | In house method (COX1 PCR and **sequencing**) | **Negative** |
| **A10** | 2 | 2 | 0 | 0 | EURLP method 1 (**PCR_RFLP**) | **Positive** |
| **A12** | 2 | 2 | 0 | 0 | EURLP method 1 (**PCR_RFLP**) | **Positive** |
| **A15** | 2 | 2 | 0 | 0 | EURLP method 1 (**PCR_RFLP** and **sequencing**) | **Positive** |
| **A16** | 2 | 2 | 0 | 0 | EURLP method 2 (**multiplex PCR**) | **Positive** |
| **A20** | 2 | 2 | 0 | 0 | EURLP method 1 (**PCR_RFLP**) | |
| **A26** | 2 | 2 | 0 | 0 | EURLP method 1 (**PCR_RFLP**) | **Positive** |
| **A28** | 2 | 2 | 0 | 0 | In house method (COX2 PCR and **sequencing**) | **Positive** |
| **A31** | 2 | 2 | 0 | 0 | EURLP method 1 (**PCR_RFLP** and **sequencing**) | **Positive** |
| **A39** | 2 | 2 | 0 | 0 | EURLP method 1 (**PCR_RFLP** and **sequencing**) | **Positive** |
| **A40** | 2 | 2 | 0 | 0 | EURLP method 2 (**multiplex PCR**) | **Positive** |

# PT trend

| Lab code | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 |
|---|---|---|---|---|---|---|
| A1 | na | | | | | |
| A3 | | | | | | |
| A6 | | | | | | |
| A7 | | | | | | |
| A8 | | | | | | |
| A10 | | | | | | |
| A11 | | na | | | | |
| A12 | | | | | | |
| A15 | | | | | | |
| A16 | | | | | | |
| A17 | | | | | | |
| A20 | | | | | | |
| A26 | | | | | na | |
| A28 | | | | | | |
| A31 | | na | | | | |
| A38 | | | | | | |
| A39 | | | | | | |
| A40 | | | | na | | |



# Conclusions



- 12/14 reporting laboratories passed the PT.
- The PCR-RFLP was the preferred method.
- The PT trend is stable and quite positive over time.

Overall, participant laboratories proved to be competent in the identification of Anisakidae at the species level, irrespectively of the molecular method used.

# Questions?

*See you in 2023!*

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