

XX Workshop of National Reference Laboratories for Parasites

Istituto Superiore di Sanità

28-29 October 2025

**PT-01: Artificial digestion to
detect *Trichinella* larvae in
meat samples according to
ISO 18743:2015/Amd 1:2023
or Annex III Reg UE 2015/1375**

**PT-03: Identification of
Trichinella larvae at the
species level by a
molecular method**

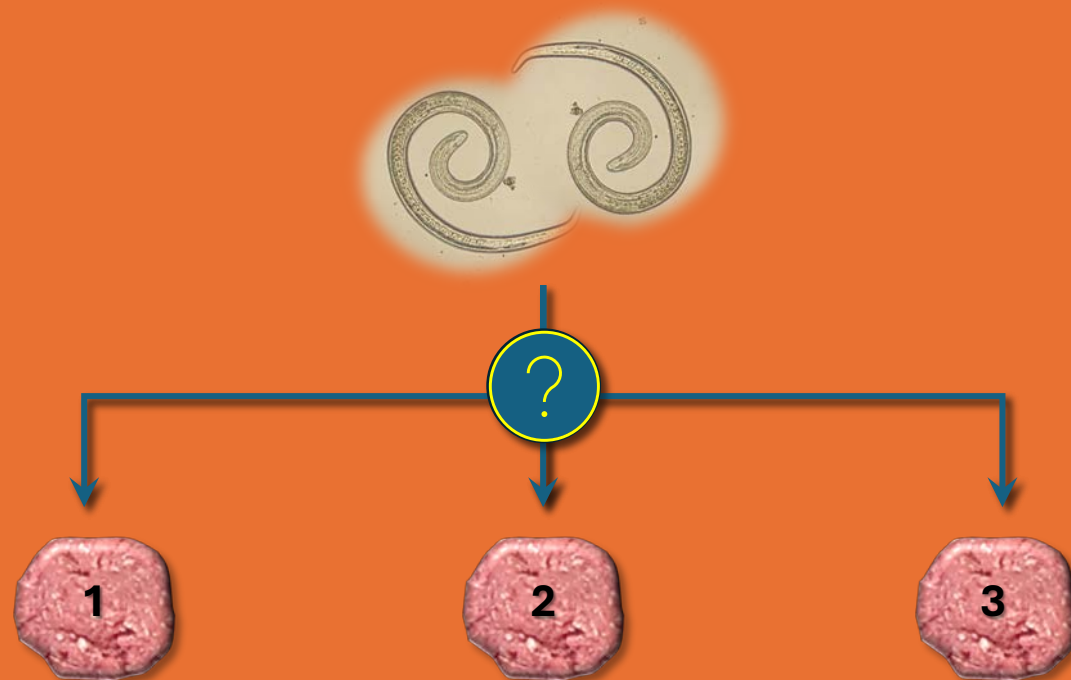


Dr. Irene Tartarelli

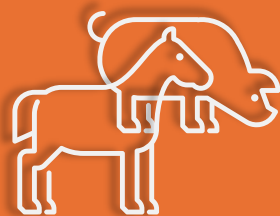


PT-01: Artificial digestion to detect *Trichinella* larvae in meat samples according to ISO 18743:2015/Amd 1:2023 or Annex III Reg UE 2015/1375

Participating laboratories must correctly identify positive and negative samples in a panel consisting in three meatballs



PT-01: Test material



Meat type:

- pork meat
- horse meat

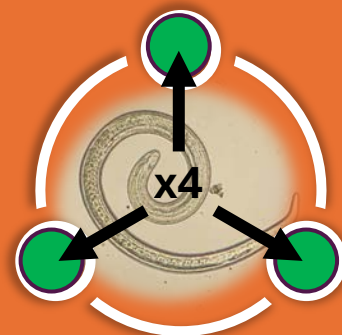


Samples weight:

- 100g
- 35g

2025 PT panel composition:

- 3 positive samples
(4 *T. spiralis* larvae/sample)
- NO negative sample

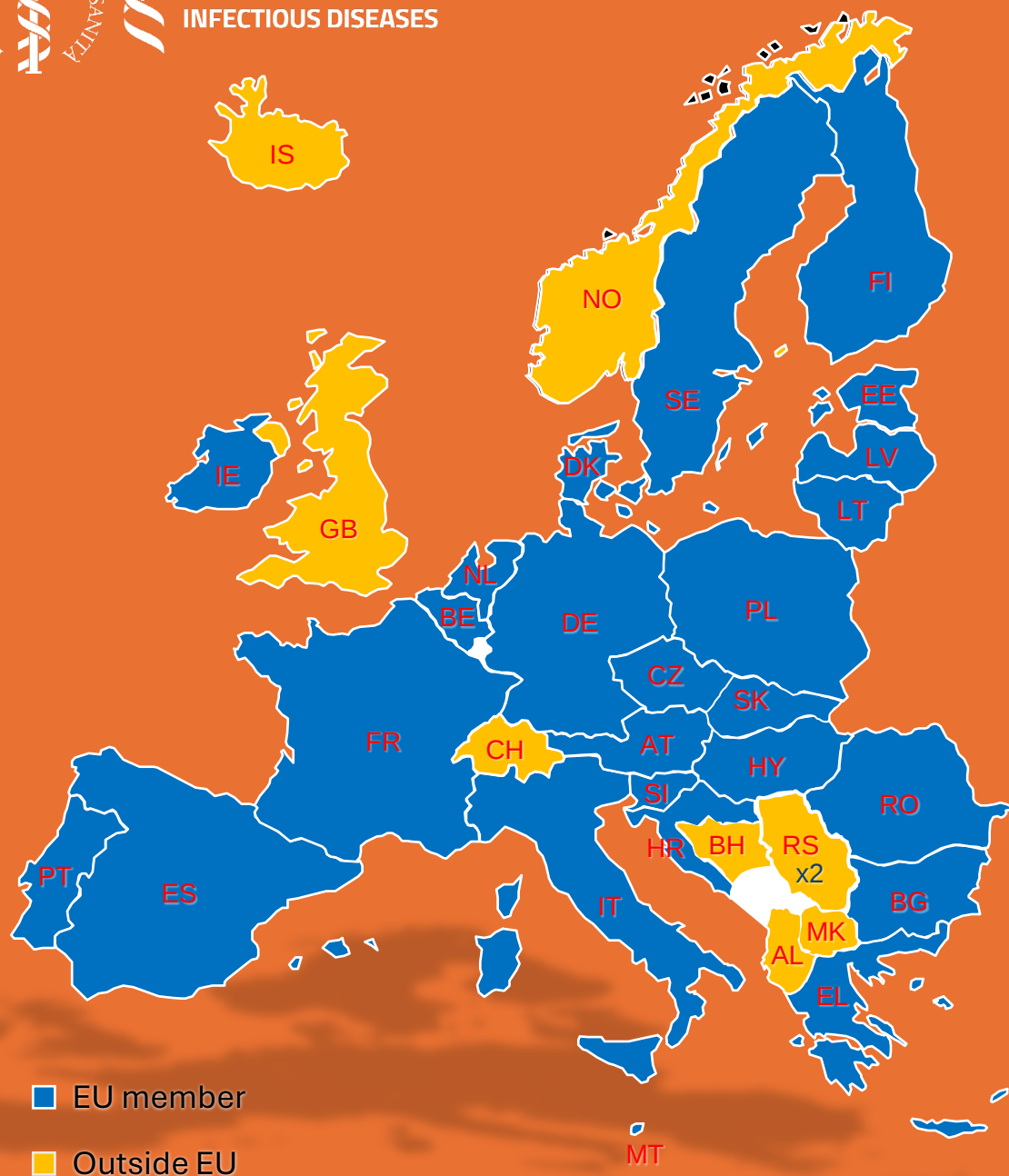
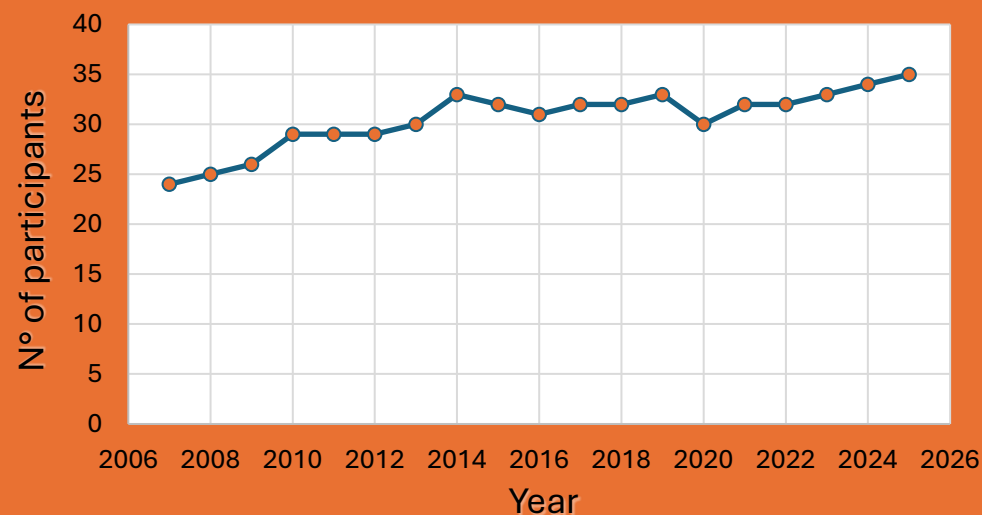


PT-01

2025 Participants

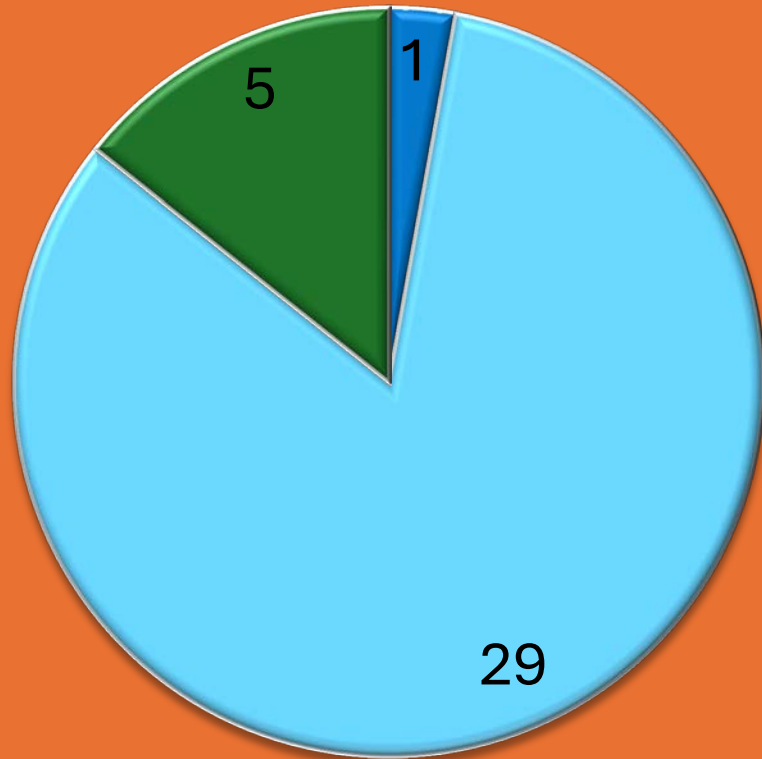
35 NRLs (including 9 outside EU)

Number of participants for PT-01 over time



PT-01: Samples

Sample Type:



- Pork 35g
- Pork 100g
- Horse 100g

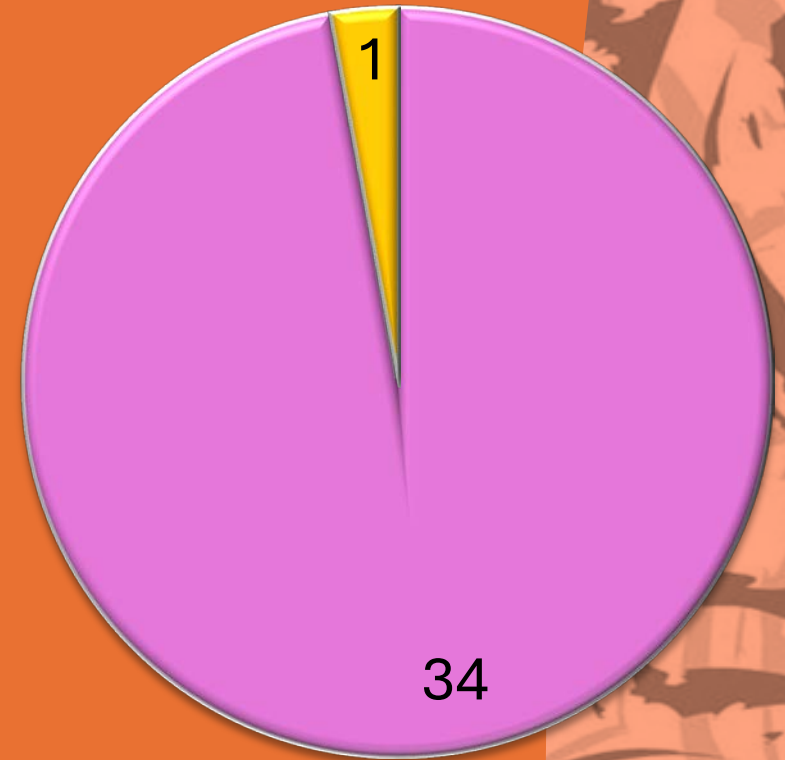


PT-01: Methods



- ISO 18743
- Trichomatic 35

Method used:



PT-01: RESULTS – Qualitative evaluation

Lab code	False negatives	False positives	PT final evaluation
NRL56	0	0	positive
NRL80	0	0	positive
NRL76	0	0	positive
NRL70	0	0	positive
NRL64	0	0	positive
NRL61	0	0	positive
NRL65	0	0	positive
NRL58	0	0	positive
NRL66	0	0	positive
NRL74	0	0	positive
NRL50	0	0	positive
NRL53	0	0	positive
NRL62	0	0	positive
NRL82	0	0	positive
NRL79	0	0	positive
TLE32	1	0	negative
NRL83	0	0	positive
NRL51	0	0	positive

Lab code	False negatives	False positives	PT final evaluation
NRL68	0	0	positive
NRL84	0	0	positive
NRL63	0	0	positive
NRL67	0	0	positive
NRL55	0	0	positive
NRL72	0	0	positive
NRL60	0	0	positive
NRL57	0	0	positive
NRL69	0	0	positive
NRL78	0	0	positive
NRL73	0	0	positive
NRL71	0	0	positive
NRL59	0	0	positive
TLE7	0	0	positive
NRL52	0	0	positive
NRL77	0	0	positive
NRL75	0	0	positive

PT-01: RESULTS – Causes of failure

Why TLE32 Lab failed the PT-01?

1° attempt: failure

TLE32 explanation

"A large portion of the meat remained undigested, I decided to filter it anyway, but I didn't observe anything on the plate. I'm not sure what caused the issue, and I'm currently running tests in the lab to figure it out."

prevented proper filtration of the samples

Larvae were lost

2° attempt: failure again

TLE32 explanation

"The meatballs were digested without first homogenizing them"

The same problem

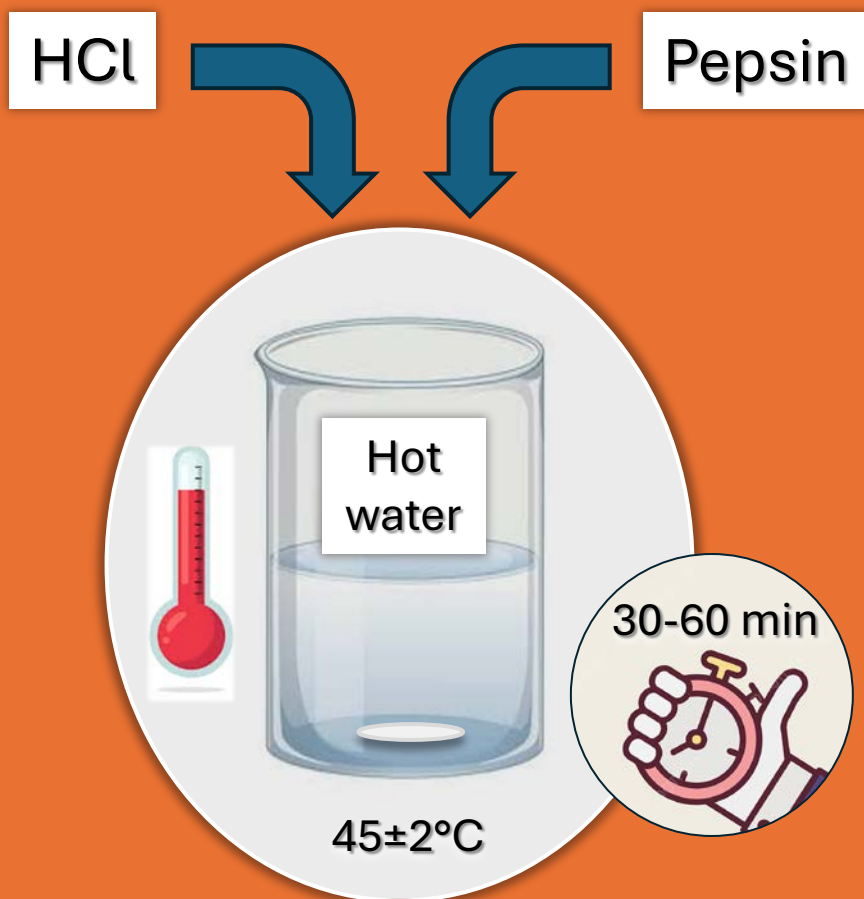
False negative

Only 1 larva out of 4 found

PT-01: RESULTS – Causes of failure

How to avoid problems during the digestion phase

ISO 18743:2015/Amd 1:2023



Check:

- The reagents have not expired
- The volumes of the different reagents are correct and proportional
- The digestive solution is maintained at the right temperature throughout the entire process
- The magnetic stirrer operates throughout the entire process
- Digestion time 30-60 minutes

Tips:

- Thoroughly homogenize the meatball before starting the digestion
- Meat should not be processed while still cold

No more than 5% of the initial weight

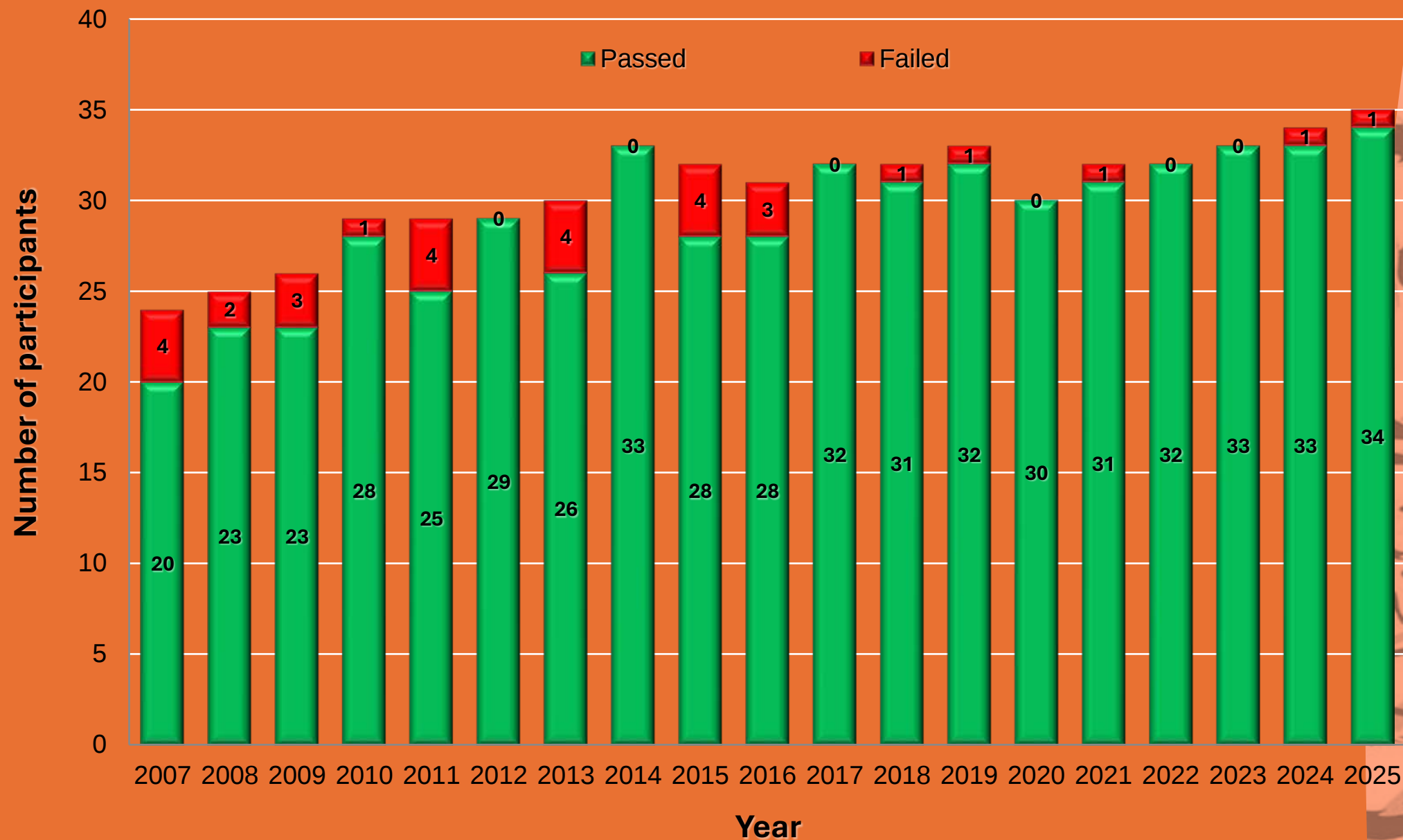
PT-01: Overtime comparison

Last five years results

Laboratory code		NRL56	NRL80	NRL76	NRL70	NRL64	NRL61	NRL65	NRL58	NRL66	NRL74	NRL50	NRL53	NRL62	NRL82	NRL79	TLE32	NRL83	NRL51	NRL68	NRL84	NRL63	NRL67	NRL55	NRL72	NRL60	NRL57	NRL69	NRL78	NRL73	NRL71	NRL59	TLE7	NRL52	NRL77	NRL75
Year	2021	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	-	P	P	P	-	P	P	P	P	P	P	P	P	P	P	P	-	P	P	P
	2022	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	-	P	P	P	-	P	P	P	P	P	P	P	P	P	P	P	-	P	P	P
	2023	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	-	P	P	P	P	P	P	P	P	P	P	P	-	P	P	P
	2024	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	F	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	-	P	P	P
	2025	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	F	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

P = passed F = failed - = no participation

PT-01: Overtime comparison



PT-03: Identification of *Trichinella* larvae at the species level by a molecular method

«Who are you?»



PT-03: Test material and Evaluation criteria



x10



In 96% EtOH

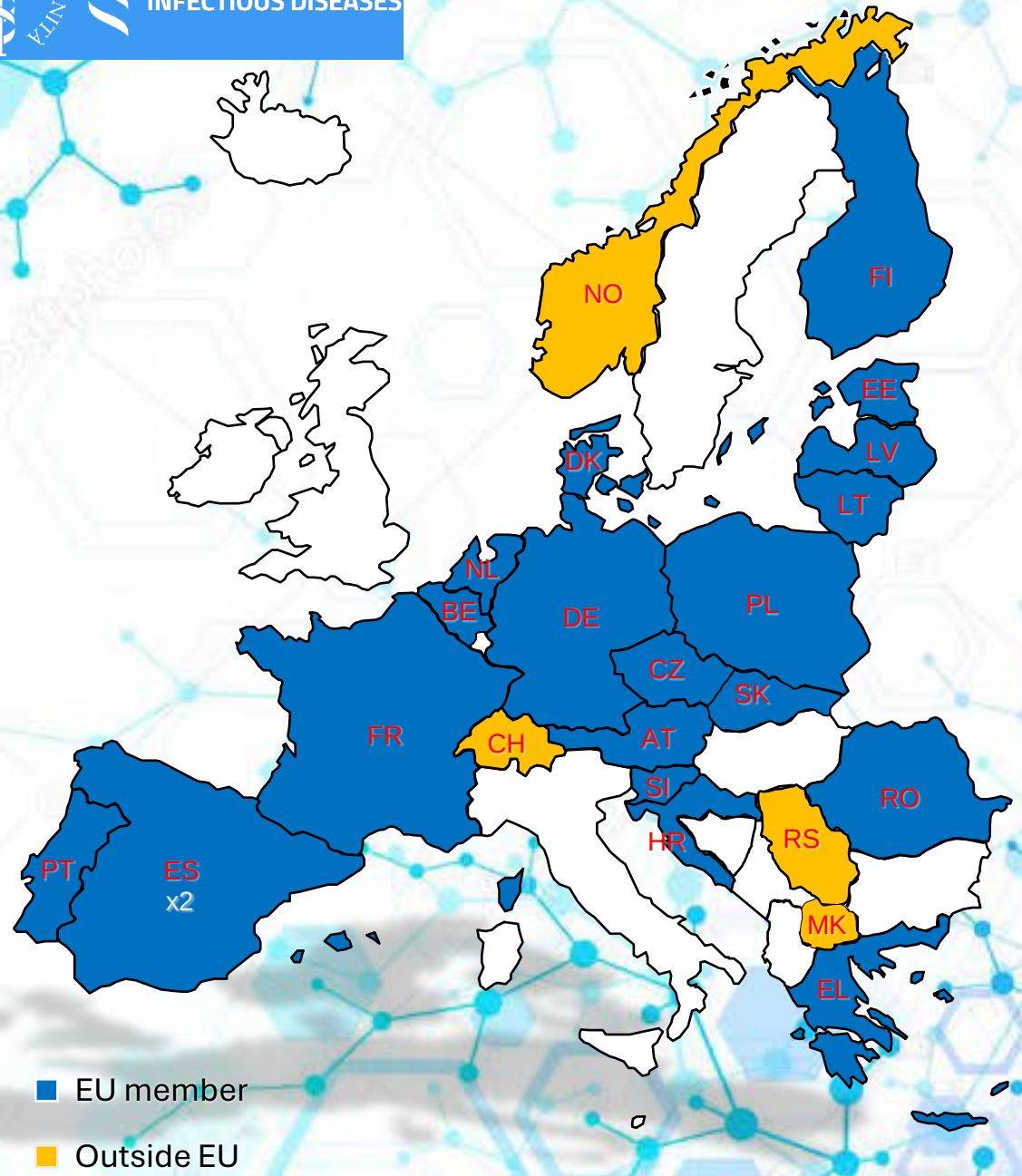
Participating laboratories must correctly
identify the *Trichinella* species in all samples

Test method:

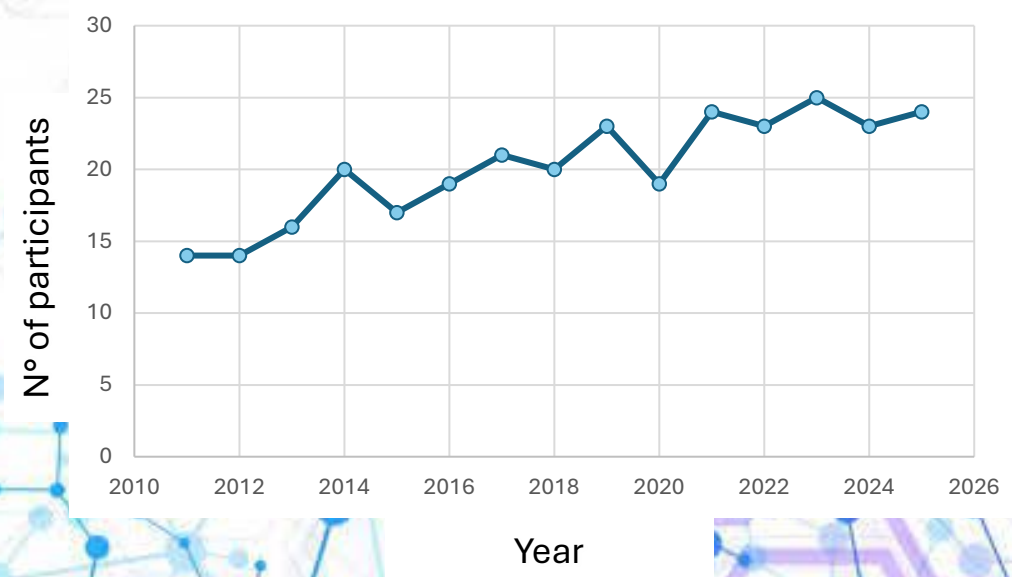
- Any molecular method able to discriminate the *Trichinella* species or genotype was allowed
- Possibility to analyse larvae singularly or as pool, depending on the sensitivity of the method used or on the experience of the technical staff

PT-03: 2025 Participants

24 NRLs (including 4 outside EU)



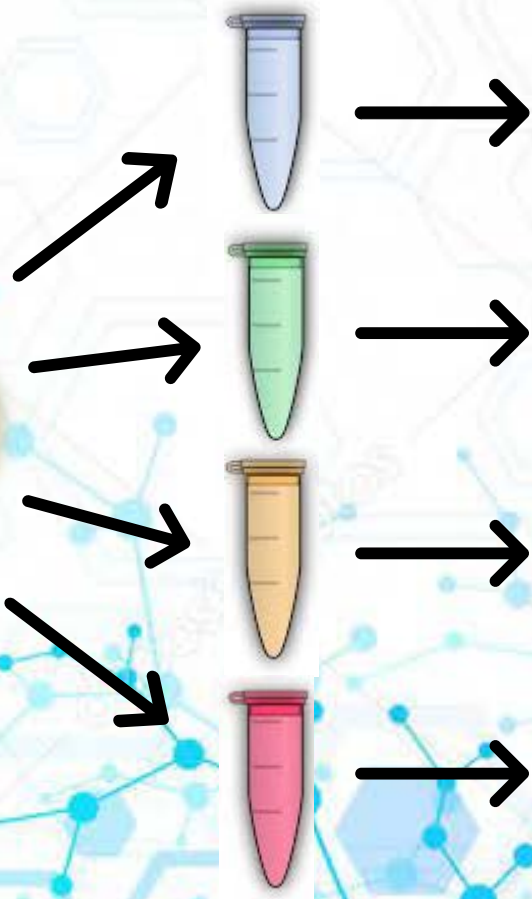
Number of participants for PT-03 over time



PT-03: Samples



x10



In 96% EtOH

2025 Samples

T. spiralis

T. nativa

T. britovi

T. pseudospiralis

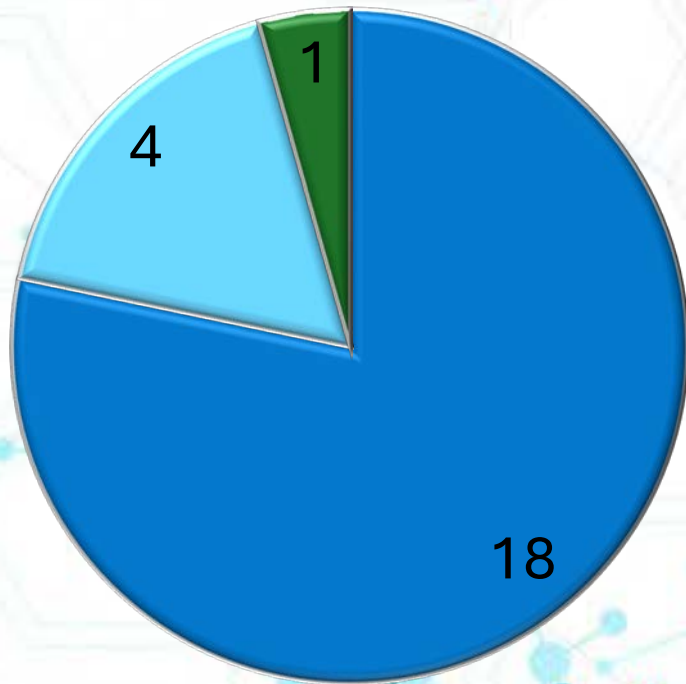
1) DNA purification

2) DNA detection

3) Species identification

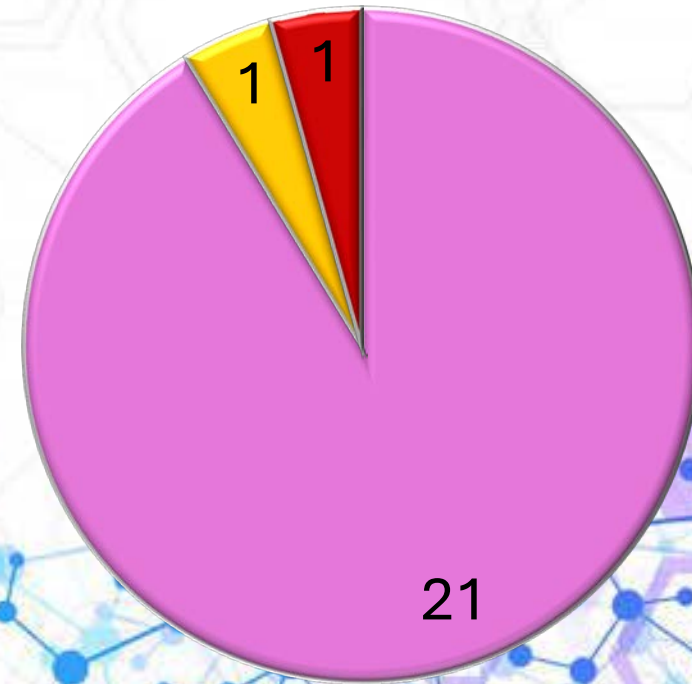
PT-03: Methods

1) DNA purification method:



- Magnetic resin
- pK incubation
- Other kit

2) DNA detection method:



- Multiplex PCR
- 5S rDNA (PCR and sequencing)
- In house (PCR and sequencing)

PT-03: Results – Qualitative evaluation

Lab code	Wrong identification	Missed identification	PT final evaluation	Lab code	Wrong identification	Missed identification	PT final evaluation
TM60	0	0	positive	TM72	0	1	negative
TM61	0	0	positive	TM73	0	0	positive
TM62	0	0	positive	TM74	0	0	positive
TM63	0	0	positive	TM75	0	0	positive
TM64	0	0	positive	TM76	0	0	positive
TM65	0	0	positive	TM77	0	0	positive
TM66	0	0	positive	TM78	-	-	NA
TM67	0	0	positive	TM79	0	0	positive
TM68	0	0	positive	TM80	0	0	positive
TM69	1	0	negative	TM81	0	0	positive
TM70	0	0	positive	TM82	0	0	positive
TM71	0	0	positive	TM83	0	0	positive

PT-03: Results – Causes of failure



Missed identification

Hypothesis 1:

Ethanol remained in the sample, inhibiting DNA extraction

Hypothesis 2:

Errors or skipped steps during the extraction phase

Hypothesis 3:

Errors or skipped steps during the amplification phase



How to avoid

Follow the protocol carefully



Wrong identification

Hypothesis 1:

There was a non-specific amplification, whose fragments coincidentally had the same size as the wrongly identified species

Hypothesis 2:

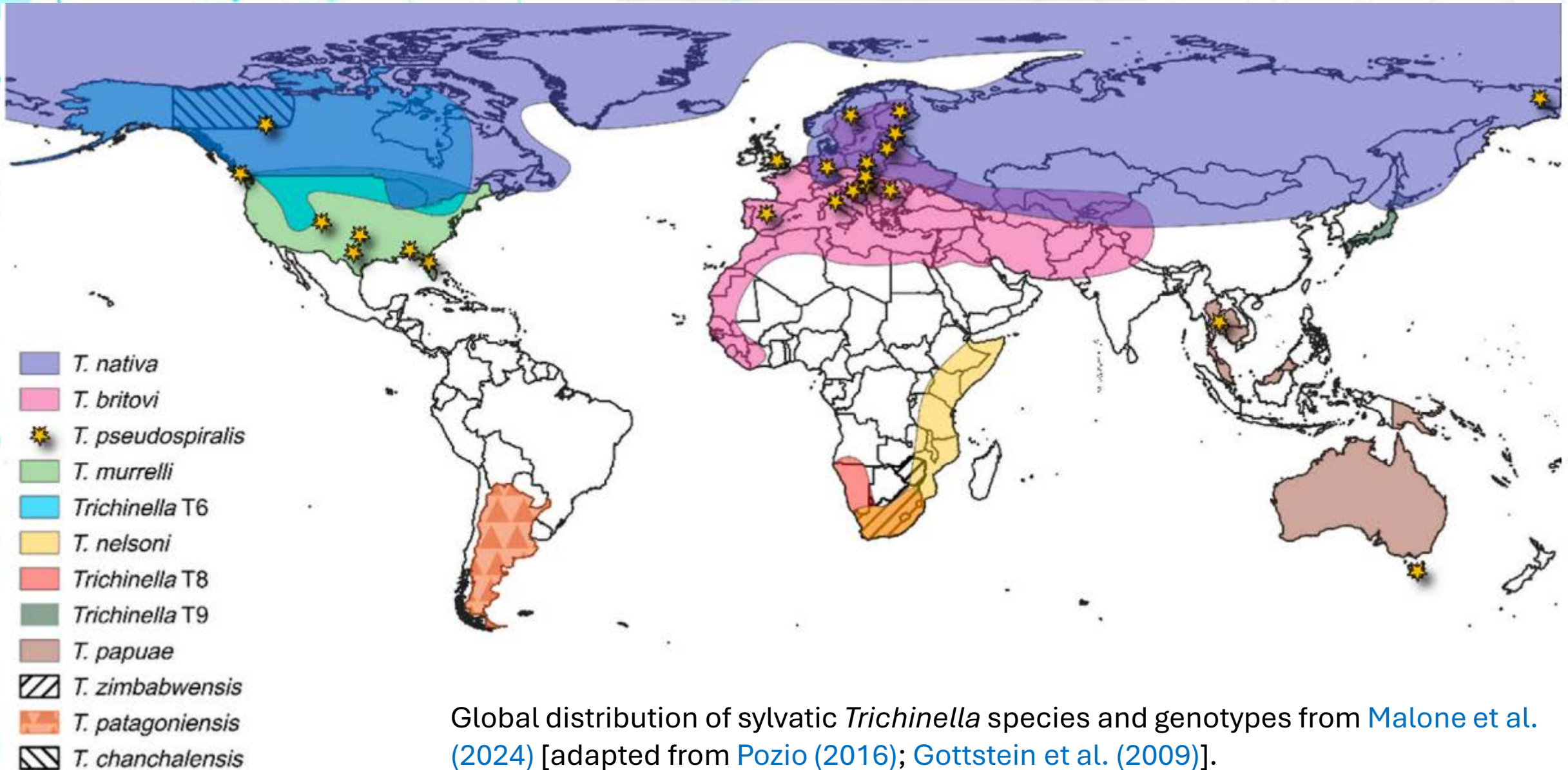
Incorrect interpretation of DNA fragment sizes



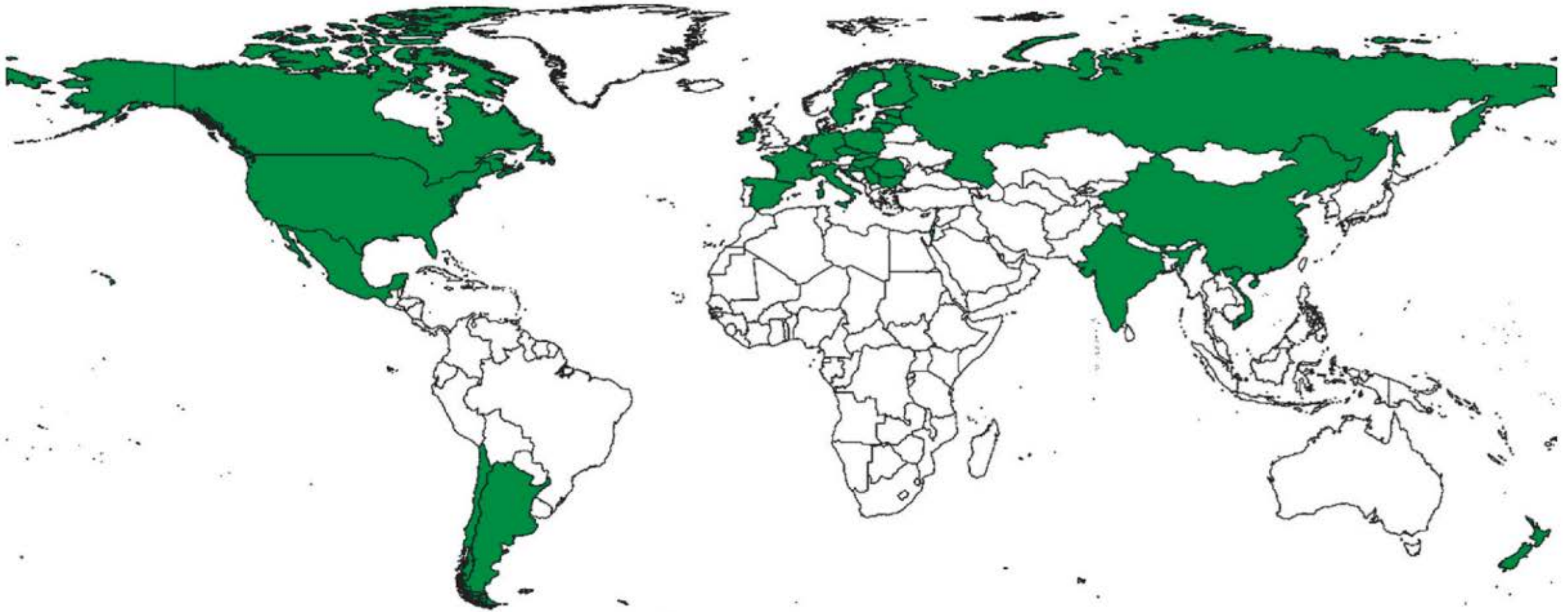
How to avoid

Consider the geographic origin of the larval sample (for routine analysis)

Geographic distribution of *Trichinella* species and genotypes



Geographic distribution of *Trichinella* species and genotypes



Confirmed reports of *T. spiralis* in wildlife from 1991-2023

Global distribution of *Trichinella spiralis* in wildlife reported
in [Malone et al. \(2024\)](#)

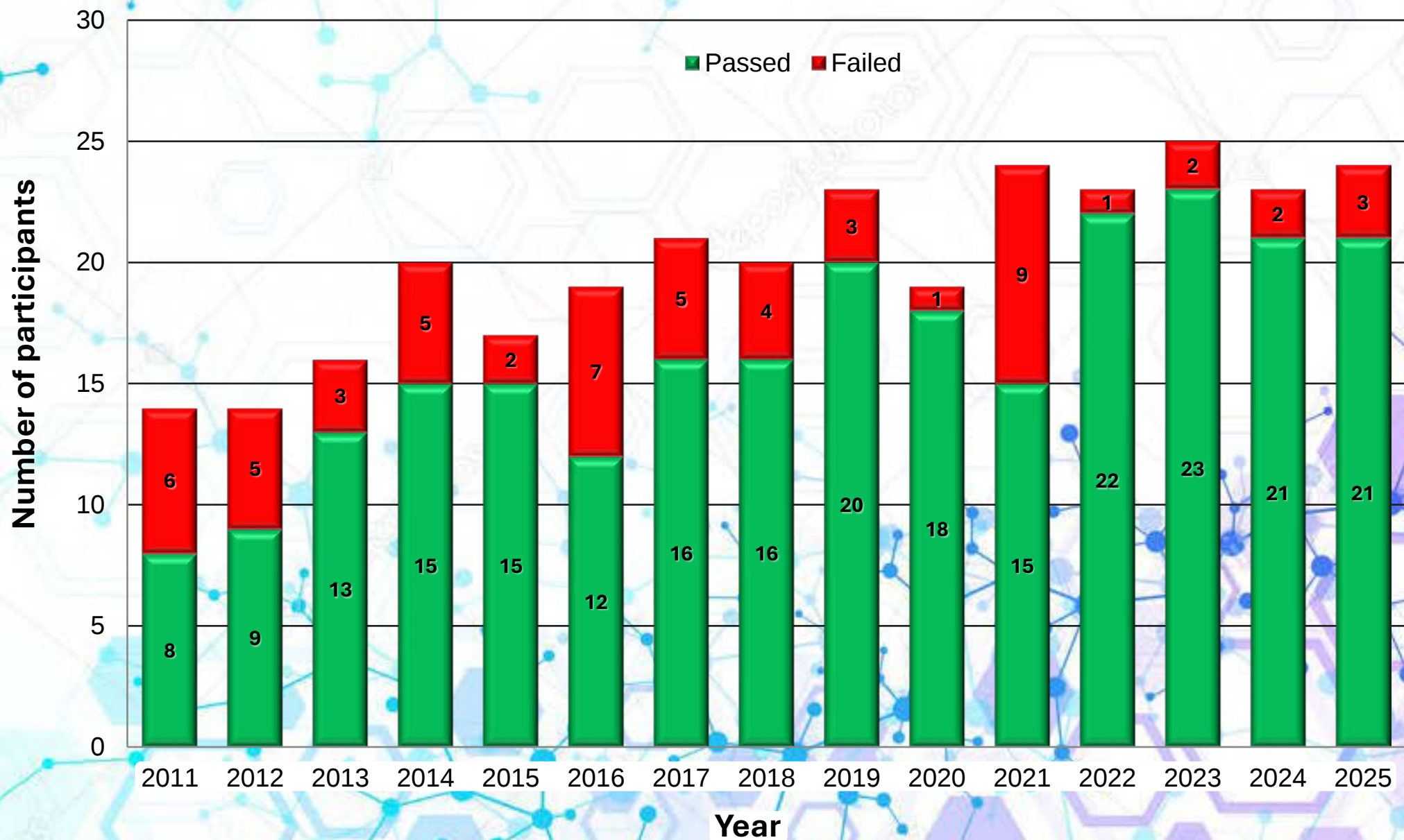
PT-03: Overtime comparison

Last five year results

Laboratory code		TM60	TM61	TM62	TM63	TM64	TM65	TM66	TM67	TM68	TM69	TM70	TM71	TM72	TM73	TM74	TM75	TM76	TM77	TM78	TM79	TM80	TM81	TM82	TM83
Year	2021	F	P	P	P	P	P	F	P	P	F	F	P	P	P	P	F	F	P	P	F	P	P	P	P
	2022	P	P	P	P	P	P	P	P	P	P	F	P	P	P	P	P	P	P	-	P	P	P	P	P
	2023	P	P	P	P	P	P	P	P	P	P	F	P	P	P	P	P	P	P	P	P	P	P	P	F
	2024	P	P	P	P	P	P	P	P	P	P	F	P	P	P	P	P	P	P	P	-	P	F	P	P
	2025	P	P	P	P	P	P	P	P	P	F	P	P	F	P	P	P	P	P	NA	P	P	P	P	P

P = passed F = failed NA = not applicable - = No participation

PT-03: Overtime comparison

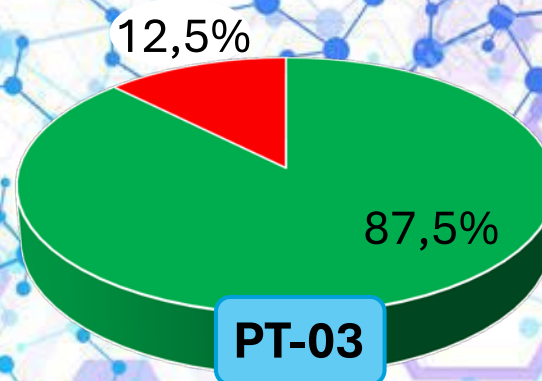
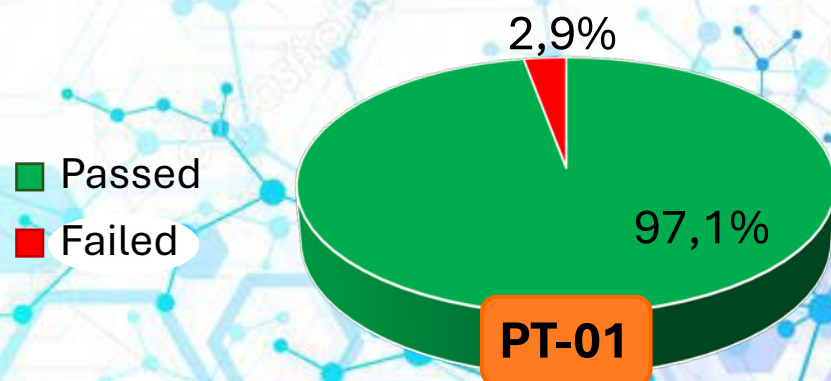


PT-01 and PT-03 Conclusion

- ★ The number of participants in both the PT-01 and PT-03 has been slowly but steadily increasing over the years



- ★ This year, the following labs passed the test:
 - 97.1% of PT-01 participants
 - 87.5% of PT-03 participants



If you fail (any) PT...

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

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



MICROSOFT FORMS





G. Marucci - RPVI



S. Cherchi



“Trichinella Team”



I. Tartarelli



F. Celani



A. Di Grazia

Thanks for your attention