XXI Workshop of National Reference Laboratories for Parasites

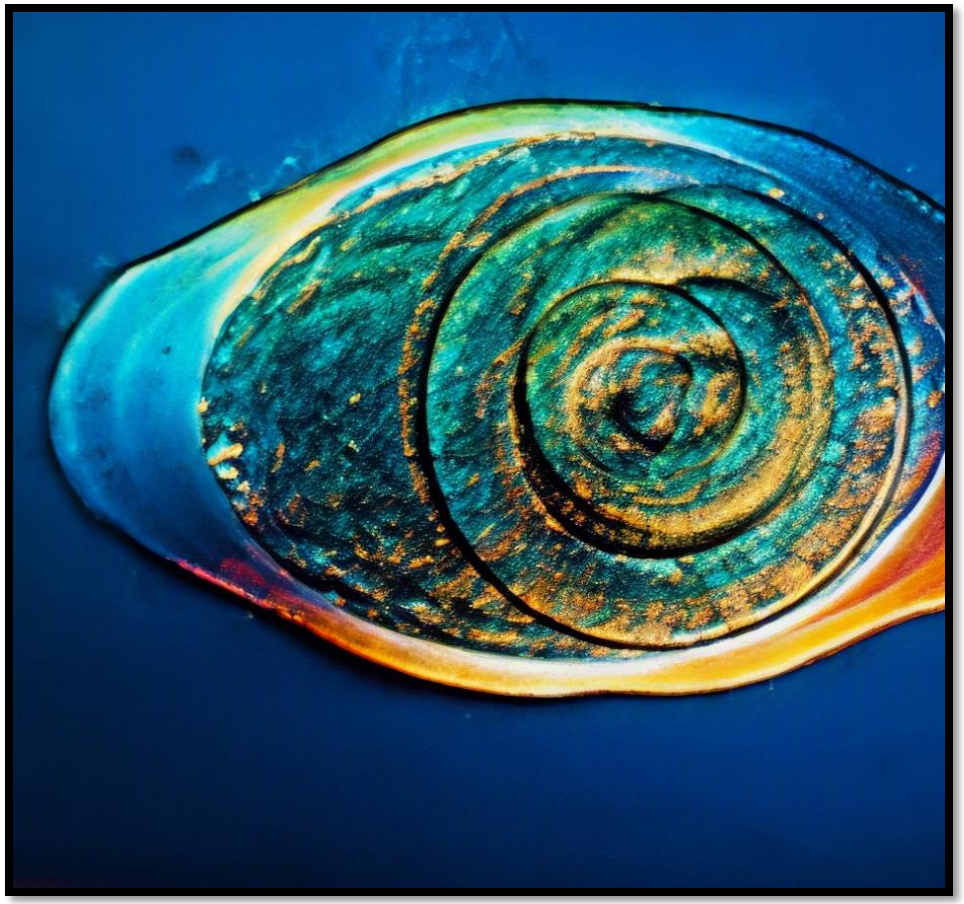
Istituto Superiore di Sanità

28-29 October 2026

DEPARTMENT INFECTIOUS DISEASES

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

Introduction regarding all PTs

Something new this year

No more Individual Reports



All the information

Shortly after the PTs

Accreditation body mark
(for accredited schemes)

Only Final Reports

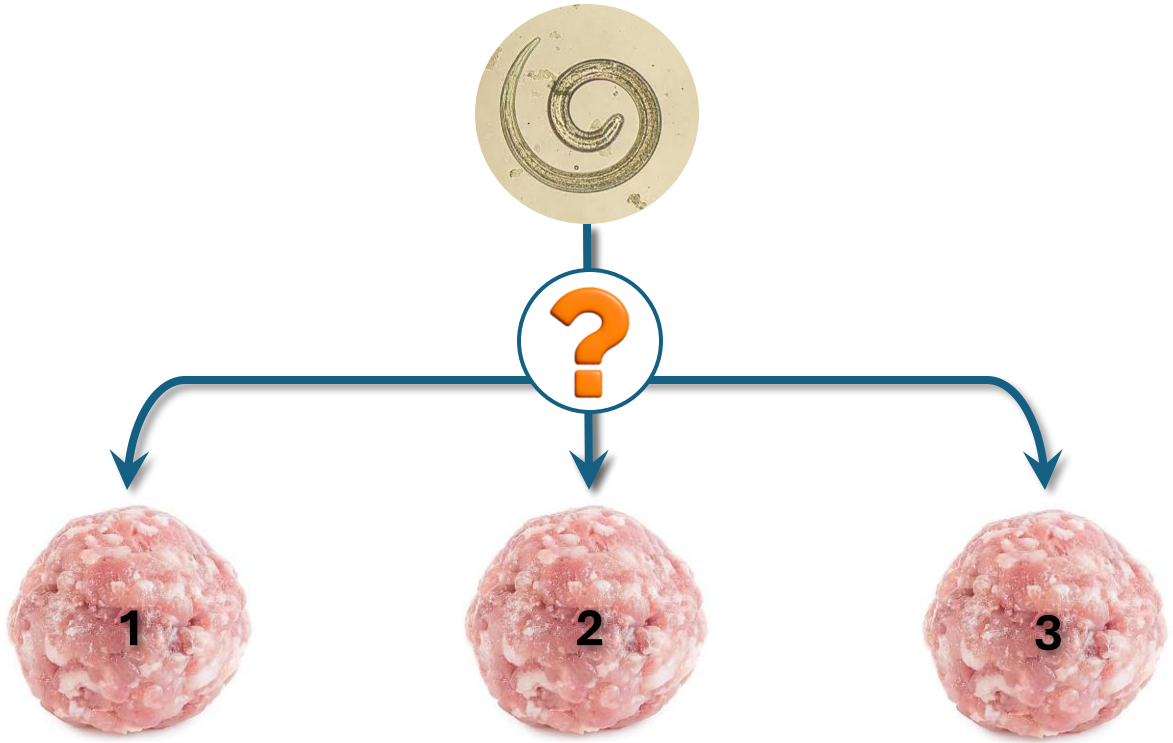


PT-01 | Artificial digestion to detect Trichinella larvae in meat samples according to ISO 18743:2015/Amd 1:2023 or Annex III Reg UE 2015/1375

DEPARTMENT INFECTIOUS DISEASES

Test objective

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



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

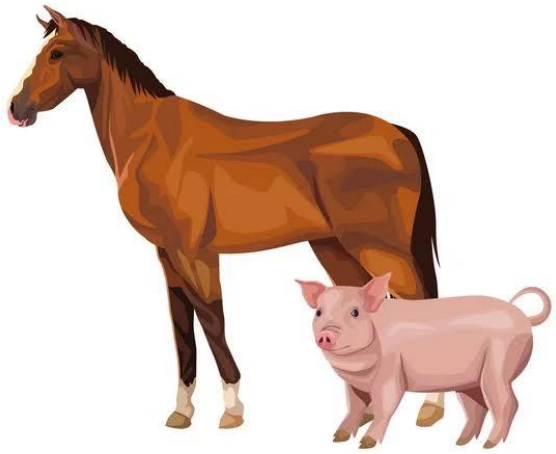
DEPARTMENT INFECTIOUS DISEASES

Test material

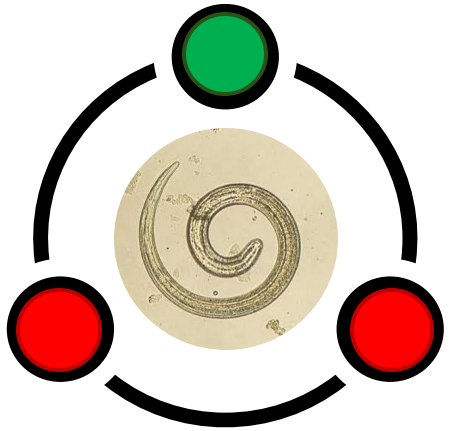
Meat type:
- pork meat
- horse meat

Samples weight:
- 100g
- 35g

2026 PT panel composition:
- 1 positive PT item (5 larvae)
- 2 negatives PT items





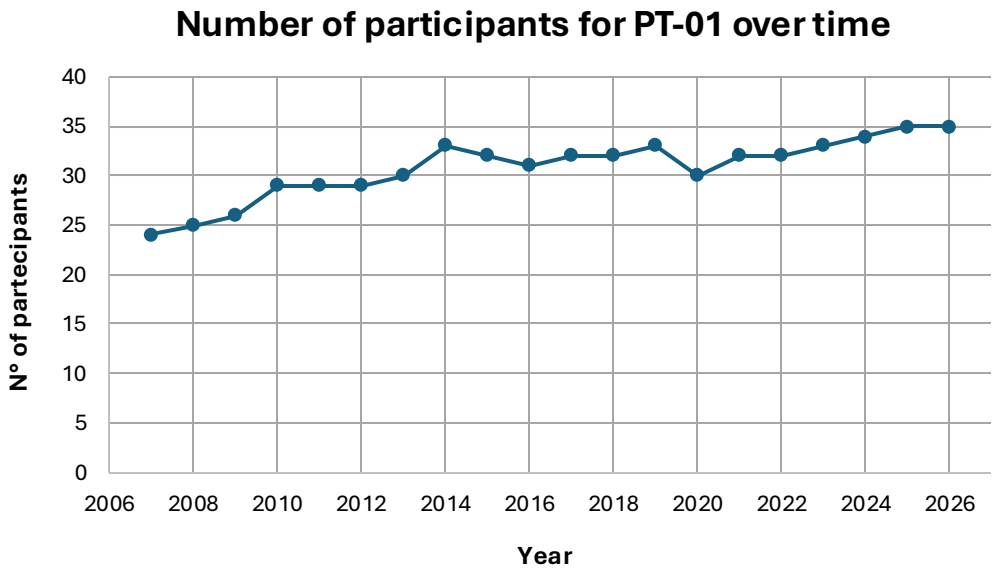


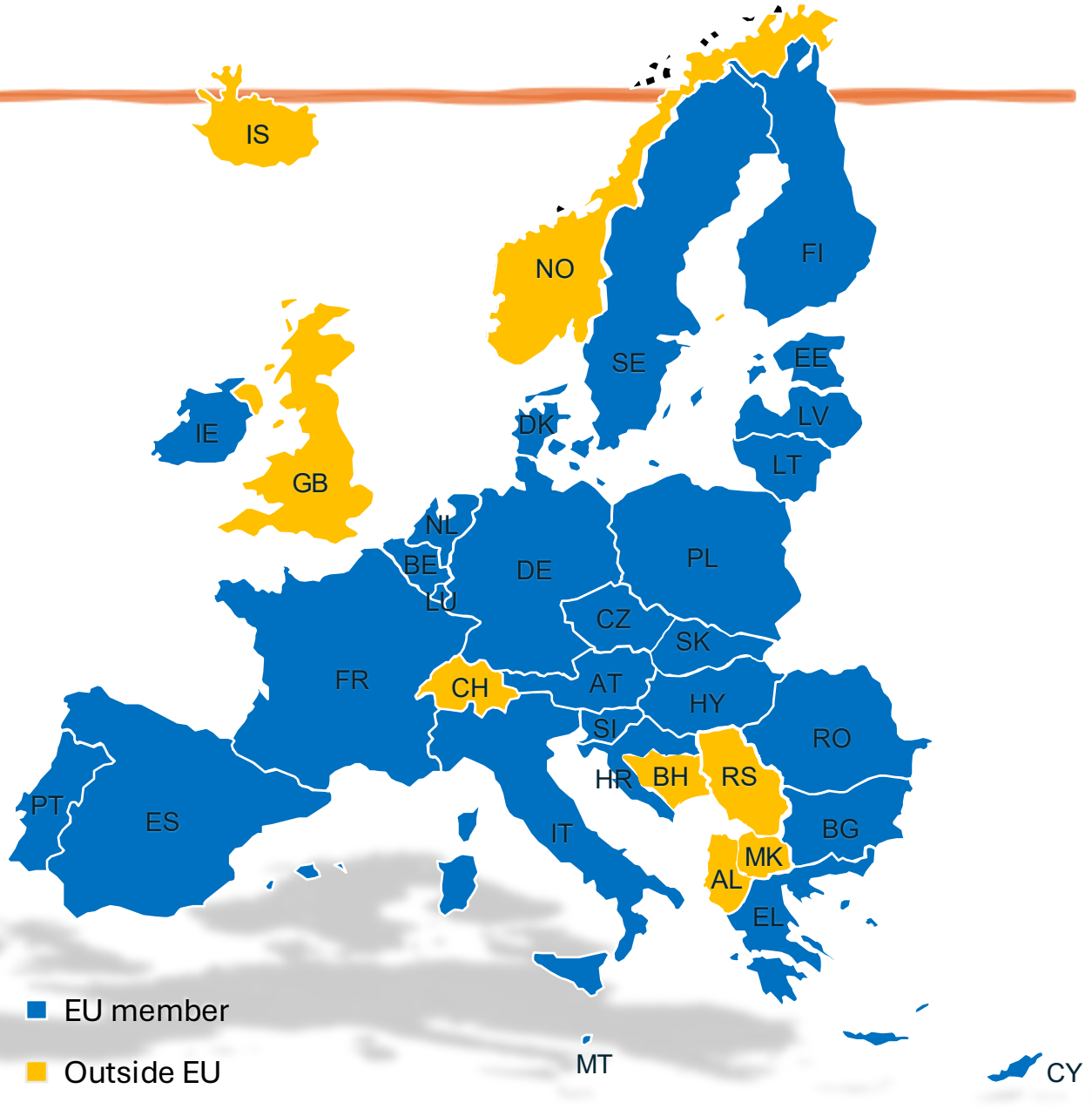
PT-01 | Artificial digestion to detect Trichinella larvae in meat samples according to ISO 18743:2015/Amd 1:2023 or Annex III Reg UE 2015/1375

DEPARTMENT INFECTIOUS DISEASES

2026 Participants

35 NRLs (including 8 outside EU)

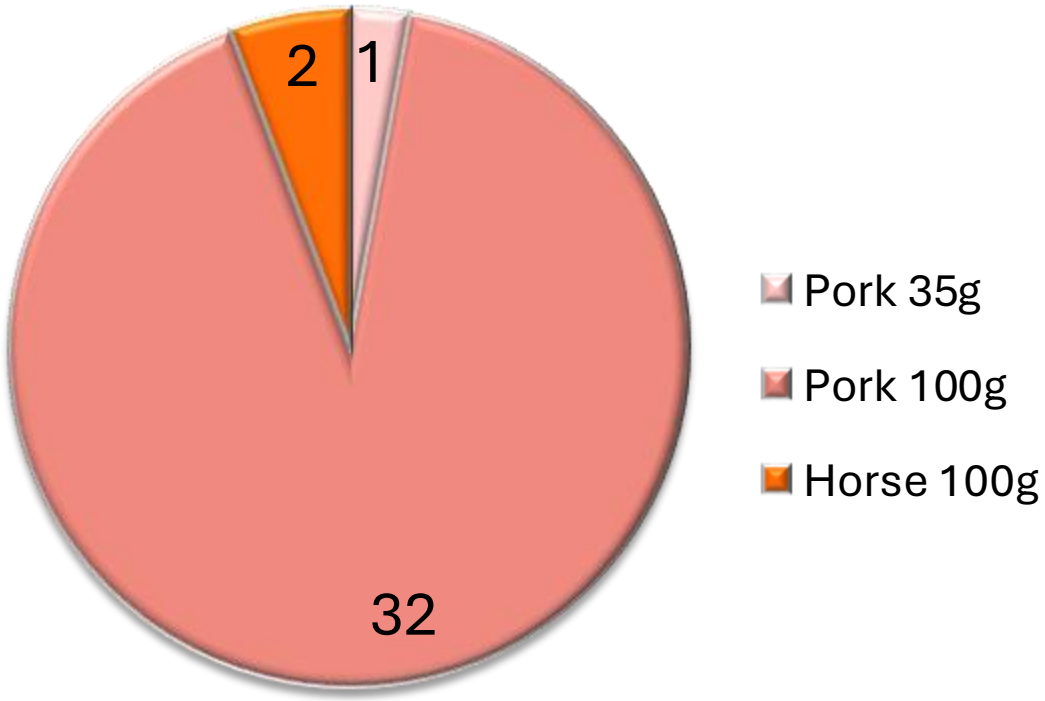




PT-01 | Artificial digestion to detect Trichinella larvae in meat samples according to ISO 18743:2015/Amd 1:2023 or Annex III Reg UE 2015/1375

DEPARTMENT INFECTIOUS DISEASES

Type of samples required





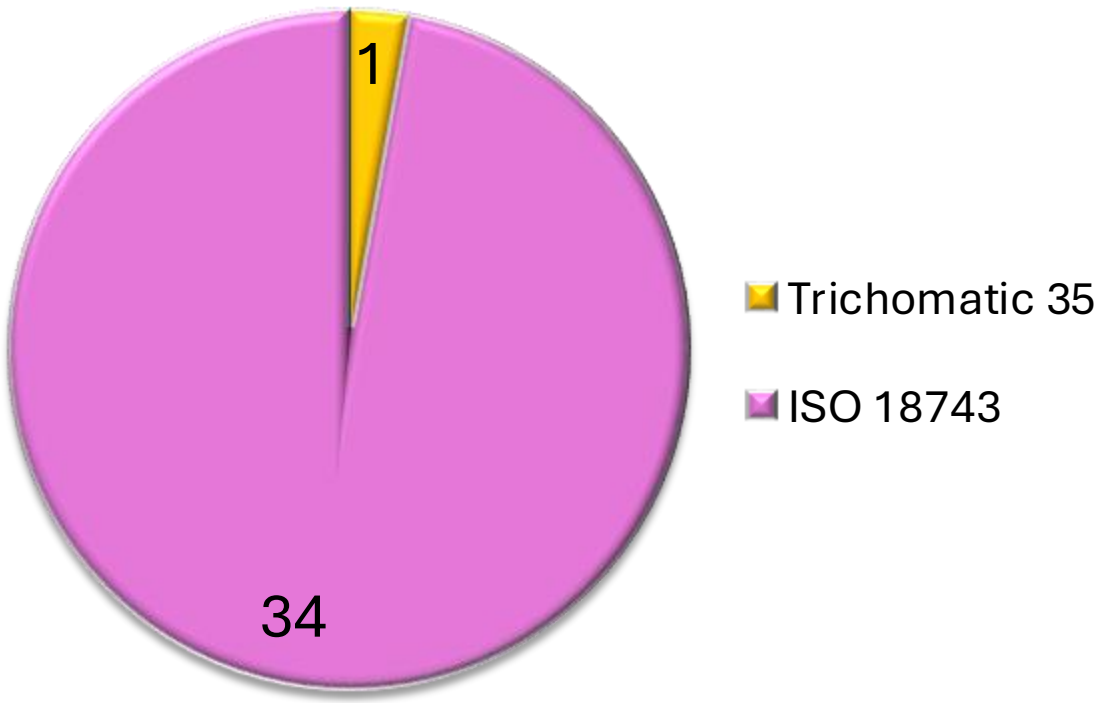




PT-01 | Artificial digestion to detect Trichinella larvae in meat samples according to ISO 18743:2015/Amd 1:2023 or Annex III Reg UE 2015/1375

DEPARTMENT INFECTIOUS DISEASES

Methods used for the analysis







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

DEPARTMENT INFECTIOUS DISEASES

RESULTS – Qualitative evaluation

Lab code	False negatives	False positives	PT final evaluation
NRL63	0	0	positive
NRL64	0	0	positive
NRL67	0	0	positive
NRL68	0	0	positive
NRL74	0	0	positive
NRL78	0	0	positive
NRL61	0	0	positive
NRL56	0	0	positive
NRL55	0	0	positive
NRL57	0	0	positive
NRL76	0	0	positive
NRL52	0	0	positive
NRL65	0	0	positive
NRL66	0	0	positive
NRL70	0	0	positive
NRL75	0	0	positive
NRL80	0	0	positive



Lab code	False negatives	False positives	PT final evaluation
NRL72	0	0	positive
NRL73	0	0	positive
NRL62	0	0	positive
NRL79	0	0	positive
NRL58	0	0	positive
NRL53	0	0	positive
NRL50	0	0	positive
NRL69	0	0	positive
NRL60	0	0	positive
NRL59	0	0	positive
NRL82	0	0	positive
NRL83	0	0	positive
NRL71	0	0	positive
TLE32	0	0	positive
NRL77	0	0	positive
NRL84	0	0	positive
NRL51	0	0	positive
NRL85	0	0	positive

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

DEPARTMENT INFECTIOUS DISEASES

OVERTIME COMPARISON – Last five years results

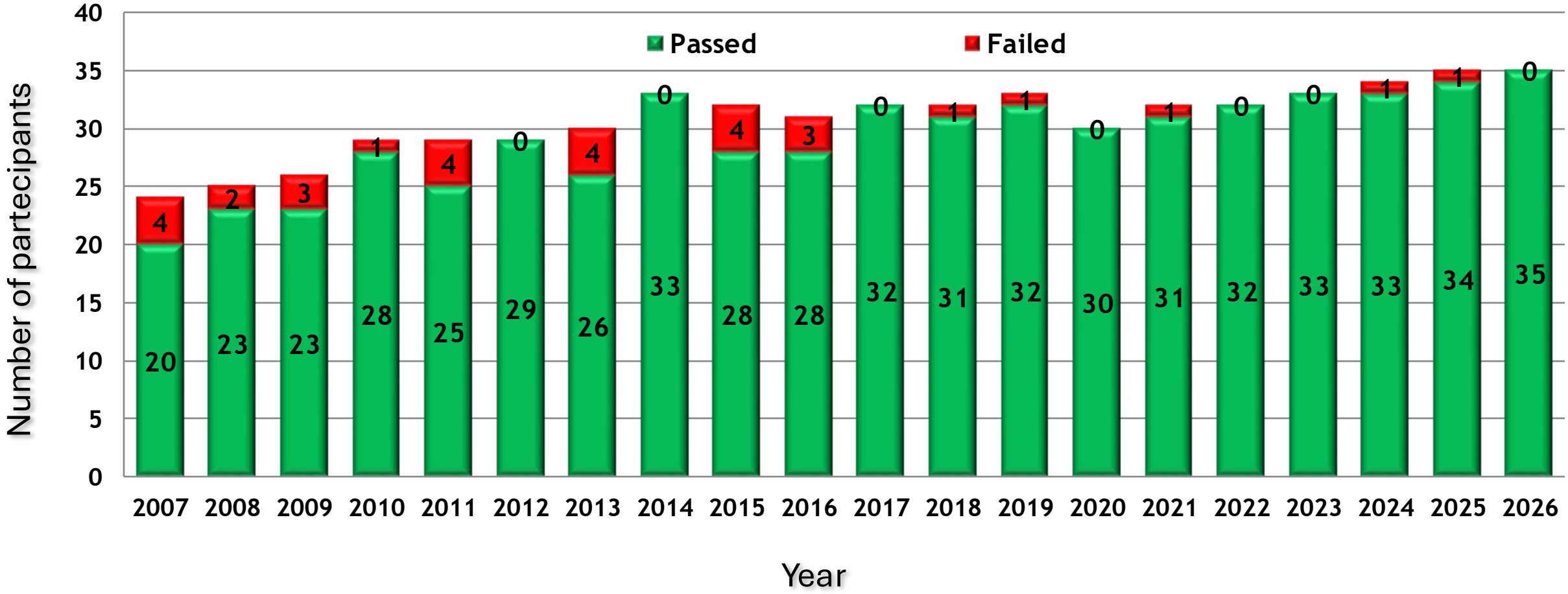
Laboratory code		NRL63	NRL64	NRL67	NRL68	NRL74	NRL78	NRL61	NRL56	NRL55	NRL57	NRL76	NRL52	NRL65	NRL66	NRL70	NRL75	NRL80	NRL72	NRL73	NRL62	NRL79	NRL58	NRL53	NRL50	NRL69	NRL60	NRL59	NRL82	NRL83	NRL71	TLE32	NRL77	NRL84	NRL51	NRL85
Year	2022	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	-	P	-	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	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	F	P	P	P	-
	2025	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	F	P	P	P	-
	2026	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	P	P

P = passed **F** = failed **-** = no partecipation

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

DEPARTMENT INFECTIOUS DISEASES

OVERTIME COMPARISON – From 2007 to the present



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

Test objective

Participating laboratories must correctly identify the Trichinella species in all samples



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

DEPARTMENT INFECTIOUS DISEASES

Test material



x10



hidden identity sample n°1

hidden identity sample n°2

hidden identity sample n°3

hidden identity sample n°4

In 96% EtOH

Procedural steps

1) DNA purification

↓

2) DNA amplification

↓

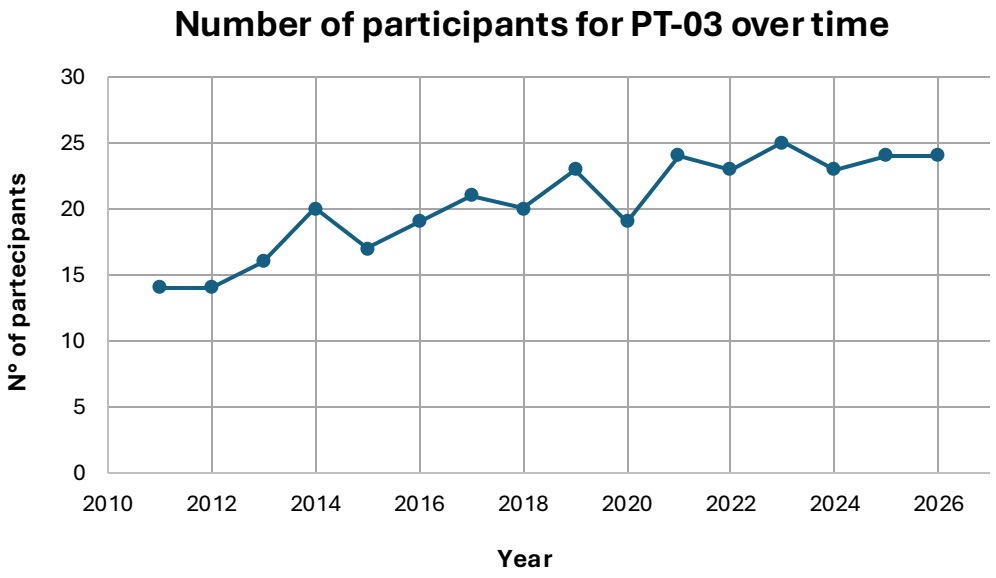
3) Interpretation of results

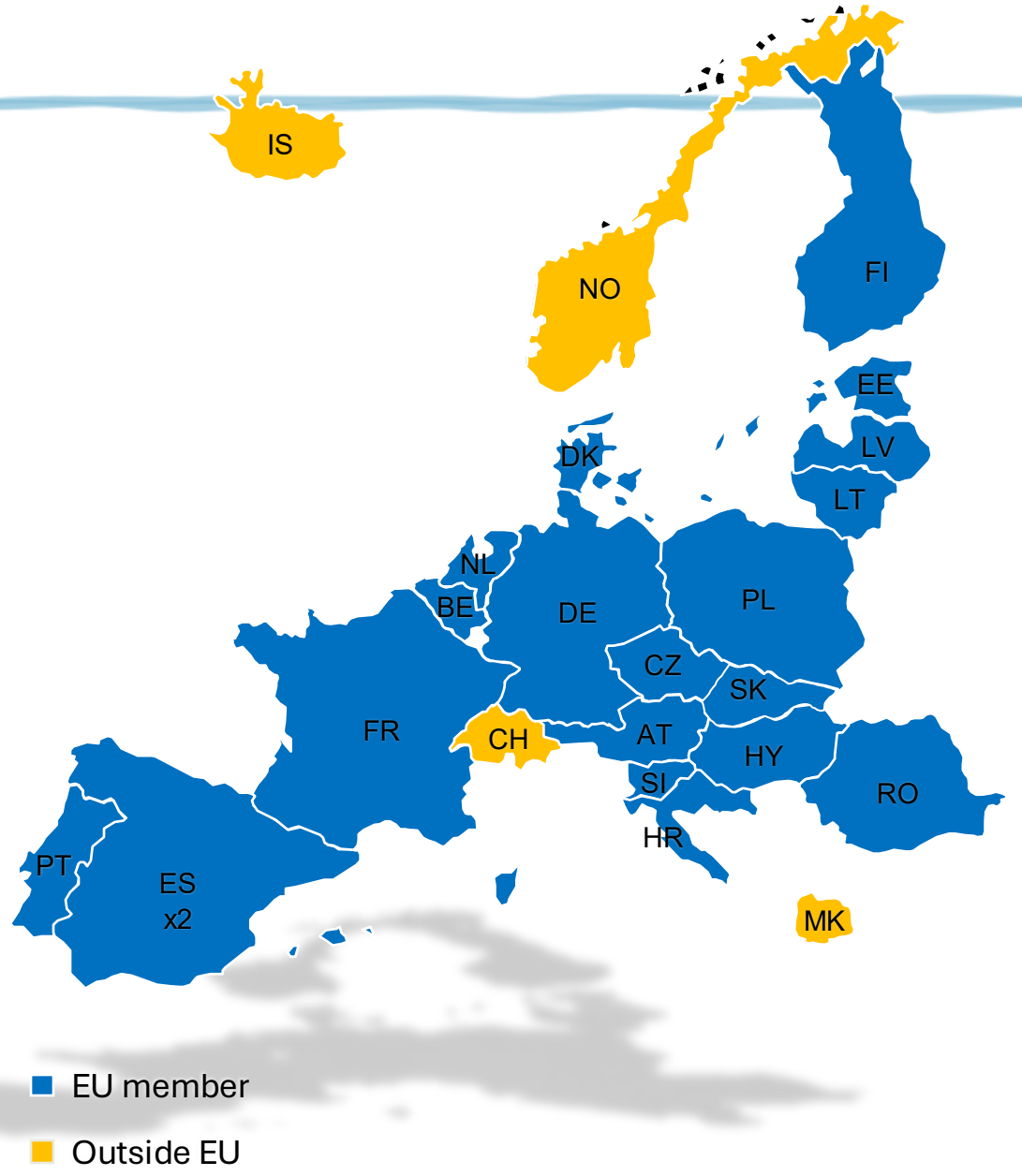
PT-03 | Identification of Trichinella larvae at the species level by a molecular method

DEPARTMENT INFECTIOUS DISEASES

2026 Participants

24 NRLs (including 4 outside EU)



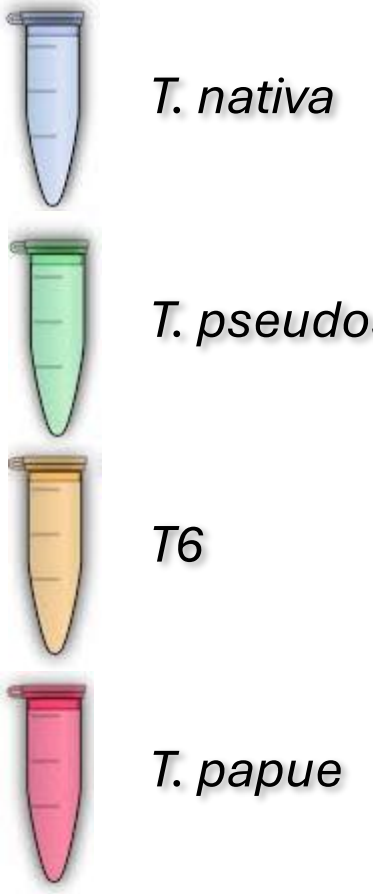


PT-03 | Identification of Trichinella larvae at the species level by a molecular method

2026 Samples supplied



x10



In 96% EtOH

How to approach the test:

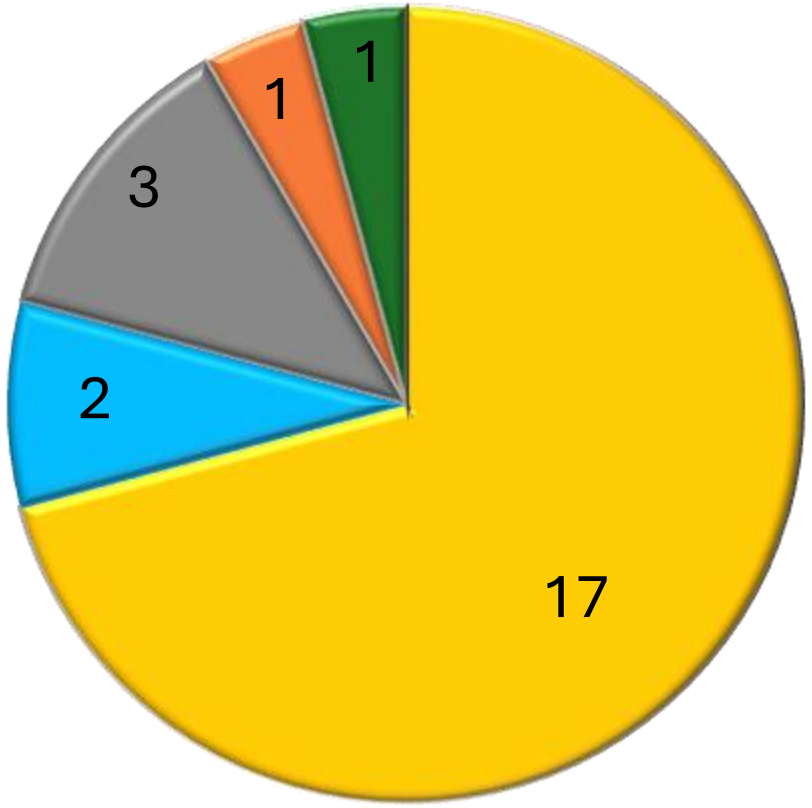
- Any molecular method able to discriminate the *Trichinella* species or genotype was allowed
- Possibility to analyse larvae singularly or as pool, depending on the sensitivity of the method used or on the experience of the technical staff

All methods used were considered equivalent and were evaluated in the same way

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

DEPARTMENT INFECTIOUS DISEASES

Methods used for the analysis



- EURL-P method (MI-02: magnetic resin and multiplex PCR)
- EURL-P method (MI-02) and sequencing
- La Rosa and Pozio 2003 (pK incubation and multiplex PCR)
- Rombout et al. 2001 (PCR and sequencing of 5S rDNA)
- in house PCR and sequencing of 16S rDNA and COI



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

DEPARTMENT INFECTIOUS DISEASES

RESULTS – Qualitative evaluation

Lab code	Wrong identification	Missed identification	PT final evaluation
TM01	0	0	positive
TM02	0	0	positive
TM03	0	0	positive
TM04	0	0	positive
TM05	0	0	positive
TM06	0	0	positive
TM07	0	0	positive
TM08	0	0	positive
TM09	0	0	positive
TM10	2	0	negative
TM11	0	1	negative
TM12	1	0	negative

Lab code	Wrong identification	Missed identification	PT final evaluation
TM13	0	0	positive
TM14	0	0	positive
TM15	0	0	positive
TM16	1	0	negative
TM17	2	0	negative
TM18	0	0	positive
TM19	0	0	positive
TM20	0	0	positive
TM21	0	0	positive
TM22	0	0	positive
TM23	0	0	positive
TM24	0	0	positive

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

DEPARTMENT INFECTIOUS DISEASES

RESULTS – Details regarding test failures (part 1)

Participant code	Applied method	PT item code	Result	Assigned value	Outcome
TM10	EURLP method	Trich37	*T. nativa*	*T. nativa*	correct
		Trich38	*T. pseudospiralis*	*T. pseudospiralis*	correct
		Trich39	*T. papuae*	*T6*	incorrect
		Trich40	*T. britovi*	*T. papuae*	incorrect
TM11	EURLP method	Trich41	*T. nativa*	*T. nativa*	correct
		Trich42	*impossible*	*T. pseudospiralis*	incorrect
		Trich43	*T6*	*T6*	correct
		Trich44	*T. papuae*	*T. papuae*	correct
TM12	EURLP method	Trich45	*T. nativa*	*T. nativa*	correct
		Trich46	*T. pseudospiralis*	*T. pseudospiralis*	correct
		Trich47	*T6*	*T6*	correct
		Trich48	*T. zimbabwensis*	*T. papuae*	incorrect

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

DEPARTMENT INFECTIOUS DISEASES

RESULTS – Details regarding test failures (part 2)

Participant code	Applied method	PT item code	Result	Assigned value	Outcome
TM16	EURLP method	Trich61	*T. nativa*	*T. nativa*	correct
		Trich62	*T. pseudospiralis*	*T. pseudospiralis*	correct
		Trich63	*T. britovi*	*T6*	incorrect
		Trich64	*T. papuae*	*T. papuae*	correct
TM17	EURLP method	Trich65	*T. nativa*	*T. nativa*	correct
		Trich66	*T. murrelli*	*T. pseudospiralis*	incorrect
		Trich67	*T6*	*T6*	correct
		Trich68	*T. britovi*	*T. papuae*	incorrect

The participants who failed the PT all applied the EURL-P method, but there is no direct correlation between failing the test and the method used for species identification.

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

DEPARTMENT INFECTIOUS DISEASES

RESULTS – Investigating the causes of mistakes (Part 1)

Type 1 error causing a wrong identification	Labs involved	Cause of these mistakes
T6 → *T. papuae (10)*	TM10	All these laboratories reported having problems during the electrophoresis phase
T. papuae (10) → *T. britovi (T3)*		
T. pseudospiralis (T4) → *T. murrelli (T5)*	TM17	
T. pseudospiralis (T4) → *impossible*	TM11	

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

DEPARTMENT INFECTIOUS DISEASES

RESULTS – Investigating the causes of mistakes (Part 1)

Type 1 error causing a wrong identification	Labs involved	Cause of these mistakes
T6 → *T. papuae (10)*	TM10	The laboratories reported problems during electrophoresis
T. papuae (10) → *T. britovi (T3)*	TM10	
T. pseudospiralis (T4) → *T. murrelli (T5)*	TM17	
T. pseudospiralis (T4) → *impossible*	TM11	

TM10



1) Lab TM10 reported that only the 210 bp band was clearly visible, while the expected 127 bp band for *T6* appeared as a faint smear and could not be confidently interpreted. So, they interpreted the sample as *T. papue.*

2) The lab reported that a very faint band corresponding to *T. britovi* was observed, while the second diagnostic band was not visible. Anyway, they interpreted the sample as *T. britovi.*

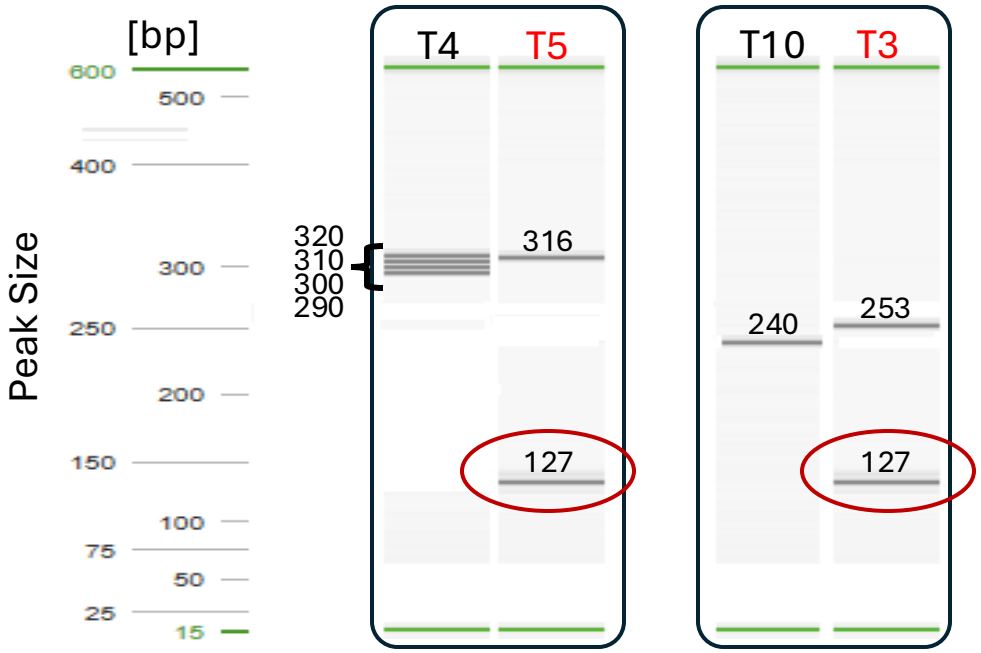
PT-03 | Identification of Trichinella larvae at the species level by a molecular method

DEPARTMENT INFECTIOUS DISEASES

RESULTS – Investigating the causes of mistakes (Part 1)

Type 1 error causing a wrong identification	Labs involved	Cause of these mistakes
T6 → T. papuae (10)	TM10	The laboratories reported problems during electrophoresis
T. papuae (10) → T. britovi (T3)	TM17	
T. pseudospiralis (T4) → T. murrelli (T5)	TM17	
T. pseudospiralis (T4) → impossible	TM11	

TM17



Lab TM17 observed the presence of nonspecific bands in the PCR, probably due to incorrect primer synthesis, which led to the misidentification of the two PT items

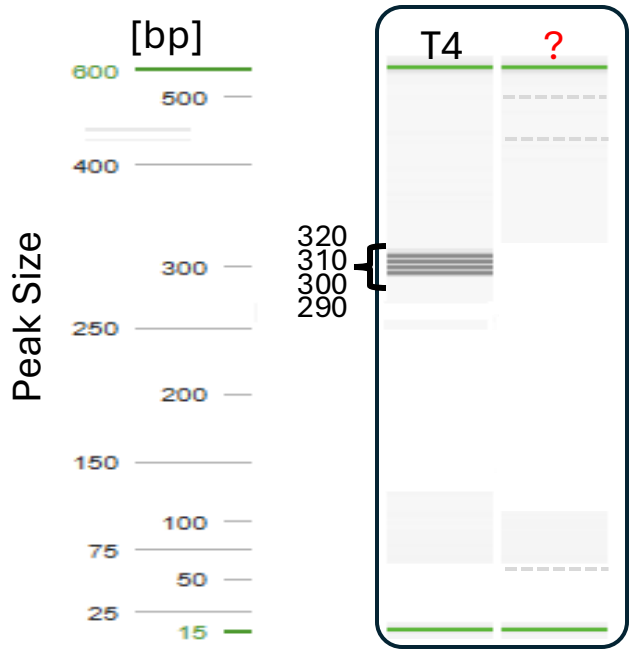
PT-03 | Identification of Trichinella larvae at the species level by a molecular method

DEPARTMENT INFECTIOUS DISEASES

RESULTS – Investigating the causes of mistakes (Part 1)

Type 1 error causing a wrong identification	Labs involved	Cause of these mistakes
T6 → T. papuae (10)	TM10	The laboratories reported problems during electrophoresis
T. papuae (10) → T. britovi (T3)	TM10	
T. pseudospiralis (T4) → T. murrelli (T5)	TM17	
T. pseudospiralis (T4) → impossible	TM11	

TM11



Lab TM11 laboratory observed bands that were not very well-defined and did not correspond to any of the bands expected for the various species. Consequently, they decided to classify the sample as "impossible."

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

DEPARTMENT INFECTIOUS DISEASES

RESULTS – Investigating the causes of mistakes (Part 2)

Type 2 error causing a wrong identification	Labs involved	Cause of the mistake
T6 → *T. britovi (T3)*	TM16	These species have been confused with each other because they exhibit similar banding patterns.
T. papuae (T10) → *T. zimbabwensis (T11)*	TM12	



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

DEPARTMENT INFECTIOUS DISEASES

OVERTIME COMPARISON – Last five years results

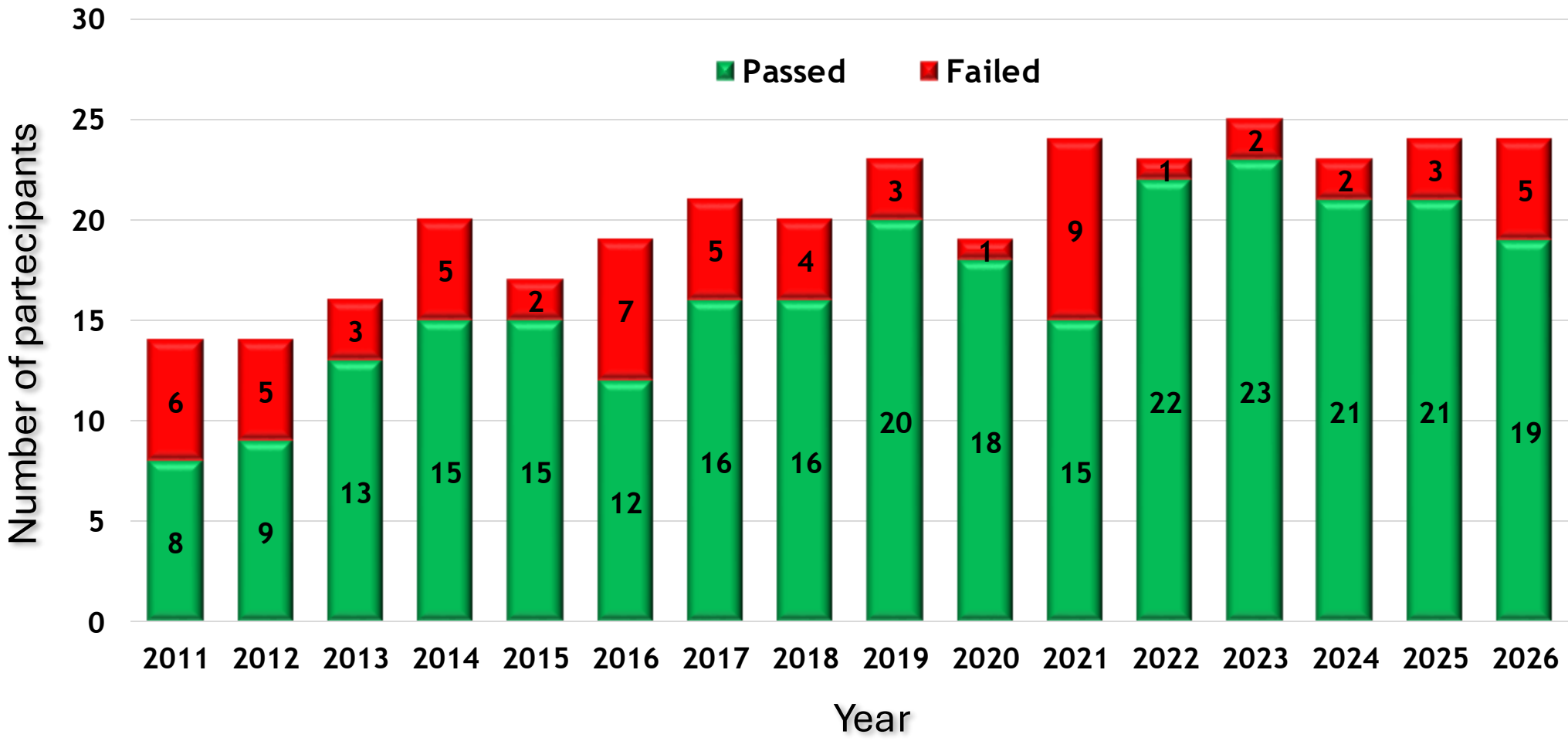
Laboratory code		TM01	TM02	TM03	TM04	TM05	TM06	TM07	TM08	TM09	TM10	TM11	TM12	TM13	TM14	TM15	TM16	TM17	TM18	TM19	TM20	TM21	TM22	TM23	TM24
Year	2022	P	P	P	P	P	P	P	P	P	-	-	F	P	P	P	P	P	P	P	P	P	P	P	P
	2023	P	P	P	P	P	P	P	P	P	-	P	F	P	P	P	P	P	P	P	P	P	P	P	F
	2024	P	P	P	P	P	P	P	P	P	-	-	F	P	P	P	P	P	P	P	-	P	F	P	P
	2025	P	P	P	P	P	P	P	P	P	-	-	P	P	F	P	P	P	P	P	P	P	P	P	P
	2026	P	P	P	P	P	P	P	P	P	F	F	F	P	P	P	F	F	P	P	P	P	P	P	P

P = passed **F** = failed **-** = no partecipation

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

DEPARTMENT INFECTIOUS DISEASES

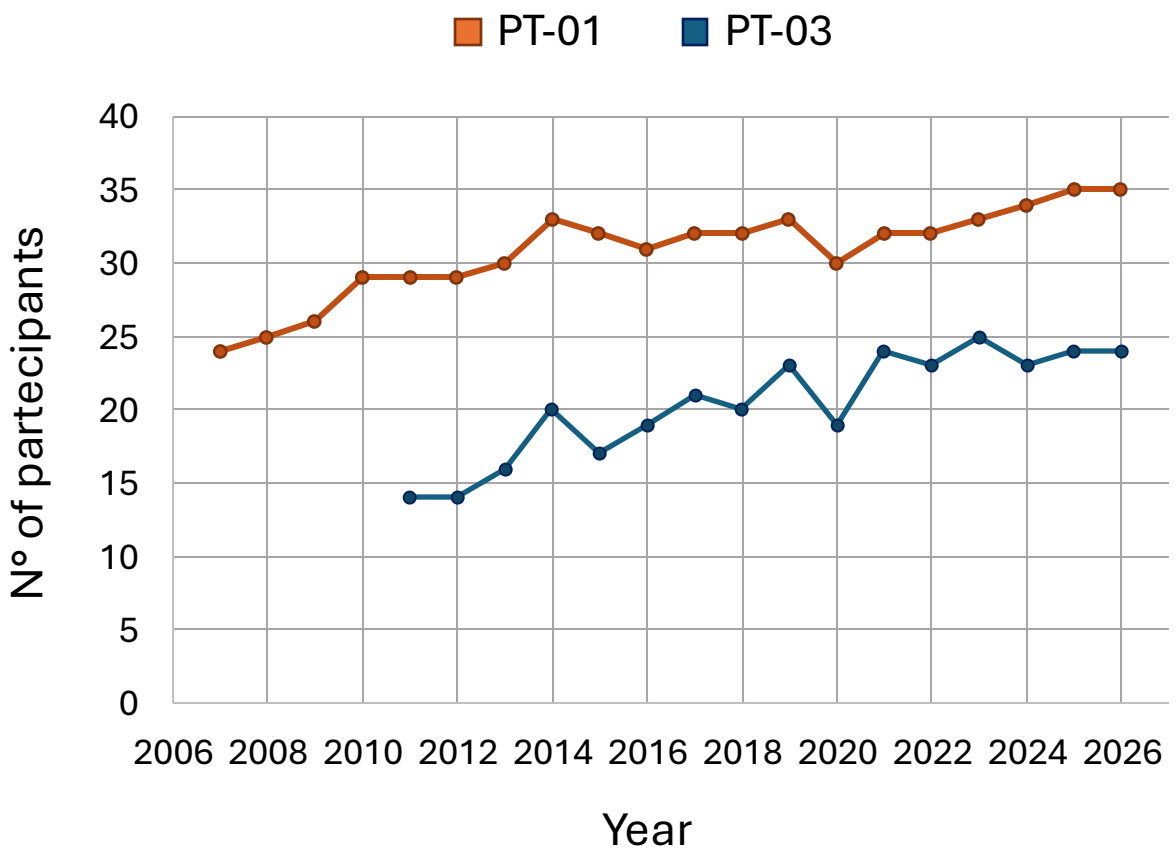
OVERTIME COMPARISON – From 2011 to the present



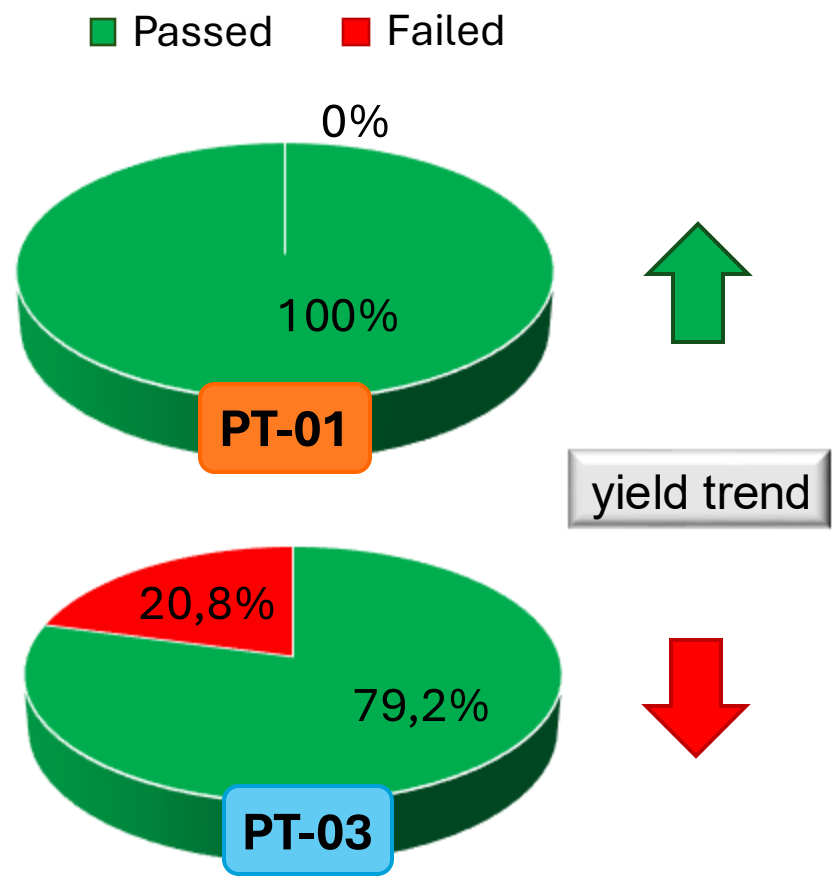


PT-01 and PT-03 Conclusion

Participation rate



Performance





G. Marucci
- RPVI -



S. Cherchi
- technician -

"Trichinella Team"



I. Tartarelli
- technician -



F. Celani
- technician -



A. Di Grazia
- technician -

