DEPARTMENT
INFECTIOUS DISEASES

XVII Workshop of National Reference Laboratories for Parasites
Istituto Superiore di Sanità
15- 16 September 2022

Proficiency testing on artificial digestion to detect *Trichinella* larvae in meat samples according **to ISO 18743:2015**

DEPARTMENT
INFECTIOUS DISEASES

What changed in PT 2022

ISO/TC34/SC9 aims at ensuring that all standardized reference methods are validated for their scope, which is needed for the implementation of ISO 16140-3:2021 (Method validation — Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory)

The ISO Committee highlighted the need for validation data for the *Trichinella* standard ISO 18743:2015

Additionally, the European Commission raised an issue about the reduction of the time required to examine the digestive fluid reported in the ISO

then

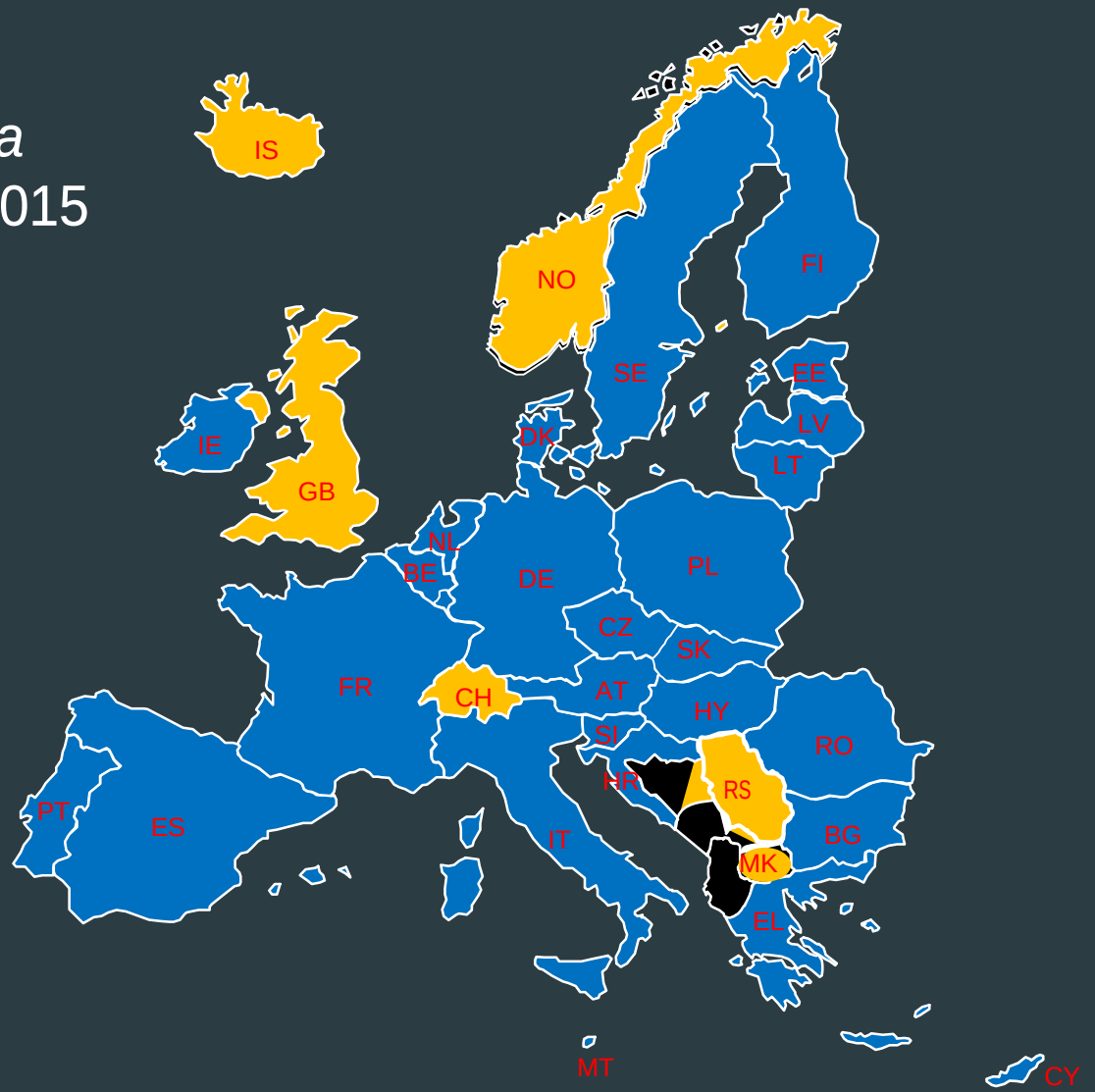
The 2022 PT was planned to ensure and satisfy the validation requirements for an inter-laboratory study (e.g., sample panel composition, homogeneity of the panel, homogeneity of the applied test)

Purpose and participants

Purpose:

- test the capacity of NRLs to identify *Trichinella* muscle larvae in meat by applying ISO 18743:2015
- acquire data for the ISO validation

Participants: **32 NRLs** (including 6 outside EU)



DEPARTMENT
INFECTIOUS DISEASES

Test material

Meat type: pork

Samples weight: 100 g

PT panel composition: 2 positive samples (3 and 5 larvae) and 1 negative sample

DEPARTMENT
INFECTIOUS DISEASES

Results

qualitative evaluation

Lab code	False negatives	False positives	PT final evaluation
NRL50	0	0	positive
NRL51	0	0	positive
NRL52	0	0	positive
NRL53	0	0	positive
NRL55	0	0	positive
NRL56	0	0	positive
NRL57	0	0	positive
NRL58	0	0	positive
NRL59	0	0	positive
NRL60	0	0	positive
NRL61	0	0	positive
NRL62	0	0	positive
NRL63	0	0	positive
NRL64	0	0	positive
NRL65	0	0	positive
NRL66	0	0	positive

Lab code	False negatives	False positives	PT final evaluation
NRL67	0	0	positive
NRL68	0	0	positive
NRL69	0	0	positive
NRL70	0	0	positive
NRL71	0	0	positive
NRL72	0	0	positive
NRL73	0	0	positive
NRL74	0	0	positive
NRL75	0	0	positive
NRL76	-	-	NA*
NRL77	0	0	positive
NRL78	0	0	positive
NRL79	0	0	positive
NRL80	0	0	positive
NRL82	0	0	positive
NRL83	0	0	positive

*The lab was not able to test PT samples due to a technical problem

DEPARTMENT
INFECTIOUS DISEASES

Overtime comparison

Last five years

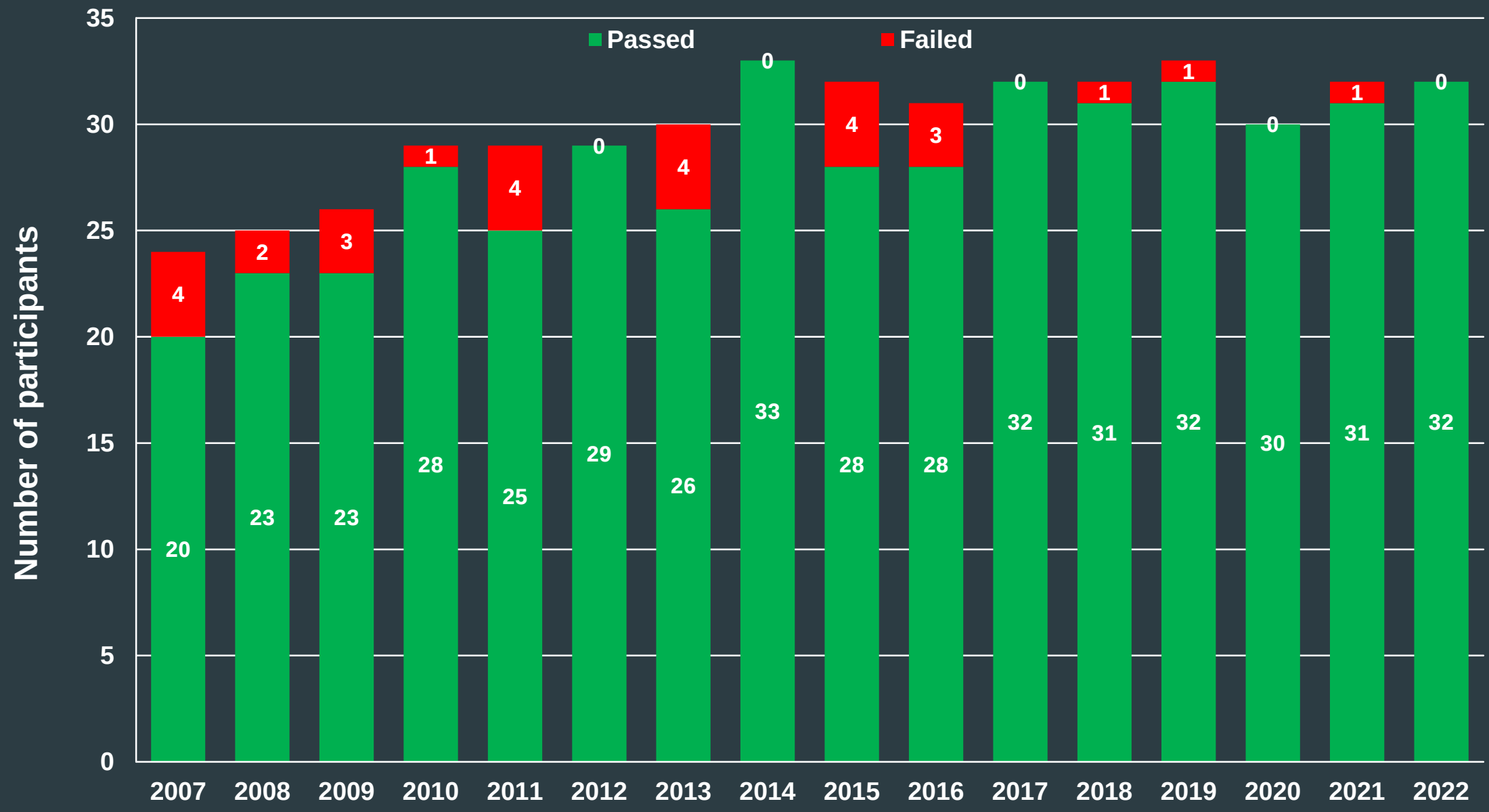
Laboratory code		50	51	52	53	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	82	83
Year	2018	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	F	P	P	P	-	P	P	P	P	P	-
	2019	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	F	P	P	P	P	P	P	P
	2020	P	P	P	P	P	P	P	P	P	P	P	P	-	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	-
	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	F	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	NA	P	P	P	P	P	P

P = passed F = failed

Sporadic failures involving no more than one participant

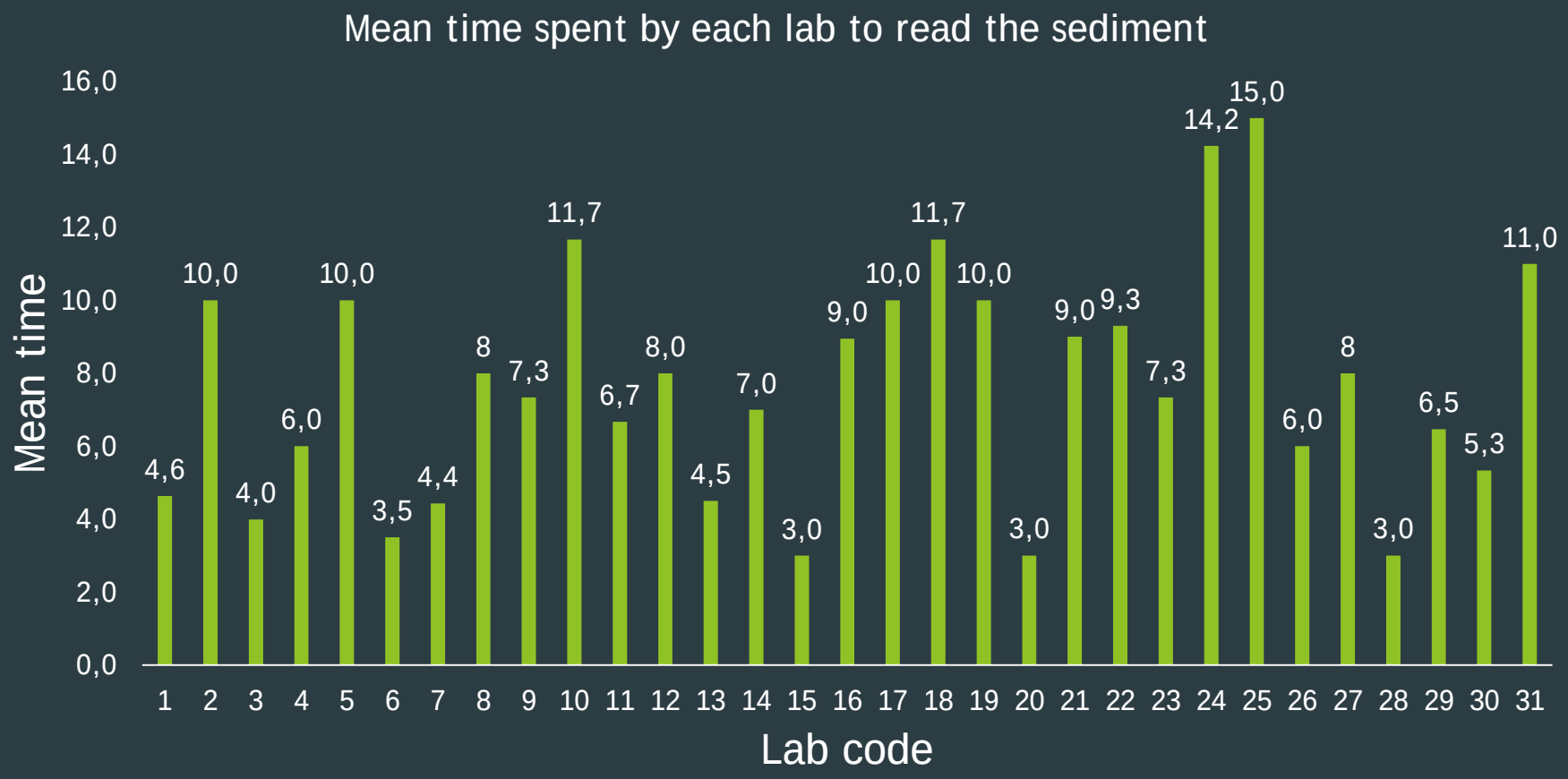
DEPARTMENT
INFECTIOUS DISEASES

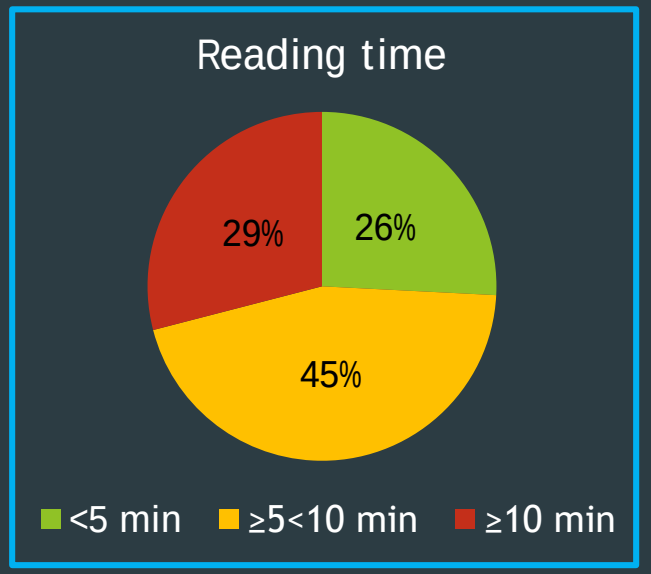
Overtime comparison



DEPARTMENT
INFECTIOUS DISEASES

Sediment reading time





Mean reading time calculated for all labs 7.65 minutes

DEPARTMENT
INFECTIOUS DISEASES

Quantitative results

Quantitative results for 3 larvae samples

17 labs (54.8%) recovered 3/3 larvae
8 labs (25.8%) recovered 2/3 larvae
6 labs (19.3%) recovered 1/3 larvae

Quantitative results for 5 larvae samples

14 labs (45.1%) recovered 5/5 larvae
12 labs (38.7%) recovered 4/5 larvae
4 labs (12.9%) recovered 3/5 larvae
1 lab (3.2%) recovered 2/5 larvae

10 labs found 100% of the larvae in both positive samples

For 2 labs reading time was ≥10 minutes

For 8 labs reading time was between 4.4 and 9 minutes

DEPARTMENT
INFECTIOUS DISEASES

Conclusions

All participants successfully passed the PT

The laboratory was not able to test the PT samples, successfully analysed a new set of samples as EQA scheme

The most part of participants spent less than 10 minutes to read the sediment

No correlation was observed between an extended reading time and an increased accuracy in larvae detection

DEPARTMENT
INFECTIOUS DISEASES

XVII Workshop of National Reference Laboratories for Parasites
Istituto Superiore di Sanità
15- 16 September 2022

Proficiency testing on *Trichinella* larvae identification at species level by a molecular method

DEPARTMENT
INFECTIOUS DISEASES

Purpose and participants

Purpose: to test the capacity of NRLs to identify *Trichinella* larvae at the species level

Participants: **23 NRLs** (including 3 outside EU)

DEPARTMENT
INFECTIOUS DISEASES

Test material

PT item: *Trichinella* muscle larvae preserved in 96% ethanol

PANEL COMPOSITION (4 samples)		
Species	**Larvae per vial**	**Evaluation criteria**
T. nativa	10	Correct identification of all four species
T. britovi	10	
T. pseudospiralis	10	
T6 genotype	10	

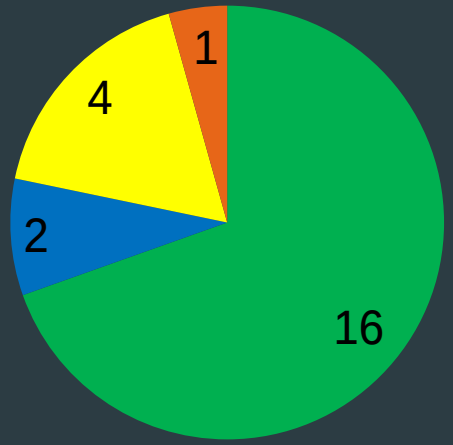
Test method:

- 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

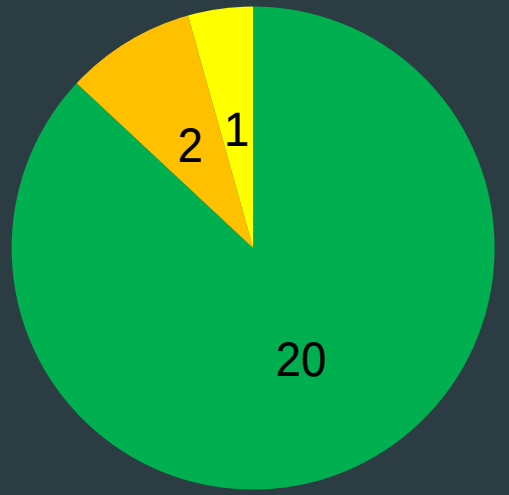
DEPARTMENT
INFECTIOUS DISEASES

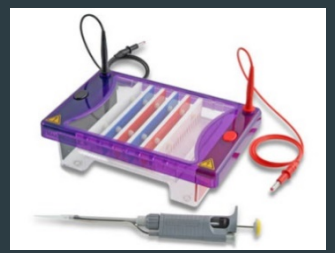
Samples and methods

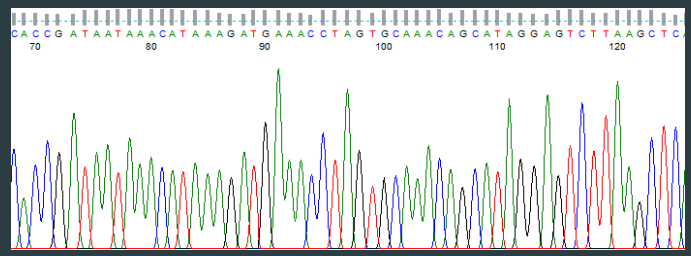
DNA purification methods



Species identification methods







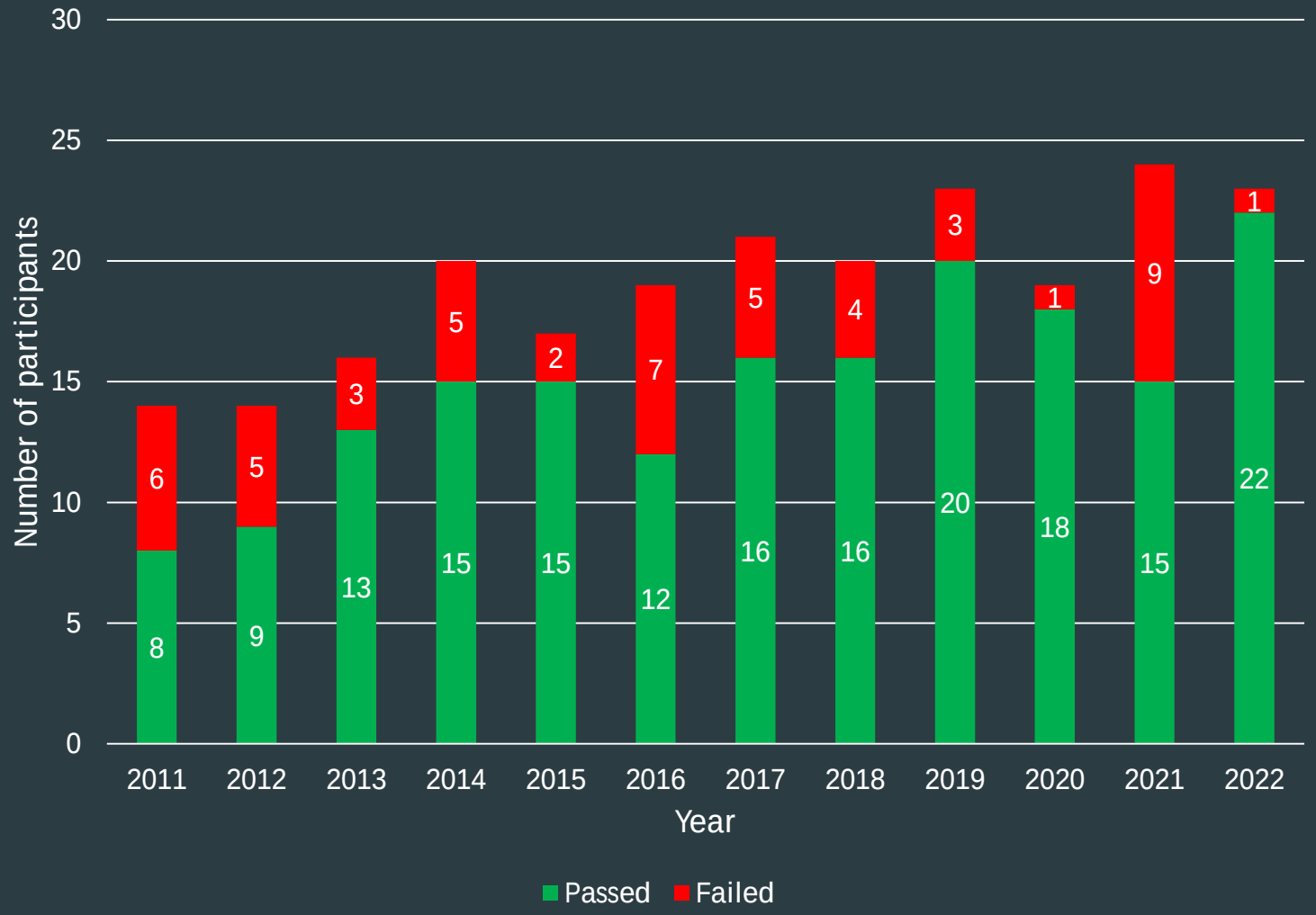
DEPARTMENT
INFECTIOUS DISEASES

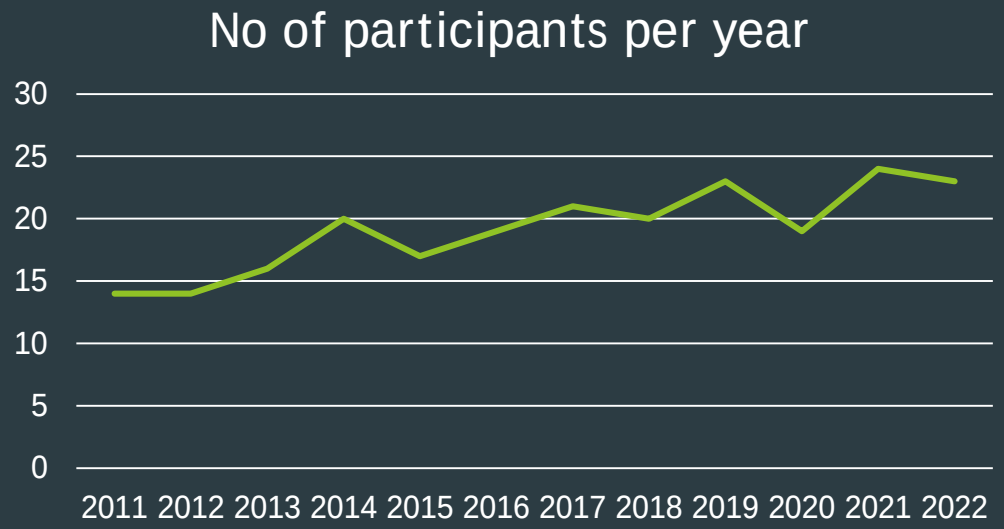
Results

Laboratory code	Right identification	Wrong identification	Missed identification	Final evaluation
NRL50	4	0	0	positive
NRL51	4	0	0	positive
NRL52	2	2	0	negative
NRL55	4	0	0	positive
NRL56	4	0	0	positive
NRL57	4	0	0	positive
NRL59	4	0	0	positive
NRL61	4	0	0	positive
NRL62	4	0	0	positive
NRL63	4	0	0	positive
NRL65	4	0	0	positive
NRL66	4	0	0	positive
NRL70	4	0	0	positive
NRL71	4	0	0	positive
NRL72	4	0	0	positive
NRL73	4	0	0	positive
NRL74	4	0	0	positive
NRL77	4	0	0	positive
NRL78	4	0	0	positive
NRL79	4	0	0	positive
NRL82	4	0	0	positive
NRL83	4	0	0	positive
NRL84	4	0	0	positive
NRL50	4	0	0	positive
NRL51	4	0	0	positive

DEPARTMENT
INFECTIOUS DISEASES

Overtime comparison





Increased number of participants linked to augmented number of NRLs that validated molecular method for *Trichinella* species identification

Failure reasons:

- First participation of little experienced labs
- Technical staff turnover
- Presence of non-European *Trichinella* species

DEPARTMENT
INFECTIOUS DISEASES

Conclusions

- DNA purification was done mainly by commercial kits based on magnetic beads or silica gel, and the multiplex PCR was the most used method to identify larvae at species level

- The laboratory that failed the PT misidentified *T. pseudospiralis* and T6 genotype larvae because of lack of experience of the technical staff who did the analysis

Thanks for your attention