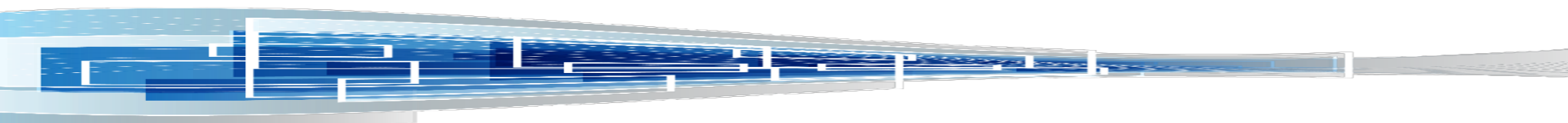# 11th Proficiency Test on the detection of *Anisakis spp.* L3 larvae in fish fillets

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Virtual XVII Workshop of National Reference Laboratories for Parasites
15th and 16th september 2022
Istituto Superiore di Sanità

Aim of the PT

PT-04: Detection of Anisakidae L3 larvae in fish fillets

✓ Identification of the presence of Anisakidae L3 larvae in fish fillets

✓ The PT have been organized following the NRL request during the virtual annual workshop in 2021

✓ PT is accredited according to the ISO 17043

INTERNATIONAL STANDARD

ISO/IEC 17043

First edition 2010-02-01

PT timing 2022

PT-04: Detection of Anisakidae L3 larvae in fish fillets

January 25th

PTs REQUEST FORM 2022

https://www.iss.it/en/web/iss-en/eurlp-proficiency-testing

March 14th

March 21st

DEADLINE RESULT REPORTING

April 27th

Individual Report PT-04

May 31st

Final Report PT-04: An 1/2022

Test material

✓ A panel of 3 items (fish fillet sandwiches) has been prepared
✓ Each fillet sandwiches was spiked with 1 Anisakidae larva

✓ Anisakidae L3 larvae were recovered from the body cavity of a heavily parasitized European horse mackerel

European horse mackerel

✓ Fillets of farmed rainbow trout were freshly prepared and used to guarantee an Anisakidae-free matrix
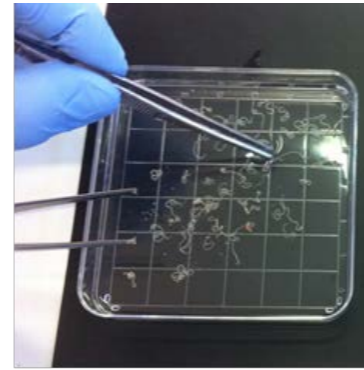
Rainbow trout

Test material

PT-04: Detection of Anisakidae L3 larvae in fish fillets
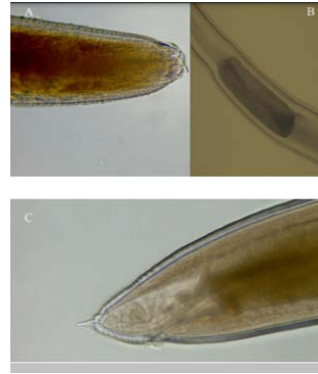

✓ The L3 identification at genus level was assessed by microscopic examination

✓ The correct number of larvae was transferred in the pockets by tweezers

✓ Fish sandwiches were sealed individually in a plastic bag under vacuum

✓ The parcel were sent to participants by international courier

PT-04: Detection of Anisakidae L3 larvae in fish fillets

Instructions to participants and Detection Methods

The laboratories were allowed to use one (or a combination) of the following methods

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

Tests suggested to detect Anisakidae larvae in fish fillets

- ✓ Artificial digestion
- ✓ UV on squeezed and frozen
- ✓ Candling by lighting
- ✓ Compression system

NORME INTERNATIONALE   ISO 23036-2
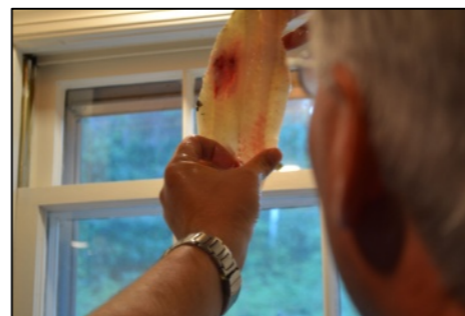
Première édition 2021-04

Microbiologie de la chaîne alimentaire — Méthodes de recherche des larves L3 d'Anisakidae dans les poissons et produits de la pêche —

Partie 2:
Méthode de digestion artificielle

Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products —
Part 2: Artificial digestion method

Artificial digestion

INTERNATIONAL STANDARD   ISO 23036-1
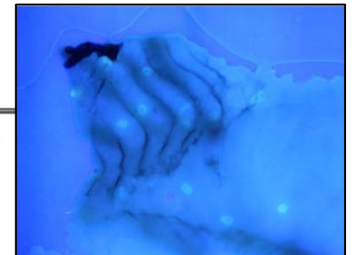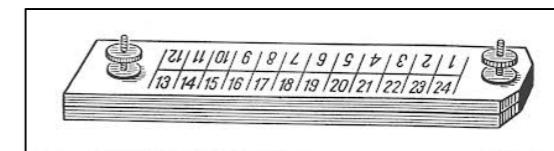
First edition 2021-04

Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products —

Part 1:
UV-press method

Microbiologie de la chaîne alimentaire — Méthodes de recherche des larves L3 d'Anisakidae dans le poisson et les produits de la pêche —
Partie 1: Méthode presse/UV

UV examination

Candling

Compressorium

PT Evaluation criteria

The PT evaluation is <u>qualitative</u> (presence or absence of larvae)

The result is "correct" if the laboratory detected Anisakidae larvae in the three spiked samples
The result is "incorrect" if the laboratory did not detect any larva in the spiked samples

Lab code	expected	observed	Result (correct/incorrect)	evaluation (positive/negative)
Ax	3	3	correct	Positive
	1	1	correct	
	3	3	correct	
Axx	3	0	incorrect	Negative
	1	1	correct	
	3	3	correct	

The PT is considered "POSITIVE" if "correct" results were obtained
The PT is considered "NEGATIVE" if at least one "incorrect" result was obtained

PT Results

Lab code	N° of spiked/detected larvae[1]			Method(s)	Final Evaluation
	1	**1**	**1**		
A1	1	1	1	Artificial digestion	Positive
A2	1	1	1	Artificial digestion	Positive
A3	1	1	0	Artificial digestion	Negative
A4	1	1	1	Artificial digestion	Positive
A5	1	1	1	Artificial digestion	Positive
A6	1	1	1	Candling; Artificial digestion	Positive
A7	1	1	1	Candling; Artificial digestion	Positive
A9	1	1	1	Artificial digestion	Positive
A10	1	1	1	Candling; Artificial digestion	Positive
A12	1	1	1	Artificial digestion	Positive
A13	1	1	1	Artificial digestion	Positive
A15	1	1	1	UV examination after freezing (UV-Press)	Positive
A16	1	1	1	UV examination after freezing (UV-Press)	Positive
A18	1	1	1	Artificial digestion	Positive
A19	1	1	1	Artificial digestion	Positive
A20	1	1	1	Artificial digestion	Positive
A21	1	1	1	Artificial digestion	Positive
A25	1	1	1	Artificial digestion	Positive
A26	1	1	1	Artificial digestion	Positive
A28	0	1	1	UV examination after freezing (UV-Press)	Negative
A29	1	1	1	UV examination after freezing (UV-Press)	Positive
A30	1	1	1	Candling; Artificial digestion	Positive
A31	1	1	1	Artificial digestion	Positive
A32	1	0	1	Artificial digestion	Negative
A35	0	0	0	Artificial digestion	Negative
A36	1	1	1	Artificial digestion	Positive
A38	1	0	1	Compressorium; Artificial digestion	Negative
A39	1	1	1	UV examination after freezing (UV-Press)	Positive
A43	26	1	1	Artificial digestion	Positive
A44	1	1	1	Candling	Positive

[1]Number of recovered larvae are reported

PT Results

Participation
30/30 labs sent the results

Methods

- 19 Artificial digestion alone (63%) (4 labs followed ISO 23036-2)
- 5 UV-Press (16%) (3 labs followed ISO 23036-1)
- 4 Candling + Artificial digestion
- 1 Artificial digestion + compressorium
- 1 Candling
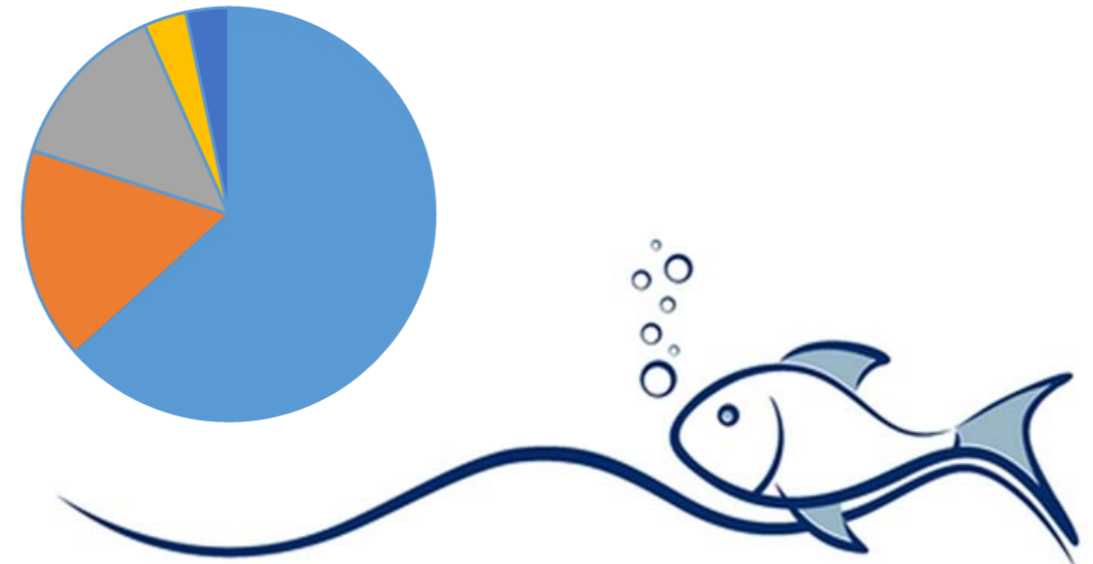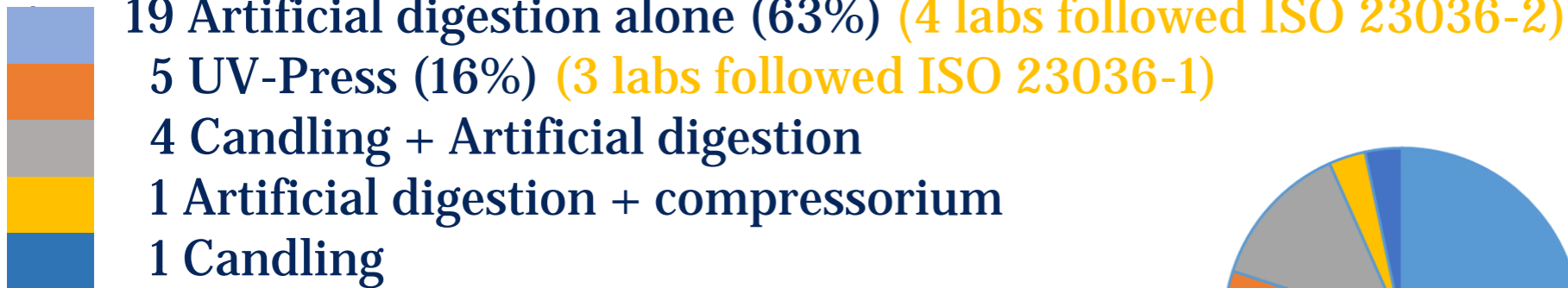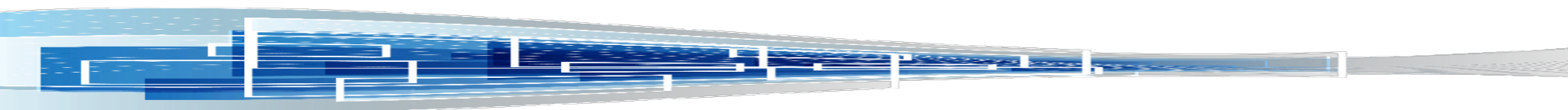
Detection

- 25 labs of 30 passed the PT
- 5 labs failed: 4 using the digestion method, 1 UV-press method
- 4 labs reported one false negative and one reported all false negative
- 1 lab overestimated the number of spiked larvae (n=26)

PT04 Trend

PT-04: Detection of Anisakidae L3 larvae in fish fillets

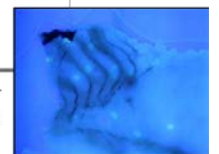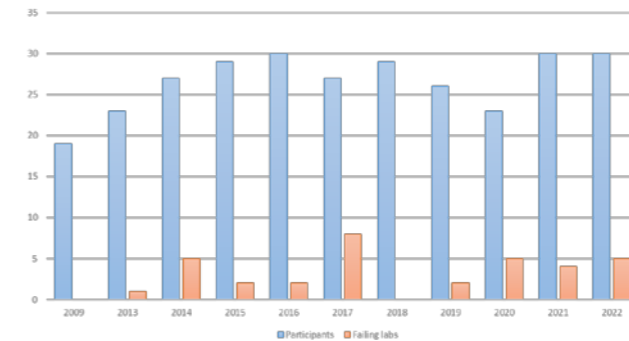| 2009	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
0%	4%	18,5%	7%	7%	30%	0%	7,5%	22%	13%	16%

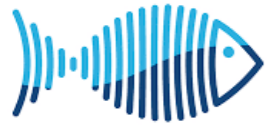Percentage of participants failing the PT overtime

Conclusions
11th PT on the detection of *Anisakis spp.* L3 larvae in fish fillets

✓ A stable number of PT participants was recorded in 2022 compared to previous years

✓ 16% of laboratories failed the PT
✓ Only one lab overestimated (n=26) the number of spiked larvae
✓ all other labs that passed the PT reported the exact number of larvae

✓ Among the methods adopted the most widespread is artificial digestion followed by UV examination and candling used in combination with artificial digestion

NORME INTERNATIONALE   ISO 23036-2

Première édition 2021-04

Microbiologie de la chaîne alimentaire — Méthodes de recherche des larves L3 d'Anisakidae dans les poissons et produits de la pêche —

Partie 2: Méthode de digestion artificielle

Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products — Part 2: Artificial digestion method

INTERNATIONAL STANDARD   ISO 23036-1

First edition 2021-04

Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products —

Part 1: UV-press method

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Thanks for your attention