Report of the 3rd NRL proficiency testing on "Detection of Anisakidae L3 larvae in fish fillets"

March - May, 2014

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1 Introduction

Nematode worms of the Anisakidae family are zoonotic parasites circulating in most marine fish and cephalopods at the larval stage, and in marine mammals at the adult stage (Pozio, 2013). Humans acquire the infection by the consumption of raw, undercooked or marinated fish (Audicana and Kennedy, 2008). According to the Commission Regulation (EC) No 853/2004, food business operators must ensure that fishery products have been subjected to a visual examination for the purpose of detecting visible parasites before being placed on the market. They must not place on the market fishery products for human consumption that are obviously contaminated with parasites.

2 Scope

The organization of proficiency testings (PTs) falls in the duties of the EURL for Parasites, as it is stated in the Regulation (EC) No 882/2004 of the European Parliament and of the Council (Commission Regulation No. 882/2004). The PT aims to evaluate the competence of the appointed NRLs to detect Anisakidae larvae in fish fillets. The third PT on detection of Anisakidae larvae in fish fillets has been organized in agreement to the request of NRLs during the 2013 EURLP annual workshop, and following the second PT on detection of Anisakidae larvae in fish fillets performed in 2013.

3 Time frame

The PT was announced to NRLs by email on February 3th, 2014 and the deadline to send the participation form (see Annex 1; MO/MAQPVI/01.PT Request Form) was February 21st, 2014. On March 17th, 2014, the samples were dispatched to participants by an international courier. Reporting deadline was 24th March, 2014.

4 Test material

The test material forwarded to each laboratory, consisted of 3 fish fillets containing in the core a different number of third stage Anisakidae larvae (L3). To ensure that fish was free from L3 infestation, fillets from farmed rainbow trout (*Oncorhynchus mykiss irideus*) were used. A panel of three fish fillet sandwiches were prepared. The panel includes a negative sample, one sample spiked with a L3 and one sample spiked with three L3. Live L3 larvae of *Anisakis pegreffii,* free of their capsule, were recovered from the body cavity of heavily parasitized silver scabbardfish (*Lepidopus caudatus*) from the Mediterranean sea and used to spike samples. Ten L3 were identified at genus level by microscopic examination, and at species level by PCR-RFLP of the ITS region according to D'Amelio et al., 2000. L3 larvae were carefully transferred by tweezers in pockets made in the middle of fish fillets. Fish sandwiches were then put individually in a plastic bag sealed under vacuum, identified by an alphanumeric code stuck on the plastic bag, without any indication of the level of contamination or any information on the identity of the testing laboratory, and stored at +4 °C. The same code with the number of larvae was reported in an Excel file. Samples were forwarded to each participant laboratory by an international courier in a polystyrene box with cooler packages to maintain a temperature lower than +15 °C during shipping. Packages were forwarded according to the international forwarding regulations. The stability of the samples in the package has been evaluated by ad hoc experiments made at EURLP. Larvae in samples sealed under vacuum and stored between +4 °C and +15 °C, survive at least 7 days from the date of preparation.

5 Instructions to participants

From February 3th, 2014, general information on PT organization were made available to NRLs on EURLP web site (http://www.iss.it/crlp/index.php?lang=2&id=137&tipo=28).
Electronic copies of the forms which had to be filled in and send back to EURLP, were sent by e-mail on March 17th, 2014, (see Annex 1; MO/POPVI/00 from 01 to 04):

1) "Check package", to inform about the content and the condition of the PT material at arrival in the lab;
2) "Instruments and Materials List" needed to perform the suggested methods;
3) "Procedure", step by step description of the four suggested methods;
4) "Result", to report the number of larvae detected in each sample.

The code assigned to the laboratory was sent individually to each participant by a separate e-mail on March 17th 2014.

To make comparable the results obtained by laboratories, all participants should follow the protocol step by step or, if the case, describe any variation. Four tests were suggested and described: 1. candling; 2. compressorium; 3. UV examination after freezing; and 4. artificial digestion.

6 Criteria for result evaluation and statistical analysis

The evaluation was only qualitative (presence or absence of larvae). The result was "correct" if the laboratory detected Anisakidae larvae in the two spiked samples and no larvae in the non-spiked sample. The result was "incorrect" if the laboratory did not detect any larva in the spiked samples. The PT was considered "positive" if no "incorrect" results were obtained; the PT was considered "negative" if at least one "incorrect" result was obtained.

Due to the low number of samples in the panel, no statistical analysis of the results was performed.

Ten days after the deadline of result submission, the EURLP provided an Individual PT Report including: i) the number of spiked larvae per sample; ii) the number of larvae detected in each sample by the laboratory; iii) the final evaluation; and iv) recommendation based on laboratory performance.

Moreover, when applicable, an updated summary of laboratory performance over successive PT rounds was provided. The Individual PT Reports (see Annex 2) were delivered via e-mail to each laboratory as .pdf file on March 28th 2014.

The EURLP also provided a Final PT Report, including results obtained by all participant labs. The final report was presented at the NRL workshop and published on the EURLP website (see Annex 2).

To guarantee confidentiality in the final report, laboratories were identified only by alphanumeric codes.

7 Participating laboratories

Twenty-seven NRLs (two NRLs from France) from 25 MS and from one Associated Country agreed to participate in the PT. Croatia, Denmark, Hungary, and Switzerland participated in the PT for the first time (Annex 3).

8 Results

8.1 Delivery of the package to NRLs

All packages were delivered to NRLs within 24-72 hours (1 day for 23 NRLs, 2 days for 4 NRLs, and 3 days for 1 NRL) (see Annex 4). At delivery, the internal temperature of packages was ≤ 10 °C in 25 parcels (90%), 11 °C in one and only "cool" was reported by another NRL. The elapsed time from package delivery at the NRL and its control was ≤ 1 h for 24 NRLs, approximately 4 hs for 1 NRL, 16 hs for 1 NRL and 22 hs for 1 NRLs.

8.2 Test methods

Eighteen laboratories used the digestion method only, five laboratories used the digestion method in combination with at least one other method (UV, candling or compressorium) (see Annex 5). Two labs used the UV method only, one lab used the compressorium only, and one lab used the candling only.

Significant deviations from the suggested protocols were reported by six participants only for the digestion method (two labs chopped fish fillets (Form 3, 1.4) by stomacher; two labs extended the digestion time (Form 3, 1.8) up to 40-90 min; two labs followed an internal SOP for which digestion is done in a 2L glass beaker (Form 2, 1.4) using 0.2 % HCl and liquid pepsin in a final volume of 1.5L (Form 2, 5.3 and 5.4; Form 3, 1.1) at 35 °C for 1 h (Form 3, 1.7; 1.8).

One laboratory also performed molecular analysis of the recovered larvae by an accredited PCR-RFLP method. Larvae were correctly typed as *A. pegreffii*.

8.3 Evaluation

All participant labs sent the results. One laboratory sent the result after the deadline since troubles with samples handling occurred, as reported by the participant in the dedicated Claim/Appeal Form, and a second PT panel was sent on Monday 24th of March and received on 26th of March. Three out of 27 labs reported the presence of motile larvae irrespective of the method used (2 digestion, 1 candling).
Five out of 27 participants failed to pass the PT. In four cases, the laboratory did not find any larva in the sample spiked with one larva, and in one case the laboratory reported a false positive. The misdetection could be due to lack of experience of the analysts and, in at least two cases, by the sole use of compressorium or UV examination not followed by the digestion method, which is considered the gold standard by the Codex stan 244-2004 (http://www.codexalimentarius.org/standards/list-of-standards/en/?provide=standards&orderField=fullReference&sort=asc&num1=CODEX)

8.4 Corrective Actions

According to the Commission request, corrective actions were planned for the 5 laboratories which failed the PT. First, an e-mail was sent on April 2nd, 2014 to the involved laboratories, asking them to carefully check the possible reason/s of the failure and send back their comments. These five labs were also informed that a second panel of sample would be sent on May 26th, 2014. Only two labs sent a feedback reporting as cause of PT failure the lack of experience of the analysts in performing the test and L3 detection.
The laboratory of Finnish Food Safety Authority Evira, located in Helsinki, Finland, requested to be included in the new panel forwarding. All six participating labs were informed that the new panel was part of a corrective action for those labs which failed the 2014 annual PT round. This corrective action was considered as an External quality assessment (EQA) scheme, therefore only anIndividual Report was provided to each participant.
The PT Participation form, the Instruction form (MO/POPVI/04 03. PT Anisakis) and the Procedure form (MO/POPVI/04. 05) were sent to all 6 labs by email on May 15th, 2014, and the deadline to send the signed participation form was established to be May 21st, 2014. Sample preparation was as reported in paragraph 4.
On May 26th, 2014, samples were dispatched to participants by an international courier. Reporting deadline was 3th June, 2014. The procedure and applicable methods were the same as detailed for the PT, and the 4 Forms and the individual lab codes were sent individually to each participant by a separate e-mail on May 26th, 2014.
Delivery of the package to NRLs, test methods used and results are summarized in Annex 6.
Three laboratories received the samples within 24 hs and 3 laboratories within 48 hs. At delivery, the internal temperature of all packages was ≤ 10 °C in 4 parcels, 12.5 °C in another parcel and 19 °C in the last parcel. No claim or appeal were done in this last case.
Four laboratories used the digestion method only, 2 labs used the UV method only, one lab used the compressorium only. No deviation from the indicated procedures were reported.
The evaluation was only qualitative (presence or absence of larvae) as detailed in the paragraph 6. All labs correctly identified all samples and passed the round. An individual report was send by e-mail to each laboratory on June 4th, 2014.

9 Conclusions

In agreement with previous PTs, these results suggest that most of participating labs have a high level of competence in the identification of Anisakidae larvae, irrespective of the detection method used. The artificial digestion is still the most used method, followed by UV examination after freezing, which is often used in combination with the previous method.

10 References

Pozio E. 2013. Integrating animal health surveillance and food safety: the example of Anisakis. Rev Sci Tech 32, 487-496.

Audicana MT, Kennedy MW. 2008. *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. Clin Microbiol Rev 21, 360-379.

D'Amelio S, Mathiopoulos KD, Santos CP, Pugachev ON, Webb SC, Picanço M, Paggi L. 2000 Genetic markers in ribosomal DNA for the identification of members of the genus Anisakis (Nematoda: ascaridoidea) defined by polymerase-chain-reaction-based restriction fragment length polymorphism. Int J Parasitol. 30, pp. 223-226.

Annex 1

ISTITUTO SUPERIORE DI SANITA'
EUROPEAN UNION REFERENCE LABORATORY FOR PARASITES

PT REQUEST FORM

DETECTION OF ANISAKIDAE L3 LARVAE IN FISH FILLETS

Person in charge: dr. Marco Lalle - e-mail: marco.lalle@iss.it

Participant information:

Name _____

Affiliation _____

Address _____

Tel. _____ *Fax* _____ *e-mail* _____

PT samples will be sent on	17th March, 2014
Results shall be sent	**Within 4 working days** from the PT samples arrival and in any case the due date is 24th March 2014
The PT report will be sent	**Within 10 working days** after the due date for PT result submission

Notes:

1. PT panels are delivered within 72 hs from shipping
2. The original raw data and a copy of the test report are kept for 10 years at this Institute.
3. For any complain or appeal, you can download the form here: http://www.iss.it/binary/crlp/cont/Claim_Appeal_Form.pdf
4. For any particolar need, please use the lines below:

_____

_____

_____

Date _____ Signature of the applicant _____

Do not fill in this section

Request received on _____

Accepted ☐ ***Non accepted*** ☐

PT samples sent on _____

Notes

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Unit of Gastroenteric and Tissue Parasitic Diseases
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Form 1 Laboratory code ____

PT on "DETECTION OF ANISAKIDAE L3 LARVAE IN FISH FILLETS"

Check of the package content and its condition of preservation

3 fish fillet sandwiches under vacuum ☐

The content of the package has been forwarded refrigerated/frozen

- When did you receive the package? Date _____hour____
- When did you open it? hour____
- Which temperature did you measure inside the package when you opened it? _____

Additional instructions:

The fish sample should be stored refrigerated at 4°C or better on ice before testing.

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Form 2 Laboratory code _____

PT on "DETECTION OF ANISAKIDAE L3 LARVAE IN FISH FILLETS"

List of instruments, reagents and materials required to perform the test

Instruments

The following instruments are needed to perform the test. To make comparable the results obtained by laboratories involved in the PT, we have to verify which instruments have been used. Thus, you are requested to mark the column YES in case of use of the relevant instrument or, on the contrary, describe the variation in the following column.

1 DIGESTION OF FISH FILLETS

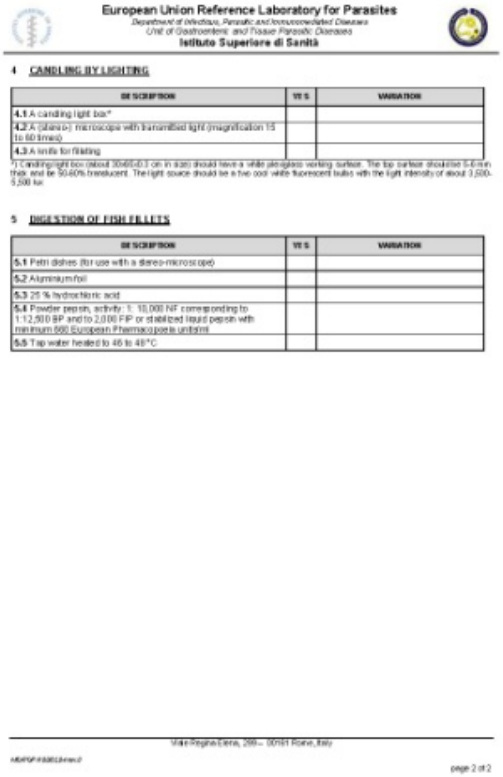
DESCRIPTION	YES	VARIATION
1.1 A blender with a sharp chopping blade or scissors		
1.2 Magnetic stirrers with thermostatically controlled heating plate and teflon-coated stirring rods approximately 5 cm long		
1.3 Sieves, mesh size 180-1000 microns, with stainless steel mesh		
1.4 Glass beakers, capacity 3 litres		
1.5 A stereo-microscope, with a substage transmitted light source of adjustable intensity		
1.6 A balance accurate to at least 0.1 g		
1.7 Pipettes of different sizes (1, 10 and 25 ml) and pipette holders		
1.8 A thermometer accurate to 0,5 °C within the range 1 to 100 ·C		
1.9 A knife for filleting		

2 UV ON SQUEEZED AND FROZEN FISH FILLET

DESCRIPTION	YES	VARIATION
2.1 An UV-light transilluminator		
2.2 A – 20°C freezer		
2.3 A (stereo-) microscope with transmitted light (15X to 60X magnification)		
2.4 A knife for filleting		

3 COMPRESSION OF THE FILLET

DESCRIPTION	YES	VARIATION
3.1 A compressorium system		
3.2 A (stereo-) microscope with transmitted light (15X to 60X magnification)		
3.3 A knife for filleting		



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4 CANDLING BY LIGHTING

DESCRIPTION	YES	VARIATION
4.1 A candling light box*		
4.2 A (stereo-) microscope with transmitted light (magnification 15 to 60 times)		
4.3 A knife for filleting		

*) Candling light box (about 30x60x0.3 cm in size) should have a white plexiglass working surface. The top surface should be 5-6 mm thick and be 50-60% translucent. The light source should be a five cool white fluorescent bulbs with the light intensity of about 3,500-5,500 lux

5 DIGESTION OF FISH FILLETS

DESCRIPTION	YES	VARIATION
5.1 Petri dishes (for use with a stereo-microscope)		
5.2 Aluminium foil		
5.3 25 % hydrochloric acid		
5.4 Powder pepsin, activity 1: 10,000 NF corresponding to 1:12,500 BP and to 2,000 FIP or stabilized liquid pepsin with minimum 600 European Pharmacopoeia units/ml		
5.5 Tap water heated to 46 to 48°C		

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Form 3 Laboratory code _____

PT on "DETECTION OF ANISAKIDAE L3 LARVAE IN FISH FILLETS"

Procedure

The procedure is described step by step in the following table. To make comparable the results obtained by laboratories involved in the PT, the operative protocol must be carefully followed. Thus, you are requested to mark the column YES if you strictly followed the indications (ex. pH, volume, incubation temperature, time) or, alternatively, describe in the column VARIATION any variation you brought to the step.

When opening each sample, carefully check the plastic bag for the presence of larvae outside the fish fillet sandwich.

Before proceeding with any of the following methods, fish fillets should be skinned (if necessary) using a knife.

1. DIGESTION FOR 100 g OF FISH FILLETS

Step	DESCRIPTION	YES	VARIATION
1.1	15 ± 0.5 ml of hydrochloric acid is added to a 3 litre beaker containing 2.0 litre of tap water, preheated to 46 to 48 °C; a stirring rod is placed in the beaker, the beaker is placed on the preheated plate and the stirring is started.		
1.2	10 ± 0.2 g of pepsin is added		
1.3	The sample is chopped in the blender for 1-2 seconds or by scissors or by hands		
1.4	The chopped flesh is transferred to the 3 litre beaker containing water, pepsin and hydrochloric acid		
1.5	The mincing insert of the blender is immersed repeatedly in the digestion fluid in the beaker and the blender bowl is rinsed with a small quantity of digestion fluid to remove any fish muscles still adhering		
1.6	The beaker is covered with aluminium foil		
1.7	The magnetic stirrer must be adjusted so that it maintains a constant temperature of 44 to 46 °C throughout the operation. During stirring, the digestion fluid must rotate at a sufficiently high speed to create a deep whirl without splashing		
1.8	The digestion fluid is stirred until the fish muscles disappear (approximately 15-20 min). The stirrer is then switched off and the digestion fluid is poured through the sieve into a beaker		
1.9	The Anisakidae larvae can be detected on the sieve		
1.10	Larvae can be collected and examined under the stereomicroscope with transmitted light		

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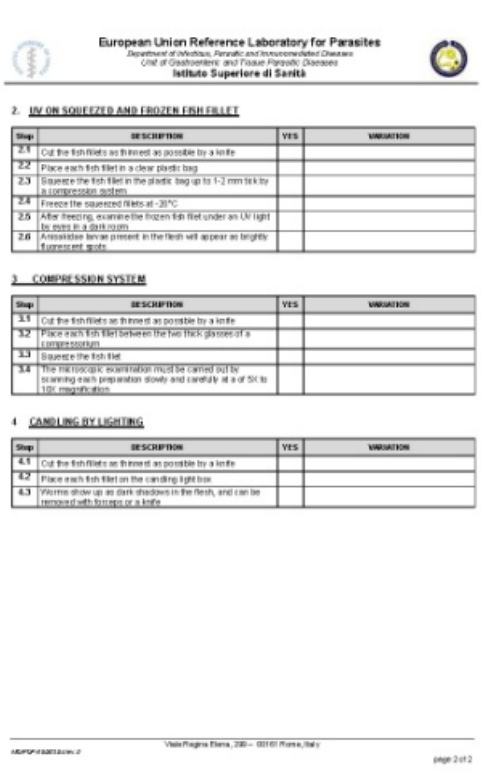

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2. UV ON SQUEEZED AND FROZEN FISH FILLET

Step	DESCRIPTION	YES	VARIATION
2.1	Cut the fish fillets as thinnest as possible by a knife		
2.2	Place each fish fillet in a clear plastic bag		
2.3	Squeeze the fish fillet in the plastic bag up to 1-2 mm tick by a compression system		
2.4	Freeze the squeezed fillets at -20°C		
2.5	After freezing, examine the frozen fish fillet under an UV light by eyes in a dark room		
2.6	Anisakidae larvae present in the flesh will appear as brightly fluorescent spots		

3. COMPRESSION SYSTEM

Step	DESCRIPTION	YES	VARIATION
3.1	Cut the fish fillets as thinnest as possible by a knife		
3.2	Place each fish fillet between the two thick glasses of a compressorium		
3.3	Squeeze the fish filet		
3.4	The microscopic examination must be carried out by scanning each preparation slowly and carefully at a of 5X to 10X magnification		

4. CANDLING BY LIGHTING

Step	DESCRIPTION	YES	VARIATION
4.1	Cut the fish fillets as thinnest as possible by a knife		
4.2	Place each fish fillet on the candling light box		
4.3	Worms show up as dark shadows in the flesh, and can be removed with forceps or a knife		

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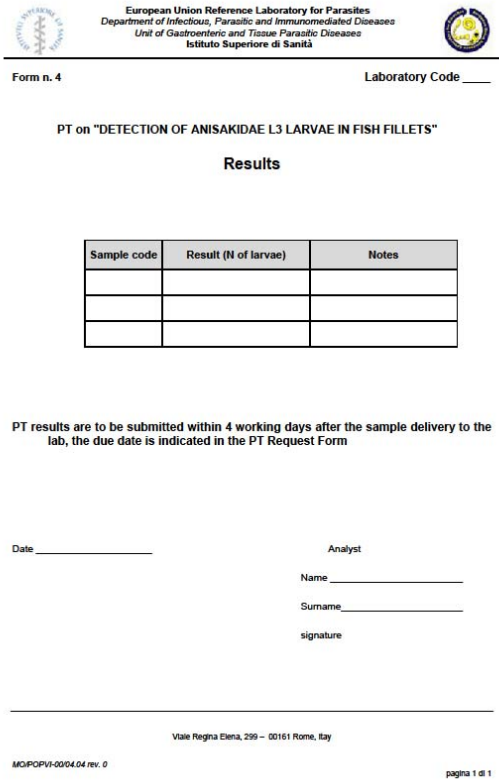

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Form n. 4 Laboratory Code ____

PT on "DETECTION OF ANISAKIDAE L3 LARVAE IN FISH FILLETS"

Results

Sample code	Result (N of larvae)	Notes

PT results are to be submitted within 4 working days after the sample delivery to the lab, the due date is indicated in the PT Request Form

Date _____ Analyst

Name _____

Surname _____

signature

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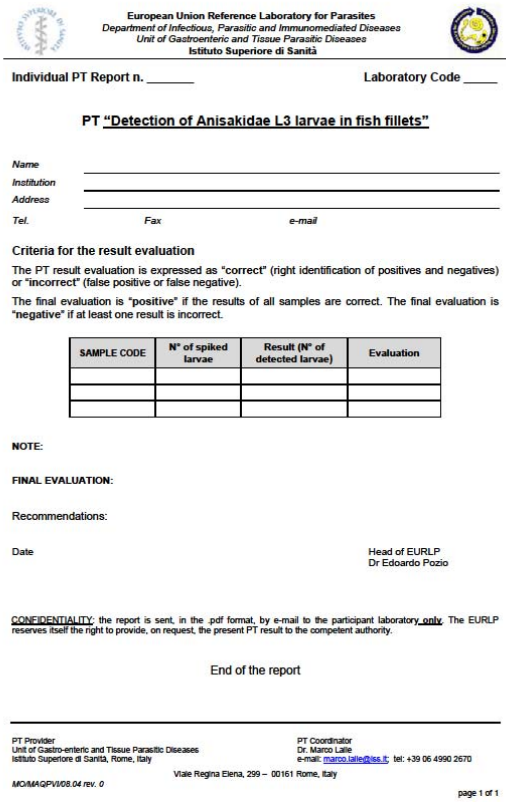
Annex 2



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Individual PT Report n. _____ **Laboratory Code _____**

PT "Detection of Anisakidae L3 larvae in fish fillets"

Name _____
Institution _____
Address _____
Tel. *Fax* *e-mail*

Criteria for the result evaluation

The PT result evaluation is expressed as "correct" (right identification of positives and negatives) or "incorrect" (false positive or false negative).

The final evaluation is "positive" if the results of all samples are correct. The final evaluation is "negative" if at least one result is incorrect.

SAMPLE CODE	N° of spiked larvae	Result (N° of detected larvae)	Evaluation

NOTE:

FINAL EVALUATION:

Recommendations:

Date

Head of EURLP
Dr Edoardo Pozio

CONFIDENTIALITY: the report is sent, in the .pdf format, by e-mail to the participant laboratory only. The EURLP reserves itself the right to provide, on request, the present PT result to the competent authority.

End of the report

PT Provider
Unit of Gastro-enteric and Tissue Parasitic Diseases
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PT Coordinator
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Final PT report n._____

PT report on "Detection of Anisakidae L3 larvae in fish fillets"

Design

Purpose	Evaluation of laboratories in charge for official control on food	
Scheme type	Single	
Participants	Public and private, European laboratories	
N. of participants	Depending on request	
Method	not regulated	
Test method	chosen by the participant	
PT items	Matrix	fresh water farmed fish fillet
	Item	Anisakidae live larvae
	N. of samples	3 for each participant
	Distribution	Immediate shipment after preparation
Subcontracted activities	PT item transport and delivery	
Results evaluation	Qualitative	

Implementation

N. of participants		PT items	fish fillet sandwiches	
Public laboratories				
Private laboratories			PT panel composition	
NRL			Subcontractor	TNT Express
Shipping dates				

Qualitative results

The PT final evaluation was qualitative only. The PT was considered passed if all positive and all negative samples were correctly identified by the participant. The PT was considered negative if at least one sample was not correctly identified (false positive or false negative).

Organizzatore PoT
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Laboratory code	N° False positives	N° False negatives	Final evaluation

Legend:

- Laboratories that failed the PT are marked in bold
- Laboratories that didn't send the PT result are indicated by N/A (not applicable)

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Summary of qualitative results:

Total number of PT items	
Number of participant laboratories	
Number of participants that passed the PT	
Number of participants that failed the PT	

Comments:

The causes of laboratories' failure have been analyzed and can be attributed to: i) use of the sole "compression method", that has a low sensitivity, without further analysis by "artificial digestion method"; ii) limited experience of either the laboratory or the single analyst in performing the methods, and/or in morphological identification of Anisakidae larvae.

The Director
Dr. E. Pozio

Data ______________________

Notes:

1. To guarantee confidentiality, participant laboratories are identified by alphanumeric codes. PT participant identity is kept confidential and bound by professional secrecy. If PT results have to be provided directly to a competent authority, the organizer shall send a written notice to inform the involved participants.
2. The organizer subcontracts PT item transport and delivery to a qualified transportation company.
3. Each participating laboratory receive a PT panel according to the PT scheme. Each PT item consists of a fish fillet sandwich spiked or not with live Anisakidae larvae. The homogeneity of PT items is ensured by an accurate control of the number of larvae spiked into each sample (item), made by two operators using a stereo microscope. PT items are stable for 7 days from the date of preparation (corresponding to the shipping date), provided that they are maintained in suitable conditions.
4. At the beginning of each year, the organizer draws up a PT program and makes it known by sending an email to the NRLs.
5. The final report issue of each PT round shows the PT program implementation.

End of the report

Annex 3

Participating NRL	Country
Institut für Veterinärmedizin, Innsbruck	Austria
Institute of Tropical Medicine, Antwerp	Belgium
National Diagnostic and Research Veterinary Institute, Sofia	Bulgaria
Cyprus Veterinary Services, Nicosia	Cyprus
Croatian Veterinary Institute, Zagreb,	Croatia
State Veterinary Institute, Olomouc	Czech Rep
Technical University of Denmark, Frederiksberg	Denmark
Estonian Animal Health, Veterinary and Food Laboratory, Tartu	Estonia
Finnish Food Safety, Evira, Oulu	Finland
ANSES Animal Health Laboratory, Maison Alfort	France
ANSES Laboratory for Food Safety, Boulogne Sur Mer,	France
Max Rubner-Institut, Institut für Sicherheit und Qualität bei Milch und Fisch, Hamburg	Germany
Institute of Infectious and Parasitic Diseases , Athens	Greece
National Food Chain Safety Office, Budapest	Hungary
Veterinary Laboratory Department of Agriculture & Food Laboratories, Celbridge	Ireland
National Reference Laboratory for Anisakiasis, Ist. Zooprof. Sper. Sicilia, Palermo	Italy
Institute of Food Safety, Animal Health and Environment "BIOR",Riga	Latvia
National Veterinary Laboratory, Albertown - Marsa	Malta
National Institute of Public Health and the Environment, RIVM, Bilthoven	Netherlands
National Veterinary Research Institute, Pulawy	Poland
Hygiene and Public Veterinary Health Institute, Bucharest	Romania
Veterinary and Food Institute in Bratislava, Bratislava	Slovak Rep.
University of Ljubljana, Ljubljana	Slovenia
Centro Nac. de Alimentación. Ag. Esp. de Seguridad Alimentaria y Nutrición, Majadahonda	Spain
National Veterinary Institute, SVA, Uppsala	Sweden
Universität Bern, Bern	Switzerland
Cefas Weymouth Laboratory, Weymouth	UK

Annex 4

Forwarding time and condition of preservation of the package content

Laboratory code	Forwarding days	Time (min/h) between package arrival and opening	Temperature (°C) inside the package at the delivery
A1	1	on arrival	7
A2	1	on arrival	5.2
A3	1	15 min	5
A4	1	on arrival	6
A5	1	onarrival	11
A6	1	30 min	4
A7	1	5 min	8
A8	2	10 min	4
A9	1	on arrival	8
A10	1	10 min	4
A11	2	22 h	4
A12	1	40 min	NA
A13	1	5 min	5
A14	1	5 min	8
A15	1	3 h 45 min	8.8
A16	2	on arrival	2
A17	1	16 h15 min	10
A18	1	30 min	4.8
A19[1]	2	on arrival	9.8
A20	3	on arrival	7
A21	1	5 min	4
A23	1	on arrival	5.9
A24	1	5 min	10
A25	1	5 min	3.1
A26	1	10 min	10
A27	1	10 min	7
A28	1	on arrival	6.7

[1]The PT panel was send to this laboratory on March 24th

Annex 5

Proficiency test results

Lab code	N° of spiked/detected larvae[1]			Method(s)[2]	Final Evaluation
	0	1	3		
A1	0	1	3	D	positive
A2	0	1	3	D	positive
A3	0	0	2	Co	negative
A4	0	1	2	Co+D	positive
A5	0	1	3	D	positive
A6	0	1	3	C+D	positive
A7	0	1(2f)	3(4f)	UV+D	positive
A8	0	1	2	D	positive
A9	0	1	3	D	positive
A10	0	1	3	C	positive
A11	0	1	1	D	positive
A12	0	1	3	D	positive
A13	0	1	3	D	positive
A14	2	4	2	D	negative
A15	0	1	1(2f)	C+UV+D	positive
A16	0	0	2	UV	negative
A17	0	1	3	UV	positive
A18	0	1	2	D	positive
A19[3]	**0**	**1**	**2**	**D**	**positive**
A20	0	1	3	D	positive
A21	0	1	3	D	positive
A23	0	0	2	D	negative
A24	0	0	2	D	negative
A25	0	1	3	D	positive
A26	0	1	2	D	positive
A27	0	1	2	D	positive
A28	0	1	3	C+UV+D	positive

[1]Number of recovered larvae are reported. Number of larval fragments are reported in brackets.
[2]D = digestion; Co = compressorium; C = candling; UV = UV examination after freezing.
[3]PT results refer to a second PT panel sent to this laboratory a second time on March 24th

Annex 6

Corrective Action results

Lab code	Forwarding days	Time (min/h) between package arrival and opening	Temperature (°C) inside the package at the delivery	N° of spiked/detected larvae[1]			Method(s)[2]	Final Evaluation
				0	1	3		
A3	2	30 min	7	0	1	3	Co	positive
A14	2	on arrival	10	0	1	2	D	positive
A16	2	20 min	19	0	1	3	UV	positive
A23	1	on arrival	5.5	0	1	3	D	positive
A24	1	on arrival	6	0	1	3	D	positive
A29	1	10 min	12.5	0	1	3	UV	positive

[1]Number of recovered larvae are reported. Number of larval fragments are reported in brackets.
[2]D = digestion; Co = compressorium; C = candling; UV = UV examination after freezing.