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

Instructions

The same day of items shipping, the participant receives a link to an on-line form where the following information must be reported:

- Package content and its condition of preservation
- Materials and Methods used to analyze PT samples
- Results

The on-line form remains active until the due date (specified in the PT request form), after this date, results will not be accepted.

At arrival in the lab, the packaging and its contents must be checked for correctness and completeness.

Before performing the test, the following remarks are to be taken into account:

1. it's necessary to treat PT items in the same manner as the routinely tested samples;
2. to prevent damaging of larvae, samples must be stored refrigerated at +4-+15°C until the test is performed;
3. to detect Anisakidae larvae in fish fillets, **four tests are suggested**: i) candling; ii) compressorium; iii) UV examination after freezing; iv) artificial digestion. Each laboratory should choose among them, the test routinely used in the lab;

NOTE: Two ISO standards are available describing UV examination after freezing and artificial digestion methods:

- ISO 23036-1:2021 Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products — Part 1: UV-press method;
- ISO 23036-2:2021 - Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products — Part 2: Artificial digestion method.

4. all tests are described below. Any deviation from the described protocols shall be specified and reported in the on-line form;
5. the test has to be performed **within 3 days** after the delivery of the samples to the lab;
6. samples handling should be performed according to routine safety procedures needed for infectious biological material, i.e. wearing individual protection devices (coat, mask and gloves). Specific safety measures must be followed according to the test procedure applied (i.e. handling of hydrochloric acid under chemical hood; use of UV-protection glasses; etc.);

Tests suggested to detect Anisakidae larvae in fish fillets

Artificial digestion

For 100g of muscle fish fillets:

Procedure:

a) 16 ± 0,5 ml of 25% hydrochloric acid (or 10.8 ± 0,5 ml 37% hydrochloric acid) is added to a 3 litre beaker containing 2.0 litres of tap water, preheated at 35 ±2°C; a stirring rod is placed in the beaker, the beaker is placed on the preheated plate and the stirring is started;
b) 10 ± 0.2 g of powder pepsin (or 30 ml of liquid pepsin) are added;
c) 25-200g samples are chopped by scissorr or knife;
d) The chopped fillets are transferred into the 3 litre beaker containing the water, pepsin and hydrochloric acid;
e) The beaker is covered with aluminium foil;
f) The magnetic stirrer must be adjusted to maintain a constant temperature of 37±2°C throughout the operation. During stirring, the digestion fluid must rotate at a sufficiently high speed to create a deep whirl without splashing;
g) The digestion fluid is stirred until the fish muscle fibers disappear (approximately 15-30 min), without exceeding 45 min. of digestion. Digestion is considered completed once fillets debris do not exceed 5% of original sample weight before digestion.
h) The stirrer is then switched off and the digestion fluid is poured through the sieve into a beaker;
i) The Anisakidae larvae can be detected on the sieve;
j) Larvae can be collected and examined under the stereomicroscope with transmitted light.

UV on squeezed and frozen fillet

Procedure:

a) Cut the fish fillets as thinnest as possible by a knife;
b) Place each fish fillet in a clear plastic bag;
c) Squeeze the fish fillet in the plastic bag up to 1-2 mm tick by a compression system ;
d) Freeze the squeezed fillets at -20°C;
e) After freezing, examine the fish fillet under an UV light by eyes in a dark room;
f) Anisakidae larvae present in the fillet will appear as brightly fluorescent spots;

Compression system

Procedure:

a) Cut the fish fillets as thinnest as possible by a knife;
b) Place each fish fillet between the two thick glasses of a compressorium;
c) Squeeze the fish fillet;
d) Microscopic examination must be carried out by scanning each preparation slowly and carefully at a 5-10X magnification.

Candling by lighting

Procedure:

a) Cut the fish fillets as thinnest as possible by a knife;
b) Place each fish fillet on the candling light box;
c) Worms show up as dark shadows in the flesh, and can be removed with forceps or a knife.

For any information or problem related to the PT participation, please address to:

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