Istituto Zooprofilattico Sperimentale della Sicilia
Ente Sanitario di Diritto Pubblico
SEDE LEGALE: Via Gino Marinuzzi, 3 – 90129 PALERMO
Codice Fiscale e Partita IVA: 00112740824
Sito Web: **www.izssicilia.it** E-mail: **DirezioneGenerale@izssicilia.it**
Tel.+39 091 6565 111 Fax. +39 091 6563 568
P.E.C.:**istzoop.sperim.sic.pa.protocollo@pa.postacertificata.gov.it**

Direttore Generale: **Antonino Salina**

DIPARTIMENTO ALIMENTI
C.RE.N.A.
Responsabile: **Dott. Vincenzo Ferrantelli**
Tel. 091 65 65 255 - Fax 091 65 65 234
E-mail: vincenzo.ferrantelli@izssicilia.it

Palermo, li 11.02.2015

Oggetto: Relazione utilizzo strumentazione Trichineasy per la rilevazione di larve di Anisakidae

In 01-13-2015 it was installed *Trichin Easy* digestion system (cod. 960), by the General Manager Armando Roggero at the National Anisakiasis Reference Centre in Palermo. This instrument is able to simulate a digestion process just citing on the EC Reg. 2075/2005 for the detection of parasite belonging to *Trichinella spp* in meat samples. *Trichin Easy* can be divided into two different sectors:
In the first sector there's a homogenization system which is composed of a blade mixer, for the sample grinding, in the second sector there's a filtration system, composed of 180 µm filter in a collecting cylinder that undergo to an aspiration system for the digestion drainage. The entire system is regulated by a fingerboard with a display. The procedural protocol is divided into two principal steps.
In the first step sample undergo to homogenization by mixer and digested by a solution of water (1L), pepsin and HCl from PLYtricons® kit. In the second step the digestion product is filtered by 180 µm filter. This procedure can be applied for the detection of Anisakidae larvae in fish products, after chemical-physical and temporal adjustments. It was planned 10 trials for the development of the method with time, temperature and reagents concentration changes to obtain optimal condition for not-altered detection of Anisakidae larvae. In the first trial it was utilized a *Sparus aurata* sample that results negative to visual inspection. 100 g of sample is weighed and put on homogenization sector with a bypass of mixer phase that can compromise the integrity of larvae. One liter of water is added to the sample. The solution underwent to a heating process by the "Heating" button press until the "Hot" signal in the display. Subsequently it was started the digestion phase for 10 min with 15 g of pepsin 1:10000 NF and 50 ml of 10% HCl. At the end of the digestion phase, the digestion product was filtered in 180 µm filter by the "START DIGESTION" button. The following test, found to be ineffective, has led to an increase of digestion time. The subsequent trials were conducted with artificial infested samples (different trials are reported in the table). A 20 min digestion with a temperature of 37± 1°C, 10 g of pepsin and 50 ml of 10% HCl, was found to be the optimum condition for the diagnostic. Filter was observed. After filtration, filter is observed at the stereomicroscope for the parasites detection. This digestion protocol is able to obtain a vital and intact larvae recovery of 100%.

Istituto Zooprofilattico Sperimentale della Sicilia
Ente Sanitario di Diritto Pubblico
SEDE LEGALE: Via Gino Marinuzzi, 3 – 90129 PALERMO
Codice Fiscale e Partita IVA: 00112740824
Sito Web: **www.izssicilia.it** E-mail: **DirezioneGenerale@izssicilia.it**
Tel.+39 091 6565 111 Fax. +39 091 6563 568
P.E.C.:**istzoop.sperim.sic.pa.protocollo@pa.postacertificata.gov.it**

Direttore Generale: **Antonino Salina**

Trial	Sample	Temperature	Time	Pepsin (g)	HCl %	N° of infested larvae	Recovery	Vitality	Outcome
1	*Sparus aurata*	45 ± 1°C	10 '	15	10	0	0	/	Inefficent digestion
2	*Engraulis encrasicolus*	40 ± 1°C	20 '	15	10	12	8 + fragments	no	Positive
3	*Scomber scombrus*	38 ± 1°C	20 '	10	10	5	5	yes	Positive
4	*Sparus aurata*	37 ± 1°C	15 '	10	10	10	10	yes	Positive
5	*Lepidopus caudatus*	37 ± 1°C	20 '	10	10	10	10	yes	Positive
6	*Sparus aurata*	37 ± 1°C	20 '	10	10	5	5	yes	Positive
7	*Lepidopus caudatus*	37 ± 1°C	20 '	10	10	4	4	yes	Positive
8	*Lepidopus caudatus*	37 ± 1°C	20 '	10	10	3	3	yes	Positive
9	*Boops boops*	37 ± 1°C	20 '	10	10	0	0	/	Positive
10	*Mullus barbatus*	37 ± 1°C	20 '	10	10	2	2	yes	Positive