REPORT ON THE VALIDATION OF THE Trichin-L ANTIGEN TEST KIT OF THE BIO-RAD COMPANY

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1. Introduction

According to the Commission regulation 2075/2005 of 5 December 2005, which specifies rules on official controls for *Trichinella* in meat, each carcass must be examined for *Trichinella* in a laboratory designated by the competent authority, using one of the detection methods set out in Chapter I of Annex I or an equivalent method set out in Chapter II of Annex I. If a new method/apparatus is to be used, prior to use it should be validated in accordance with the "Guidelines for the validation of apparatuses for the detection of *Trichinella* larvae in meat samples by digestion" (herein referred to as "the Guidelines") approved by the DG SANCO.

On April 28th, 2010, the BIO-RAD Company contacted the European Union Reference Laboratory for Parasites (EURLP) (see ANNEX 1) to request that validation be performed for the Trichin-L Antigen Test Kit. On May 2010, BIO-RAD sent the instruction manual (see Annex 2) and a description of the most important features of the apparatus. Following the evaluation of the instruction manual, the EURLP submitted an agreement to BIO-RAD and, at the same time, contacted four National Reference Laboratories (NRL) for *Trichinella*, which had shown a positive performance at the proficiency test for *Trichinella* organized by the EURLP in 2010, asking their availability in validating the Trichin-L Antigen Test Kit.

All four NRLs agreed to participate:

- NRL for *Trichinella* of Austria
- NRL for *Trichinella* of Belgium
- NRL for *Trichinella* of Estonia
- NRL for *Trichinella* of Sweden

On October 7, 2010, BIO-RAD organized a one-day training course for the NRL and EURLP personnel at the Istituto Superiore di Sanità, Rome, Italy, to present the kit and demonstrate how it works. Several proficiency samples containing a known number of *Trichinella* larvae and some without *Trichinella* larvae (negative controls), previously prepared at the EURLP, were tested. Then, BIO-RAD forwarded five Trichin-L Antigen Test Kits, including digestion apparatuses, to the EURLP. The EURLP sent the Trichin-L Antigen Test Kit to each NRL by an international courier. The detailed explanation of the Trichin-L Antigen Test Kit is shown in the Annexes 2, 3 and 4.

The EURLP invited the NRL personnel to learn about the kit before the forwarding proficiency samples of pork, some of which were spiked with *Trichinella* larvae (positive controls) and others not spiked (negative controls). When the NRL personnel felt that they had sufficient knowledge of how the kit works, they contacted the EURLP and requested that the proficiency samples be forwarded. According to the Guidelines, the proficiency samples consisted of the following:

- 10 *Trichinella*-negative samples to evaluate the digestibility of the meat and the amount of undigested meat on the sieve and the specificity
- 10 samples containing 15 *Trichinella* larvae each, to evaluate the sensitivity of the kit
- 10 samples containing 6 *Trichinella* larvae each, to evaluate the sensitivity of the kit, and
- 10 samples containing 3 *Trichinella* larvae each, to evaluate the sensitivity of the kit.

The technicians performing the tests were required to fill in the proper form (see ANNEX 1 and ANNEX 2 of the Guidelines) forwarded with the samples and to add comments and notes on whether or not the kit performed adequately, in terms of the sensitivity and user-friendliness required by the Guidelines.

The meat samples with and without *Trichinella* larvae were forwarded from EURLP to the NRLs, which had specified the most convenient times for sending the samples. According to the agreement between NRLs and EURLP, 20 samples (15 positive and 5 negative) were sent to each NRL at two different times (November 8 and 15, 2010). Additional samples (8 negative samples) were sent to two NRLs which had had some unclear results testing the negative samples. The same panel of samples (30 *Trichinella*-positive, 10 + 8 *Trichinella* negative samples) were tested at the EURLP. Within December 7, 2010, all NRLs sent to EURLP the results of the validation, with comments and notes for the preparation of the final document.

2. Sample forwarding

Each sample of 100g consisted of minced pork which was free of fat and fascia. *Trichinella*-positive samples were spiked with a known number of live larvae collected after a short period of digestion. Each sample was preserved in a plastic bag under vacuum and coded, and the samples were forwarded by an international courier in coolers placed in a polystyrene box, to maintain a temperature of less than 8°C. The parcels with the samples were delivered within 24-48 hours. Upon arrival, the polystyrene box was opened and the internal temperature was measured. The samples were then stored at +4°C until testing by the Trichin-L Antigen Test Kit within 48 hours.

3. Results

In accordance with the Guidelines, a total of 200 samples were tested in the five laboratories. Furthermore, in two NRLs and at the EURLP, 8 additional *Trichinella*-negative samples were tested. Thus a total of 150 *Trichinella*-positive samples and 74 *Trichinella*-negative samples were tested. The results were as follows:

The average undigested material on the sieve was 1.7 g (range 0.0 – < 5.0) (ANNEX 5).

3.1 Positive samples

- Pork samples spiked with 15 *Trichinella* larvae each (10 for each of the five laboratories), total 50 samples:
  - positive results were obtained for 49 samples. For a mistake, no result was obtained for one sample in one laboratory;
- pork samples spiked with 6 *Trichinella* larvae each (10 for each of the five laboratories), total 50 samples:
  - positive results were obtained for all the 50 samples;

- pork samples spiked with 3 *Trichinella* larvae each (10 for each of the five laboratories), total 50 samples:

  - positive results were obtained for all the 50 samples. In one laboratory, two samples showed a weak positive result.

3.2 Negative samples

- pork samples without *Trichinella* larvae (negative controls, 10 for each of the five laboratories), total 50 samples:

  - 37 (74%) samples tested negative;

  - 10 (20%) samples tested doubtful in three laboratories (1, 4 and 5 samples for each of the three labs);

  - 3 (6%) samples tested positive in one laboratory.

Since 26% of negative controls tested doubtful (20%) or positive (6%) and these results were mainly obtained in two out of the five laboratories, the EURLP sent additional 8 negative samples to these laboratories, requesting to strictly follow the instruction manual provided by the BIO-RAD and to avoid any contact of the samples and digestion apparatuses with other chemicals. In addition, other 8 samples were tested at the EURLP.

- Laboratory 1
  - the first three negative pork samples tested as positive, the fourth pork sample tested doubtful, whereas the other four pork samples tested negative;

- Laboratory 2
  - all the eight samples tested negative;

- EURLP
  - all the eight samples tested negative.

In the laboratory 1, it was discovered that, after digestion, the detergent traces present on the apparatus after its washing, were the cause of the false positive results. In fact, when the digestion apparatus was not anymore washed by the detergent but only with hot water, no false positive results were obtained.

4. Interpretation of the results

According to the Guidelines and Commission Regulation 2075/2005, the digestion process is considered to be satisfactory if no more than 5% of the starting sample weight (100 g) remains on the sieve. This criterion was completely fulfilled, in that the average amount of undigested material was 1.7 g, with a range from 0.0 g to 4.9 g (ANNEX 5).

According to the Guidelines, the lower acceptable detection limit for samples spiked with 10-20 larvae is 75%. The Trichin-L Antigen Test Kit correctly identified as positive, all the samples spiked with 15 larvae.

According to the Guidelines, the lower acceptable detection limit for samples spiked with 6 larvae is 3 larvae (50%). The Trichin-L Antigen Test Kit correctly identified as positive, all the samples spiked with 6 larvae.

According to the Guidelines, the lower acceptable detection limit for samples spiked with 3 larvae is 1 larva (33.3%). The Trichin-L Antigen Test Kit correctly identified as positive, all the samples spiked with 3 larvae.

Some of the negative controls were incorrectly detected as positive in two laboratories. The laboratory investigation revealed that the detection of false positive results could be due to the presence of detergent traces on the digestion apparatuses.

5. Comments

5.1 Strongnesses

- the Trichin-L Antigen Test Kit appears to be easy to use;
- the Trichin-L Antigen Test Kit shows a very highly sensibility even when very few larvae are present in the meat sample;
- in comparison with the standard digestion methods, the Trichin-L Antigen Test Kit reduces the time needed to obtain the results after digestion;
- the Trichin-L Antigen Test Kit does not need a microscope and, consequently, no personnel able to recognize the *Trichinella* larvae should be always present in the laboratory;

5.2 Weaknesses

- the seal underneath the mesh, holding the 20 µm filter is colourless; hence, it is difficult to find when dropped on the floor or in a washing basin;
- the 20 µm filter is very thin and difficult to grab out of its box;
- the specificity of the Trichin-L Antigen Test Kit can be compromised by some chemical products, even only in traces, which can contaminate the digestion apparatuses such as some detergents used to wash the apparatuses;
- there is no chance to identify the *Trichinella* larvae at the species level, since no larva DNA is available after the use of the kit; when the test is positive, larvae can be isolated and identified only using one of the traditional digestion methods, which should be used to trace back the infected animal/s present among those of the pool.
- the lid of the blender is not hermetic; consequently, during chopping some meat juice pours through the lid;
- the temperature of the magnetic stirrer is not stable and should be continuously checked during digestion;
- it is hoped for an automatic system to crush the filter and for the antigen solubilisation procedure.
- the digestion protocol suggested by the BIO-RAD is not very robust since the sample blending must be run strictly as defined in the kit insert; indeed, minor changes could impact the assay performances.

6. Conclusions

According to the results, the sensitivity of the Trichin-L Antigen Test Kit is much higher that the minimum requested. The amount of undigested material is greatly below the accepted limit (1.7 g versus 5.0 g). The Trichin-L Antigen Test Kit fulfilled the sensitivity required by the Guidelines in the 99% of positive samples.

Practical problems encountered during the validation process in two laboratories were due to the use of detergent to wash the digestion apparatus.

It is recommended that the company advises on the type of detergent to wash the equipment after use.

The overall conclusion is that Trichin-L Antigen Test Kit meets requirements for the accurate detection of *Trichinella* larvae in pork samples.

ANNEX 1

Company request to validate the Trichin-L Antigen Test Kit

Da: Gilles Nespoulous [mailto:Gilles.Nespoulous@bio-rad.com]
Inviato: mercoledì 28 aprile 2010 16.17
A: Pozio Edoardo
Oggetto: request for the CRL validation of a newly method for the detection of Trichinella spp in pork meat

Dear Dr Pozio,

Our company has recently developed a new method for the detection of Trichinella parasites in pooled meat. This new method is based on a very similar assay principle that the currently approved digestion method but with a faster and easier detection method.
The test allows the testing of one pool of 100 meat samples at a time. It is suitable for pork animal specie.
We kindly ask you if the CRL could evaluate this new method. If the CRL is satisfied with the method performances Bio-Rad will then be happy to submit it to the European Commission approval.

Please do not hesitate to contact us if you need further information on this new method.

We will be waiting for your instructions on the procedure we must follow to start the evaluation in the shortest delay.

Best regards.

Gilles NESPOULOUS
TSE Group Product Manager
Bio-Rad Laboratories
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ANNEX 2

Instruction manual provided with the Trichin-L Antigen Test Kit

Trichin-L

Trichinella Antigen Test Kit

80 Tests Ref.: 357-2120

Reagent kit for the detection of *Trichinella* Antigens in swine

For Evaluation Only

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1. GENERAL INFORMATION

Trichinellosis is the infection caused by the nematode *Trichinella*. Although the nematode may be found in a wide variety of animals worldwide, the domestic pig is the primary source of infection in humans in developed nations.

According to the European regulations EC No 2075/2005, the gold standard method to detect *larvae* in infected muscle tissue is based upon enzymatic digestion of pooled samples followed by decantation steps and a microscopic examination of *larvae*.

Trichin-L, a new alternative method to the pooled sample digestion, has been developed recently. The very first steps of the *Trichin-L* test are similar to those of the pooled sample digestion. It then differs on both decantation steps – replaced by a *larvae* filtration step – and the final detection step which consists of a latex agglutination test. It allows checking for the presence or absence of *Trichinella* antigens.

2. PRINCIPLE OF PROCEDURE

Latex particles are coated by a covalent bond with mouse monoclonal anti-*Trichinella* antibodies. This test allows the detection of the *Trichinella* antigens.

The reading of the test is very easy because of the use of suspended blue latex particles in a colorless counter stain. In the case of a negative test, a uniform blue image is obtained. In the case of a positive test, blue aggregates clearly exhibit while counter stain becomes colorless.

3. COMPOSITION OF THE KIT

Identification on label	Description	Presentation
R1	**Latex agglutination cards** with 8 reaction wells each	10
R2	**Sample diluent**: buffer with preservative (< 1.5% Proclin™ 300)	1 vial (40 ml)
R3	**Negative control:** buffer supplemented with Bovine Serum Albumin (BSA) and preservative (< 1.5% Proclin™ 300)	1 dropper vial (0.75 ml)
R4	**Positive control**: diluted *T. spiralis* crude antigen in buffer with preservative (< 1.5% Proclin™ 300)	1 dropper vial (0.75 ml)
R7	**Latex beads**: buffer containing polystyrene particles coated with Monoclonal antibody supplemented with BSA and preservative (< 1.5% Proclin™ 300)	1 dropper vial (2.5 ml)
	Pepsin: pepsin powder derived from porcine gastric mucosa	2 x 300 g
	Disposable sticks	x 80
	Filtration membranes	x 60
	Calibrated spoon	x1
	Forceps	x 1

4. PREPARATION OF REAGENTS

All reagents of the *Trichin-L* Kit are ready for use.

5. STORAGE AND HANDLING REQUIREMENTS

All reagents are stable until the expiry dates indicated on the label, if stored at +2-8°C and in absence of microbial contamination.

Store the latex reagent bottles upright.

Identification	Conservation
R2	1 month after opening when stored at +2°C to +8°C
R3	1 month after opening when stored at +2°C to +8°C
R4	1 month after opening when stored at +2°C to +8°C
R7	1 month after opening when stored at +2°C to +8°C

THE LATEX REAGENTS SHOULD NOT BE FROZEN.

6. COLLECTION AND PREPARATION OF SAMPLE

For domestic swine, a sample weighing between 1 g and 1.15 g should be collected from the pillar of the diaphragm. If the sample is collected in the diaphragm, masseter or tongue, 2 x 1g are required. . If available, special trichinae forceps could possibly be used to collect the required amount of sample.

For breeding sows and boars, a sample weighing at least 2 x1 g should be collected from the pillar of the diaphragm. If the sample is collected in the diaphragm, masseter or tongue, 4 x 1g are required. If available, special trichinae forceps could possibly be used to collect the required amount of sample.

7. PROCEDURE (domestic swine, up to 100 g of pooled samples at a time)

The *Trichin-L* assay protocol is divided in 4 steps.

All reactives should be put at room temperature [18°C – 30°C] at least 30 minutes before use.

MEAT CHOPPING/DIGESTION

1) Preparation of the digestion buffer

Successively add the following reagents in a 3 liter beaker placed on a heating plate:

- 2 liters of tap water pre-heated at 46-48°C
- 16 ml of 25 % hydrochloric acid (0.2% final)

Place a stirring rod is in the beaker and start stirring (250 rpm).

Add 10 ± 1 g of pepsin (0.5% final). The calibrated spoon included within the kit can be used to dispense the pepsin: one full level measure is required. Pepsin weight exactitude must be controlled.

Wait for the complete dissolution of the pepsin.

2) Meat chopping

Take 150 ml of the digestion buffer in a test tube.

Pour in the blender bowl:

- 100 – 115 g of meat sample collected in accordance with chap. 6.
- 150 ml of the digestion buffer from the test tube .

Note: in case of smaller pools (e.g. 50 x 1 g) always respect the 1:1,5 mass/volume ratio between meat tissue and digestion buffer. Minimum 50 g of meat sample must be tested per pool.

Chop the meat for **20 seconds at 18000 rpm (only 10 seconds for spiked samples used for ring trials)**.

3) Digestion

Keep 250 ml to 500 ml of the digestion buffer in a test tube.

Add approximately 500 ml of the digestion buffer from the beaker in the blender bowl.

Then, pour the sample homogenate (– 750 ml) into the beaker containing the remaining digestion buffer.

Cautiously rinse the blender bowl with the 250 ml to 500 ml digestion buffer kept in the test tube then pour the liquid into the beaker.

Incubate for **30 minutes at 44-46°C while stirring (250 rpm)**.

Note: a 45 minutes incubation is required when using tongue.

FILTRATION

4) Set up of the filtration unit

Position in the following order on the filtration ramp:

- one filtration membrane on the support
- the conical filtration funnel (fixed to the support with the block system)
- a stainless steel sieve (180 microns mesh size)

Start the vacuum pump few seconds before stopping the sample digestion.

5) Filtration

Stop stirring and pour the digested sample homogenate into the filtration funnel through the sieve.

Rinse the beaker with at least 250 ml of warm water. This rinsing liquid must be poured into the filtration ramp after the digested sample homogenate has been successfully filtrated, (warm water will rinse the sieve and remove residual acidity traces).

Meat chopping and digestion are efficient if less than 5 g of meat remains at the end of the process at the surface of the sieve.

With the help of the forceps (included in the kit), take the filtration membrane holding it by an edge. Fold the filtration membrane in four minimum and put it in a 15 ml Falcon® tube.

Note: All material in contact with meat (blender bowl, beaker, stirring rod, temperature sensor, conical filtration funnel, sieve and forceps) must be carefully decontaminated between runs by soaking for few seconds in warm water (60°C to 90°C). It must then be washed with tap water and a clean sponge to eliminate pieces of meat or inactivated larvae that could remains on their surface.

ANTIGEN SOLUBILISATION

If using a manual pestle for the antigen solubilisation, please follow steps 6) and 7) of the assay protocol.

If using the automated pestle, simply add 0.5 ml of Sample diluent (R2) into the 15 ml Falcon® tube and follow automatic pestle instructions.

6) Filtration membrane crushing

Push the filtration membrane at the bottom of the Falcon® tube with the help of the pestle and strongly press it by doing 20 successive back and forth movements with the pestle.

Note: the pestle should be positioned inside the filtration membrane folding to ensure optimum grinding of the larvae.

7) Antigen solubilisation

Add 0.5 ml of Sample diluent (R2) into the 15 ml Falcon® tube and homogenize the larval antigens with the help of the pestle by doing successive low amplitude back and forth movements for 30 seconds. Avoid abrupt movements to limit liquid splashes.

Note: the pestle must be carefully decontaminated between runs by soaking for few seconds in 250 ml of warm water (60°C to 90°C). It must then be rinsed with tap water to eliminate antigens that could remains on its surface and finally wiped with absorbent paper.
The warm water used for soaking must be changed every 6 runs.

DETECTION

8) Samples & controls dispensing

Dispense 50 µl of sample or 50 µl (2 drops) of negative control (R3) or 50 µl (2 drops) of positive control (R4) into a field of one agglutination card.

9) Conjugate dispensing

Add 25 µl (1 drop) of latex beads (R7), without making it come into contact, beside the first drop of sample/control.

Note: Before use, latex beads have to be cautiously homogenized (minimum 10 tube inversions) until the solution becomes homogenous.

In each field, gently mix the drops with a disposable stick until the homogeneous liquid covers all the field.

Put the agglutination card on the 3D rocker and start rocking for 10 minutes at the maximum speed (~ 30 rpm).

10) Reading

Stop rocking. Put the agglutination card on a plane surface.

Technician must be positioned at approximately 40 centimeters from the agglutination card for optimal reading.

In case of positive sample, aggregates must appear within 5-10 min. maximum.

8. RESULTS INTERPRETATION

Negative reaction: the suspension remains blue and homogenous. No beads aggregates are observed. Compare with the negative control.

Positive reaction: formation of beads aggregates (more or less clearly marked). The sample contains *Trichinella* antigens.

When a collective sample (pool) produces a positive or uncertain result, a further 20 g sample (20 x 1 g) must be taken from each pig in accordance with chap. 6. The 20 g samples are examined using the method describe above. In this way, samples from 20 groups of five pigs will be examined.

When *Trichinella* antigens are detected in a pooled sample from five pigs, further 20 g samples (20 x 1 g) are collected from the individual pigs in the group and each is tested separately using the method described above.

9. MATERIALS REQUIRED BUT NOT PROVIDED

Sample Collection
- Trichinae forceps
- Weighing scale

Sample Grinding
- Laboratory blender + 1.25 liter glass container (Cat. # 359-3980)
- Timer

Sample Digestion
- Magnetic stirrer with thermostatically controlled heating plate (Cat. # 359-3981)
- Teflon-coated stirring rods
- Glass beaker of 3 liters
- 250 ml test tube
- 20-ml test tube or 10-ml pipette
- Squeeze bottle

- Concentrate hydrochloric acid
- Timer

Sample Filtration
- One-branch vented filter system incl. single base with frit (Cat. # 359-3979), 500-ml steel funnel (Cat. # 359-3987), heavy wall red rubber tubing (Cat. # 359-3988), 180 µm sieve (Cat. # 359-3989)
- Vacuum pump IP-20T (Cat. # 359-3985)
- Closure 83B, 2 ports 1/4" (Cat. # 359-3984)
- Thick-Walled 10 L Bottle (Cat. # 359-3983)
- Forceps
- 500 ml test tube

Antigen Solubilization
- 15 ml test tubes (Cat. # 357-2123)
- Manual pestle (Cat. # 359-3986) or automated pestle (Cat. # 359-3997)
- 0.5 ml pipette

Antigen Detection
- 50 µl pipette
- 3D Gyratory Rocker (Cat. # 359-3982)
- Timer

10. PRECAUTIONS

The quality of the results depends upon the adherence to good laboratory practices:
- Do not use reagents after their expiration.
- All the reagents and the sample should be used at room temperature (18-30°C).
- Do not mix or associate reagents from kits with different lot numbers during the same manipulation.
- Do not touch the reaction fields of agglutination cards with your fingers.
- Change pipette or sampling tip for each sample tested.
- Shake the latex vial before use.
- Change the stick for each reaction.
- Discard all disposable material used in an autoclavable waste bin or disinfectant bath.

11. HYGIENE AND SAFETY INSTRUCTIONS

Generally, hygiene conditions, biosafety measures and good laboratory practices must be in agreement with recommendation of regulatory authorities of the country.
- All reagents of the kit are intended for use in *"in vitro"* veterinary diagnosis.
- All the materials directly in contact with the samples and the solutions must be considered as contaminated.
- Avoid splashing samples or solutions containing samples.

According to the European legislation, the kit containing ProClin™ 300 (< 1.5%) and Pepsin (100%) is classified as harmful.

Xn - Harmful

R43: May cause sensitisation by skin contact.
R36/37/38-42: Irritating to eyes, respiratory system and skin. May cause sensitisation by inhalation.

12. REFERENCES

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(GB) • This product contains human or animal components. Handle with care.
(FR) • Ce produit contient des composants d'origine biologique humaine ou animale. Manipuler avec précaution.
(ES) • Este producto contiene componentes humanos o animales. Manejar con cuidado.
(IT) • Questo prodotto contiene componenti umane o animali. Maneggiare con cura.
(DE) • Dieses Produkt enthält Bestandteile menschlichen oder tierischen Ursprungs. Vorsichtig handhaben.
(PT) • Este medicamento contém componentes de origem humana ou animal. Manuseie com cuidado.
(SE) • Denna produkt innehåller beståndsdelar från människa eller djur. Hantera produkten varsamt.
(DK) • Dette produkt indeholder humane og animalske komponenter. Skal behandles med forsigtighed.
(GR) • Αυτό το προϊόν περιέχει ανθρώπινα ή ζωικά στοιχεία. Χειριστείτε το με προσοχή.
(PL) • Niniejszy produkt zawiera składniki pochodzenia ludzkiego lub zwierzęcego. Należy obchodzić się z nim ostrożnie.
(LT) • Šiame produkte yra žmogiškosios arba gyvūninės kilmės sudėtinių dalių. Elgtis atsargiai.
(HU) • A készítmény emberi vagy állati eredetű összetevőket tartalmaz. Óvatosan kezelendő.
(EE) • Käesolev toode sisaldab inim-või loomseid komponente. Käsitseda ettevaatlikult.
(SK) • Tento výrobok obsahuje ľudské alebo zvieracie zložky. Narábajte s nim opatrne.
(CZ) • Tento výrobek obsahuje lidské nebo zvířecí komponenty. Zacházejte s ním opatrně.
(RO) • Acest produs conține materiale de origine umană sau animală. Manevrați-l cu grijă.
(BG) • Този продукт съдържа човешки или животински компоненти. Бъдете внимателни при работа с него.
(LV) • Šis produkts satur cilvēkiem vai dzīvniekiem paredzētas sastāvdaļas. Apieties uzmanīgi.
(MT) • Dan il-prodott fih komponenti umani jew tal-annimali. Uża b'attenzjoni.
(NL) • Dit product bevat menselijke of dierlijke bestanddelen. Breekbaar.
(SI) • Izdelek vsebuje človeške ali živalske sestavine. Rokujte previdno.
(FI) • Tässä tuotteessa on ihmisestä tai eläimistä peräisin olevia osia. Käsittele varovasti.

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(GB) Harmful
(FR) Nocif
(ES) Nocivo
(IT) Nocivo
(DE) Gesundheitsschädlich
(PT) Nocivo
(SE) Hälsoskadlig
(DK) Sundhedsskadelig
(GR) Επιβλαβές
(PL) Produkt szkodliwy
(LT) Kenksminga
(HU) Ártalmas
(EE) Kahjulik
(SK) Škodlivý
(CZ) Zdraviškodlivý
(RO) Nociv
(BG) Вреден
(LV) Kaitīgs
(MT) Jagħmel il-ħsara
(NL) Schadelijk
(SI) Zdravju škodljivo
(FI) Haitallinen

R43:

(GB) • May cause sensitisation by skin contact.
(FR) • Peut entraîner une sensibilisation par contact avec la peau.
(ES) • Posibilidad de sensibilización en contacto con la piel.
(IT) • Può provocare sensibilizzazione per contatto con la pelle.
(DE) • Sensibilisierung durch Hautkontakt möglich.
(PT) • Pode causar sensibilização em contacto com a pele.
(SE) • Kan ge allergi vid hudkontakt.
(DK) • Kan give overfølsomhed ved kontakt med huden.
(GR) • Μπορεί να προκαλέσει ευαισθητοποίηση σε επαφήμε το δέρμα.
(PL) • Może powodować uczulenie w kontakcie ze skórą.
(LT) • Gali sukelti alergiją susilietus su oda.
(HU) • Bőrrel érintkezve túlérzékenységet okozhat (szenzibilizáló hatású lehet).
(EE) • Kokkupuutel nahaga võib põhjustada ülitundlikkust.
(SK) • Môže spôsobiť senzibilizáciu pri kontakte s pokožkou.
(CZ) • Může vyvolat senzibilizaci při styku s kůží.
(RO) • Poate provoca o sensibilizare în contact cu pielea.
(BG) • Възможна е сенсибилизация при контакт с кожата.
(LV) • Saskaroties ar ādu, var izraisīt paaugstinātu jutīgumu.
(MT) • Jista' jikkaġuna sensitizzazzjoni meta jmiss il-ġilda.
(NL) • Kan overgevoeligheid veroorzaken bij contact met de huid.
(SI) • Stik s kožo lahko povzroči preobčutljivost.
(FI) • Ihokosketus voi aiheuttaa herkistymistä.

R36/37/38-42:

(GB) • Irritating to eyes, respiratory system and skin. May cause sensitization by inhalation.
(FR) • Irritant pour les yeux, les voies respiratoires et la peau. Peut entraîner une sensibilisation par inhalation.
(ES) • Irrita los ojos, la piel y las vías respiratorias. Posibilidad de sensibilización por inhalación.
(IT) • Irritante per gli occhi, le vie respiratorie e la pelle. Può provocare sensibilizzazione per inalazione.
(DE) • Reizt die Augen, Atmungsorgane und die Haut. Sensibilisierung durch Einatmen möglich.
(PT) • Irritante para os olhos, vias respiratórias e pele. Pode causar sensibilização por inalação.
(SE) • Irriterar ögonen, andningsorganen och huden. Kan ge allergi vid inandning.
(DK) • Irriterer øjnene, åndedrætsorganerne og huden. Kan give overfølsomhed ved indånding.
(GR) • Ερεθίζει τα μάτια, το αναπνευστικό σύστημα και το δέρμα. Μπορεί να προκαλέσει ευαισθητοποίηση όταν εισπνέεται.
(PL) • Działa drażniąco na oczy, drogi oddechowe i skórę. Może powodować uczulenie w następstwie narażenia drogą oddechową.

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(LT) • Dirgina akis, kvėpavimo takus ir odą. Gali sukelti alergiją įkvėpus.
(HU) • Szem- és bőrizgató hatású, izgatja a légutakat. Belélegezve túlérzékenységet okozhat (szenzibilizáló hatású lehet).
(EE) • Ärritab silmi, hingamiselundeid ja nahka. Sissehingamisel võib põhjustada ülitundlikkust.
(SK) • Dráždi oči, dýchacie cesty a pokožku. Môže spôsobiť senzibilizáciu pri vdýchnutí.
(CZ) • Dráždí oči, dýchací orgány a kůži. Může vyvolat senzibilizaci při vdechování.
(RO) • Iritant pentru ochi, sistemul respirator şi pentru piele. Poate provoca o sensibilizare prin inhalare.
(BG) • Дразни очите, дихателните пътища и кожата. Възможна е сенсибилизация при вдишване.
(LV) • Kairina acis, ādu un elpošanas sistēmu. Ieelpojot var izraisīt paaugstinātu jutīgumu.
(MT) • Jirrita l-għajnejn, is-sistema respiratorja u l-ġilda. Jista' jġib sensitizzazzjoni meta jinxtamm.
(NL) • Irriterend voor de ogen, de ademhalingswegen en de huid. Kan overgevoeligheid veroorzaken bij inademing.
(SI) • Draži oči, dihala in kožo. Vdihavanje lahko povzroči preobčutljivost.
(FI) • Ärsyttää silmiä, hengityselimiä ja ihoa. Altistuminen hengitysteitse voi aiheuttaa herkistymistä.

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ANNEX 3

Apparatuses and consumables provided by BIO-RAD to each laboratory

- Apparatuses

Table 1

Codes on figure 1 of Annex 4	Type of apparatus	Company	Model
1	Blender	Waring	HGB2WTG4
2	Magnetic stirrer with a thermometer probe	IKA	Yellow MAG HS7
3a 3b 3c	Vacuum pump + 0.5 litre steel funnel + 10 litre plastic tank	KNF Sartorius Nalgene	Laboport Stedin biotech GmbH
4	Steel sieve	Analysensieb	180 µm, 10 cm diameter
5	Rocker	Grant-bio	PS-3D

- Consumables

Trichin-L - *Trichinella* antigen test kit (80 tests)

Table 2

Codes on figure 1 of Annex 4	
6	Trichin-L Antigen Test Kit
7	Pestle

Trichin-L Antigen Test Kit

Table 3

Codes on figure 2 of Annex 4	Trichin-L Antigen Test Kit content
1	box
2	Pepsin (300 g)
3	*Trichin-L*, sample diluent
4	pestle
5	15 mL Falcon tube
6	forceps
7	20 µm nylon mesh filter
8	*Trichin-L*, negative control (green plug)
9	*Trichin-L*, positive control (red plug)
10	*Trichin-L*, latex beads (blue plug)
11	sticks
12	Latex agglutination cards

ANNEX 4

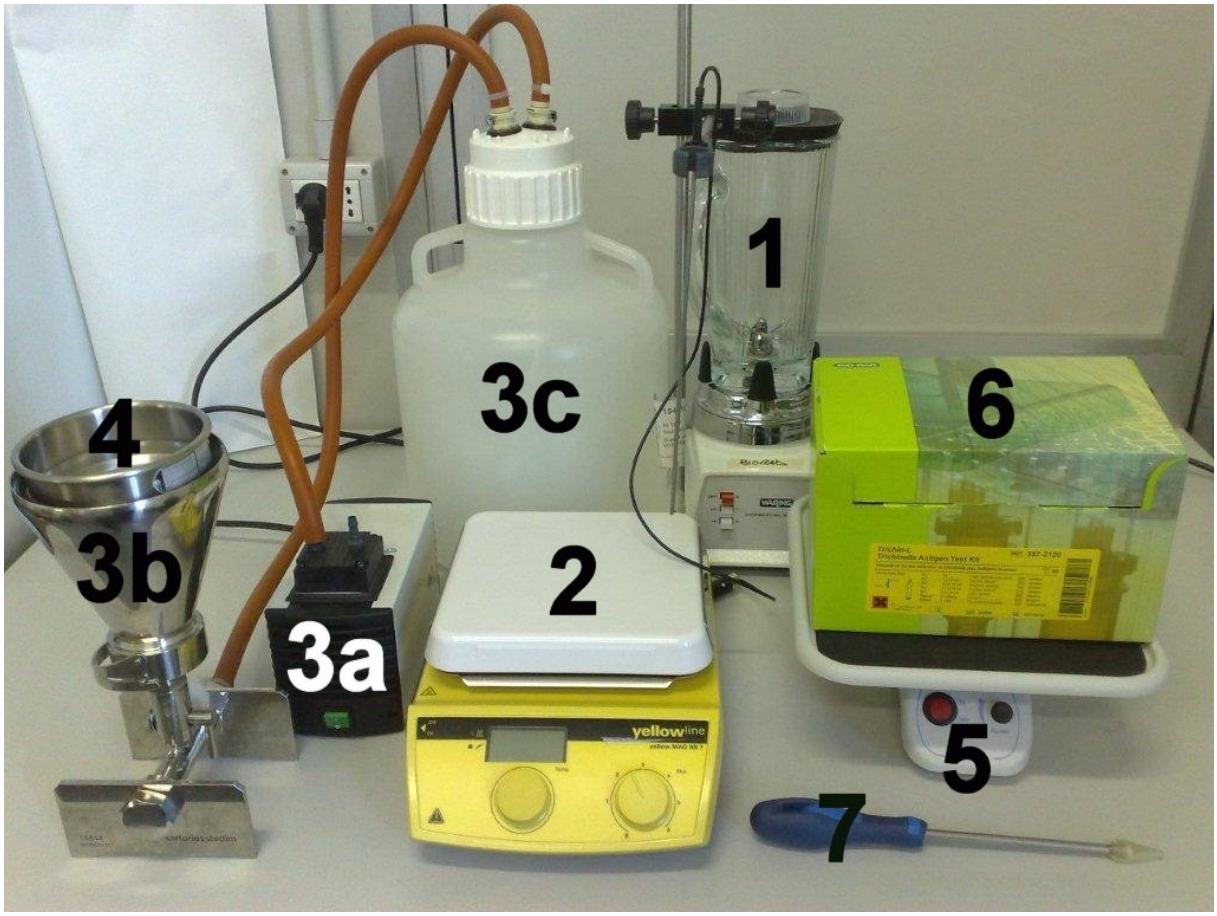

Figure 1. Apparatuses and Trichin-L Antigen Test Kit provided by the Bio-Rad Company (for the codes see ANNEX 3, Tables 1 and 2).

Figure 2. Trichin-L Antigen Test Kit (for the codes see ANNEX 3, Table 3).

ANNEX 5

Undigested material on the sieve after digestion

(total 200 digestions)

No. of g of undigested material on the sieve	No. samples (%)	No. of g of undigested material on the sieve	No. samples (%)
0.0	40 (20.0)	2.1 – 3.0	11 (5.5)
0.1 – 1.0	81 (40.5)	3.1 – 4.0	10 (5.0)
1.1 - 2.0	13 (6.5)	4.1 - < 5	45 (22.5)