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International Commission on Trichinellosis: Recommendations on methods for the control of *Trichinella* in domestic and wild animals intended for human consumption

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Abstract

This document provides a uniform set of recommendations for the control of *Trichinella* at all levels (on the farm, at slaughter and in processed meats). These recommendations are based on the best scientific information available and represent the official position of the International Commission on Trichinellosis regarding acceptable control methods. These recommendations are subject to change as new scientific information becomes available. Published by Elsevier Science B.V.

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1. Slaughter testing (individual animal inspection)

Slaughter inspection methods are designed to "prevent clinical trichinellosis in humans" and are not designed to prevent infection entirely. Currently used methods of pooled digestion using a minimum of 1 g of sample (as applied for testing of pork products) or trichinoscopy using a minimum of 0.5 g of sample are generally considered sufficient to detect infection at levels which cause clinical disease in humans. For products which are eaten without cooking or other treatments to inactivate *Trichinella*, more intensive inspection is recommended to prevent human infection.

1.1. Slaughter testing of swine

For routine examination of pork carcasses using pooled sample digestion testing, a minimum of 1 g of tissue and preferably 5 g of tissue (particularly in endemic areas) is recommended for prevention of human disease. For the same purposes, a minimum of 0.5 g of tissue, and preferably more, may be examined by use of a trichinoscope (trichinelloscope).

Examples of pooled sample digestion methods may be found in the following sources:

- OIE Manual of Standards for Diagnostic Tests and Vaccines (Article 3.5.3) Gamble (1998).
- European Union Directive 84/319/EEC, 1984, amending the annexes to Council Directive 77/96/EEC, 1977.
- Appendix A of this document.

Any of the described methods for pooled sample digestion testing must be properly validated and used in conjunction with an appropriate quality assurance system as described in Sections 1.5 and 1.6 of this document.

Although minor variations in the methodology used to perform digestion testing may not affect the outcome, there are several "critical control points" which must be monitored to assure the integrity of the testing process. These critical control points are as follows:

1. *A verifiable system of sample collection and identification must be maintained*. The process must assure that samples of 1 g or greater size originate from the appropriate number of pigs and that samples are clearly identified back to individual pigs.
2. *Digestion fluid must be consistent in quality and prepared in a manner that does not affect the activity of the pepsin*. The most critical step in preparation of digestion fluid is the addition of the hydrochloric acid to the water prior to the introduction of pepsin. This step will protect the pepsin from degradation by direct contact with concentrated hydrochloric acid. Other factors in the preparation and use of digestion fluid (the source and quality of pepsin, the amounts of pepsin and hydrochloric acid used, and the ratios of tissue to digestion fluid) should conform to published guidelines.
3. *The temperature maintained during the digestion process should not exceed* $45\pm2^{\circ}$C. Higher temperatures will result in the inactivation of pepsin, incomplete digestion and poor recovery rates. Lower temperatures will require longer digestion times or result in incomplete digestion of sample.
4. *Following digestion, no undigested muscle tissue should remain* (*as evidenced by material retained on the sieve*). Digestion must be complete to assure the integrity of the test. Remedies for incomplete digestion include adequate blending or homogenization

of samples, increasing digestion times, and, if this is not effective, verifying the quality of the pepsin.

5. *Sedimentation procedures and times should be conducted so as to maximize recovery of larvae*. Existing methods employing sedimentation times of 30 min are sufficient. Shortening the recommended times will result in reduced recovery rates. Sedimentation may be improved by periodically vibrating or tapping funnels during settling. Recovery of sediment from separatory funnels must include complete opening of the stopcock to avoid larval retention.
6. *Digest samples must be clarified sufficiently to allow visualization of larvae*. The classical measure of clarity is the ability to read newsprint through the bottom of the petri dish. Digests which are not clarified properly will result in inability to see larvae.
7. *Microscope optics must be sufficient to provide clear magnification at* 15–40×. In addition, regular microscope maintenance is required.
8. *Digests should be examined prior to the removal or release of carcasses*. This system is necessary to assure that positive carcasses are not distributed for human consumption.
9. *Records are kept which assure the accurate identification of samples and carcasses*.

1.2. Slaughter testing of horses

Due to the habits of eating horse meat without substantial cooking, and given the history of human trichinellosis resulting from eating horse meat, the following testing requirements are recommended when testing horse carcasses for *Trichinella* infection:

The ICT recommends a minimum of 5 g of tissue (10 g preferred) from the tongue or masseter of horses should be tested using a pooled sample digestion method. Diaphragm (crus muscle or *Crura diaphragmatica*) is an alternative site but harbors only from 1/4 to 1/2 as many larvae as muscle in the head region. In areas where large amounts of horse meat are eaten, greater quantities of muscle should be examined. The example of digestion testing provided in Appendix A may be followed for the testing of horses, with the exception of adjusting the size of the sample tested.

Critical control points, as described for testing swine carcasses, should receive similar attention in testing horse meat. Particular attention should be paid to clarification of digests and the interference of intact muscle fibers or debris in visualizing sediments.

Due to the history of horse meat related outbreaks of human trichinellosis, it is recommended that all countries which export horses for human consumption implement or enhance quality assurance measures within their slaughter inspection programs. An example of a quality assurance program is attached in Appendix B.

1.3. Slaughter testing of game meats

Various species of game animals are sources of *Trichinella* infection in humans. The ICT recommends that all relevant game meats intended for human consumption should be tested for *Trichinella* infection using an accepted methodology. Special sampling requirements for testing game meats include the following:

- Samples used for testing wild boar should include forearm or diaphragm musculature.
- Samples used for testing bear should include diaphragm or masseter musculature or tongue.
- Samples used for testing walrus should include tongue.

If the recommended muscles from carcasses can not be tested due to fabrication, alternative muscle cuts from the carcass or parts of the carcass should be tested using larger amounts of tissue to assure safety.

Digestion testing methods for game meats should follow methods described for swine (see Appendix A). The major process control for digestion testing of game meats is digestibility. Due to the difficulty of digesting certain game meats, test methods should be conducted to assure that complete digestion of test samples is obtained. Otherwise, appropriate adjustments to the testing procedure should be made (see Section 1.1).

1.4. Recommended actions when a positive test result is obtained

The following actions are recommended when a positive sample is detected at slaughter:

1. Established procedures should be in place that allow the positive carcass to be accurately identified. Verification of infection should be performed using a larger amount of tissue for digestion. Parasites recovered from domestic swine, horses, or game animals should be submitted to the International *Trichinella* Reference Centre, Rome, Italy [1] for species identification and/or genotyping (see Appendix E).
2. Positive carcasses should be rendered using an officially permitted procedure.
3. A regular plan should be in place which allows: trace back of positive animals to farm of origin; conduct of epidemiological studies including more extensive testing and serological surveillance; herd clean-up and management changes to avoid further infection; and, verification over time that infection no longer exists. For positive horses, it is recommended that the animal be traced and that epidemiological studies be conducted in the area of origin. Countries exporting pigs and horses for human consumption should have in place identification systems and funding which support trace back and epidemiological studies.
4. Numbers of cases occurring should be reported to the Office Internationale des Epizooties (1999) on an annual basis.

1.5. Quality assurance systems for digestion testing

The ICT recommends that all laboratories which test for the presence of *Trichinella* in swine, other livestock, or game meats, maintain a suitable quality control system. Quality assurance measures are necessary to ensure that testing processes are working properly. This includes system audits to verify that the test protocol is strictly followed and that there is proper documentation. Also, there must be provision for *Trichinella* analysts to be provided with samples from which worms can be recovered. These provisions monitor the integrity of the test system and the ability of technical personnel to accurately visualize *Trichinella*.

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An example of a quality control system for digestion testing is given in Appendix B. It is recommended that quality assurance be conducted on a regular basis (e.g. four times per year). Samples submitted for quality assurance testing should be appropriate for the test method being employed (i.e. a minimum of 0.5 g for trichinoscope (trichinelloscope) testing and a minimum of 1.0 g for pooled digestion testing).

1.6. Validation of testing systems

The ICT recommends that all slaughter testing methods, including modifications to currently accepted international methods, should be validated by standard procedures, and the results of validation studies should be available and used as a measure of the acceptability of such methods for purposes of domestic and international trade.

Validation of new methods will be monitored by the International Commission on Trichinellosis. New methods will be evaluated in a minimum of three reference laboratories, selected from a list of laboratories maintained by the ICT. Validation will consist of performing the new method on a proficiency panel, including a series of positive and negative samples. Proficiency panels should be prepared as described by Forbes et al. (1998).

Proficiency panels evaluated by each reference laboratory should consist of a minimum of 40 samples including 10 negative samples, 10 samples containing 3–5 larvae per gram of tissue (LPG), 10 samples containing 10–20 LPG and 10 samples containing 20–50 LPG. Acceptable results of testing among the three reference laboratories are: (1) a test sensitivity of 90% (with a 95% confidence level) for samples containing 3–5 LPG; and (2) recovery of a minimum of 75% of total larvae from samples containing 10–20 and 20–50 LPG.

1.7. Alternative testing systems

Indirect (serological) testing methods are not recommended as a substitute for direct (trichinoscopy or pooled digestion) testing of individual carcasses at slaughter. Improvements in direct testing methods should be evaluated against currently used digestion methods for sensitivity and specificity.

2. Processing methods to control Trichinellosis

All meat from animals which might contain *Trichinella* larvae and not tested by an acceptable method and found to be negative, should be treated by a processing method which has been proven to inactivate *Trichinella* prior to distribution for human consumption. This applies to both commercial and non-commercial sources of meat.

The ICT recognizes three acceptable means of treatment which can be used to render meat safe, if not otherwise proven to be free from *Trichinella* infection. These methods include cooking, freezing, and irradiation.

2.1. Cooking to inactivate Trichinella

When the proper equipment is available for accurately achieving and monitoring time and temperature combinations, the guidelines set forth for cooking in the United States

Department of Agriculture's Code of Federal Regulations (1990) (Appendix C) are acceptable for treatment of meat to prevent human trichinellosis.

In the absence of proper temperature and time control and monitoring systems, processors and consumers of meat should monitor the color and texture of the meat during cooking. A change in color from pink to grey throughout, and a change in texture such that muscle fibers are easily separated from each other are indicators that meat has been rendered safe to eat. (As a precautionary note, processing of meat under loosely controlled conditions creates opportunities for error. Color change is only a general indicator of safety).

2.2. Freezing to inactivate Trichinella

When the proper equipment is available for accurately achieving and monitoring time and temperature combinations, the guidelines set forth for freezing in the United States Department of Agriculture's Code of Federal Regulations (Appendix D) are acceptable for treatment of meat to prevent human trichinellosis.

In the absence of proper temperature and time control and monitoring systems, processors and consumers of meat should ensure that cuts of meat up to 15 cm in thickness are frozen solid (at least -15°C) for no less than 3 weeks and cuts of meat up to 69 cm in thickness are frozen solid (at least -15°C) for no less than 4 weeks. The requirements for freezing apply to pork and horse meat, since game meats often harbor freeze-resistance types of *Trichinella* that pose public health risks even after months or years of freezing. While freeze-resistant *Trichinella* species have low infectivity for pigs, such infections can not be ignored in areas where these parasite species are endemic (i.e. northern latitudes).

2.3. Irradiation to inactivate Trichinella

The ICT considers irradiation, at levels proven to inactivate *Trichinella* (0.3 kGy), to be an acceptable method for rendering meat safe for human consumption in those countries where irradiation of food is permitted. Irradiation is recommended for sealed packaged food only.

2.4. Curing to inactivate Trichinella

Curing and smoking processes are not recommended for control of *Trichinella* in pork, horse or game meats. Although individual validation studies have shown that various combinations of salt, temperature and drying times will inactivate *Trichinella*, curing and smoking methods are difficult to control reliably. Curing should only be used after extensive validation studies and with strict process controls. The ICT recommends that inspected or certified free meats be used in the preparation of cured or smoked products.

2.5. Consumer education

In all areas where methods of *Trichinella* control have not been fully implemented, consumers should be adequately informed by public health authorities of risk and educated

in proper meat preparation methods. Acceptable methods for consumer preparation of meats which may pose a public health risk include:

- Cooking to an internal temperature of 71°C (160°F).
- Freezing solid (−15°C or less) for 3 weeks (cuts up to 15 cm in thickness), and freezing solid (−15°C or less) for 4 weeks (cuts up to 69 cm in thickness). In areas where freeze-resistant *Trichinella* are endemic, consumers should be informed that freezing is not recommended.

Methods for preparation of meats which are not considered secure include:

- Cooking using microwaves,
- Curing, drying, or smoking.

Education of hunters for proper preparation of game meats should follow the same guidelines issued to consumers. Particular caution should be given to presence of freeze-resistant *Trichinella* in game meats.

The ICT strongly cautions against the consumption of raw meat-products (pork, horse, game) under any circumstances.

3. On-farm control

The transmission of *Trichinella* to domestic livestock is limited to a few risks including feeding of raw waste products or animal carcasses and exposure to infected rodents and wildlife. Modern swine production systems reduce or eliminate risks of swine infection with *Trichinella* and testing of individual animals raised under these conditions could be eliminated. There are minimal requirements which need to be met for livestock to be considered *Trichinella*-free based on husbandry.[2] These management requirements are summarized as follows:

3.1. Swine — requirements for Trichinella free pig production

3.1.1. Architectural and environmental barriers

- Pig buildings are constructed to prevent rodents from entering buildings.
- Openings, such as those for air ventilation or water pipes, are covered with wire (1 cm openings or less).
- Areas within 100 m of pig buildings are free from debris and rodent harborage.
- A 2 m perimeter consisting of gravel or vegetation mowed to a height of less than 10 cm is maintained around all pig buildings.

3.1.2. Feed and feed storage

- Feed is maintained in closed silos which do not allow rodents to enter.
- Purchased feed is obtained from an approved facility which produces feed by good production practices.

[2] Where statistically valid surveys have been conducted to clearly show that *Trichinella* prevalence in the highest predator populations (foxes, wolves, cats) is 0.1% or less at a 95% confidence level, biological barriers are not required for *Trichinella*-free pig production. However, all other requirements, including rodent control programs, should remain in place.

- Waste food, containing meat-products is cooked in accordance with waste food laws, and to inactivate *Trichinella*.

3.1.3. Rodent control

- A documented rodent control program is maintained by a recognized pest control provider (Appendix C).
- No evidence indicating the presence of rodents (burrows, tracks, feces) is observed by a recognized pest control provider.

3.1.4. Farm hygiene

- Dead animals are disposed of within 24 h and by sanitary means.No garbage dumps are present within a 2 km radius of the farm.

3.1.5. New animals

- New animals originate from *Trichinella*-free farms, or
- New animals are held in quarantine and are analyzed serologically after 3 weeks to assure the absence of antibodies to *Trichinella spiralis*.

3.2. Requirements for Trichinella-free production of horses

Due to the lack of knowledge concerning the transmission of *Trichinella* to horses and the general husbandry of horses, it is not possible to raise horses guaranteed free from infection. The ICT recommends that epidemiological studies be conducted in countries where horses originate for human consumption.

3.3. Certification of Trichinella-free livestock production

Programs which allow certification of pigs as free from *Trichinella*, based on good management practices which eliminate risk of exposure, should be administratively organized so as to allow proper documentation of certified herds. This administration should perform the following functions:

- Develop a system of documentation of *Trichinella*-free production practices which addresses all the points raised in Section 3.1 above.
- Issue certifications and maintain records of certified farms.
- Periodically, conduct spot audits of certified producers to assure the integrity of the system.
- Conduct periodic serology testing of pigs originating from certified farms to verify absence of infection.

Pigs raised on farms which do not meet *Trichinella*-free production, should be tested individually by approved methods (Section 1.1).

4. Regional freedom from *Trichinella* infection in pigs

The ICT does not endorse any programs (e.g. OIE International Animal Health Code, Article 3.5.3.2) for assuring pigs to be free from *Trichinella* based on region, state or country.

The ICT considers *Trichinella*-free farms to form the basis for building *Trichinella*-free regions.

5. Legislative recommendations

The ICT recommends that all countries make it illegal to distribute *Trichinella* contaminated meat, including pig meat, horse meat and game meats. Furthermore, hunters should be educated and made responsible for the safety of meat which they distribute.

It is strongly recommended that *Trichinella* infection in food animals and appropriate wildlife should be reportable, by country, to national/federal veterinary and public health organizations which in turn report occurrence to the Office Internationale des Epizooties (1999) on an annual basis.

It is recommended that human trichinellosis should be reportable, by country, to national/federal public health organizations.

The ICT recommends that an identification system (individual marking) or registration of pigs and horses be required by country. This identification system must allow reliable trace back of individual animals to their point of origin. Identification will facilitate epidemiological investigations and the implementation of corrective actions.

Appendix A. Pooled sample digestion method for *Trichinella* testing

A.1. Introduction

Digestion of muscle tissue with an acidified pepsin solution releases live *Trichinella* from muscle cysts. Various digestion procedures have been described in the scientific literature (see EEC (1984); Gajadhar et al. (1996); Gamble (1998); Tret'Yakov (1972)). The following discussion outlines steps for a generic protocol which may be used to detect *Trichinella* infection in meat. Any methods for the detection of *Trichinella* in meat should be properly validated prior to use with known positive and negative samples, then monitored for efficacy periodically using proficiency panels (see Appendix B).

A.2. Sample collection

Muscle samples should be collected from sites of predilection for the species being tested. These sites include the diaphragm pillars or tongue of pigs and the tongue or masseter muscle of horses. If *Trichinella* predilection sites are not known for the species to be tested, tongue or diaphragm are recommended.

Sample sizes should be selected to meet the sensitivity needs of the test; individual samples of 100 g may be taken from one animal, or multiple samples may be collected from a number of animals to make a pool of up to 100 g of tissue. The sensitivity of testing has been reported as follows: a 1 g sample will detect infections $\geq$3 LPG of tissue; a 3 g sample will detect infections $\geq$1.5 LPG of tissue; a 5 g sample will detect infections $\geq$1 LPG of tissue. For public health purposes, testing a 1 g sample of pig tissue (diaphragm or tongue)

has been shown to be effective in reducing the incidence of human trichinellosis in several countries. However, where meat is not intended for thorough cooking or other post-slaughter processing, testing of sample sizes sufficient to detect infection levels of 1 LPG of tissue (e.g. a minimum 5 g sample) is recommended.

A.3. Sample preparation

Samples should be trimmed free from all fat and fascia since these tissues are undigestable and do not contain *Trichinella* larvae. Samples are then blended, ground or otherwise macerated to facilitate digestion; blending is the method of choice.

For preparing sample by blending, up to 100 g of tissue is mixed with an equal volume of acidified tap water (i.e. 1% HCl) and subjected to several short (5–10 s) bursts in a Waring or Moulinet-type blender. Too little blending will result in poor digestion, while too much blending could disrupt larvae in muscle. Blending should be continued until no visible pieces of meat remain.

Preparation of sample using a meat grinder is an acceptable method provided the pore size of the grinding plate does not exceed 3 mm in diameter.

A.4. Artificial digestion

Each 100 g of tissue should be digested in a total volume of 2–3 l of an acidified pepsin solution using a validated method. A 1:30 ratio of meat to digest solution (e.g. 100 g of tissue in 3 l of digestion fluid) has been shown to facilitate rapid and complete digestion.

Care should be taken to transfer the sample in its entirety from the blender or meat grinder, into a 3–4 l beaker. Pre-warmed ($45 \pm 2^{\circ}C$) acidified (0.5–1.0% HCl) tap water should be used to thoroughly rinse all parts including blender blades or grinder plates. Then, additional pre-warmed acidified tap water should be added to achieve the appropriate volume (2–3 l).

Pepsin (1:10,000 National Standard Formulary strength) should be added to the sample/acidified tap water mixture at a ratio of 0.5–1.0% weight/volume. In the case of sample preparation by blending, the entire amount of pepsin may be added to the initial blended sample and mixed briefly to ensure even dispersion. The sample/pepsin mixture is then rinsed into a 3–4 l beaker with acidified tap water as described above.

For digestion, the sample/acidified pepsin mixture, 2–3 l volume contained in a 3–4 l beaker, is covered with aluminum foil to prevent splashing, and stirred vigorously on a magnetic stirring plate (using an 8–10 cm bar), or with an alternative stirring device, for a minimum of 30 m (longer times may be necessary to complete digestion).

Temperature during the digestion process should be maintained at $45 \pm 2^{\circ}C$ and monitored closely, using a thermometer or other thermal recording device. Temperature is controlled best by conducting the entire process in an incubator or warm room; however, a heated stirring plate or water bath is an acceptable substitute if the temperature can be controlled within the prescribed limits. Digestion is concluded only when intact pieces of meat are no longer visible in the digestion solution.

A.5. Recovery of larvae

At the conclusion of the digestion process, the entire mixture is poured from the beaker through a sieve (180–355 μm mesh) screen into an appropriate sized (2–4 l) separatory funnel, aided by a plastic funnel. The beaker, and sieve, should be rinsed with an additional volume (minimum of 100 ml) of warm tap water. No intact pieces of meat should be seen on the sieve. If so, they must be returned to fresh digestion fluid for further processing.

The digest is allowed to settle in the separatory funnel for 30 min. Several options may then be used for clarification of sample. A volume of 40 ml of fluid can be drained from the funnel directly into a 50 ml centrifuge tube. The contents of this tube are allowed to settle for an additional 10 min, after which all but 10 ml is aspirated from the top of the digest. If the remaining 10 ml appears cloudy, an additional 30 ml of warm (37°C) tap water should be added to the sediment and the settling and aspiration processes repeated until the sample appears clear. The final, clarified 10 ml is used to examine for the presence of *Trichinella* larvae.

An alternative for sample clarification is the use of a second separatory funnel step Gajadhar et al. (1996). In this procedure, approximately 125 ml of the fluid from the first separatory funnel are drained into a 500 ml separatory funnel and the volume is adjusted to 500 ml with tap water at room temperature. This mixture is allowed to settle for an additional 10 min, after which a sample of 22–27 ml is recovered for counting.

In both procedures, it is critical that fluid be recovered from separatory funnels by opening the stopcock completely. Partial opening can result in retaining worms in the separatory funnel.

A.6. Enumeration of larvae

For enumeration purposes, the clarified sediment is poured into a gridded Petri dish and examined for *Trichinella* larvae with a dissecting microscope (15–40× magnification). The fluid must be clear enough so that newsprint can be read through the fluid. If not, then further clarification and settling are required.

When larvae are detected in pooled sample digests, the entire procedure must be repeated using smaller pools or on individual samples comprising the pool until the source carcass is identified.

Appendix B. Quality assurance program for *Trichinella* testing

B.1. Introduction

Whereas the accuracy of a microbial detection method depends on consistency in sensitivity and specificity, reliability of the method requires the use of an adequate quality assurance program. A quality assurance program provides confidence that the method employed is always performed under defined conditions by competent analysts, and that the results are repeatable and dependable according to a predetermined level of sensitivity and specificity. Quality assurance programs should be used when testing for microbial hazards to ensure public health and to facilitate equivalency for international trade. The ISO Guide

25 specifies details which can be used in the development of a quality assurance program for a diagnostic test or detection method. The Guide was used to develop the following guidelines for a quality assurance program for laboratories testing meat for *Trichinella*:

B.2. Quality assurance manual

A quality assurance system based on ISO Guide 25, or a similar internationally accepted quality standard, is required to document that properly trained analysts are performing the method under controlled conditions, thereby producing reliable and consistent results. The quality assurance system should be described in a quality assurance manual or similar document which provides information on the organization's structure and describes staff qualifications, training requirements, mechanisms for monitoring adherence to written protocols, criteria for certification of analysts, equipment maintenance, reporting, record keeping, handling deviations, corrective actions, handling of complaints, documentation and audits. Other items appropriate for a QA manual for *Trichinella* testing include sampling procedures in abattoirs, sample and animal identification, trace back procedures to carcass of origin and sample acceptance/rejection criteria.

B.3. Appropriate laboratory facilities

An appropriate laboratory facility provides a controlled environment for testing and ensures the health and safety of persons working in the laboratory. At least one door should be used to separate common areas from the laboratory which must have adequate bench space, adequate lighting, hot and cold running water, a sink suitable for the glassware used in the procedure, surfaces impervious to common disinfectants, a fume hood, adequate ventilation, heating and cooling system capable of maintaining a comfortable working temperature, appropriate signage, a pest control program if necessary, and immediate access to an emergency shower and a first aid kit, staff washrooms, and appropriate laboratory wear (gloves, safety glasses and lab coats).

B.4. Validated procedure

The accuracy of the detection method must be defined through data obtained from beta testing. The sensitivity and specificity of the method must be known and must be supported by scientifically derived and statistically sound data obtained from utilizing samples obtained from known infected and non-infected animals. Comparison of new methods with existing methods not previously validated in this fashion is not an acceptable validation process. Adequate precision of the method (repeatability) must be clearly defined and then scientifically demonstrated.

B.5. Standardized protocol

A standardized protocol, in conjunction with adequate training, is necessary to ensure that accurate and repeatable results can be achieved by any laboratory performing the detection

method. A protocol for the validated method must be clearly written, and include a detailed description of all necessary equipment, reagents and procedures. The protocol must be performed exactly as written, and must include critical control points (CCPs). CCPs are defined as those procedures, equipment or reagents which could adversely affect the results of the detection method, if not used exactly as stated in the protocol. Any changes made to a standardized protocol must be supported by statistically valid parallel testing to ensure that results are not adversely affected.

B.6. Training and certification of analysts

Adequate training, in conjunction with a standardized protocol, is necessary to ensure that accurate and repeatable results can be achieved by any laboratory performing the detection method. A documented training program for analysts must be in place. This program must cover all aspects of the method including the procedure, pre- and post-testing requirements, proficiency panel analysis, responsibilities, reporting, biology of the organism and safety. The training should be provided by qualified persons in a laboratory with adequate facilities and the analysts undergoing training must demonstrate competency by written examination and successful testing of unknown samples during the training period and again at their home laboratory.

B.7. Proficiency sample program for certified analysts

To demonstrate continued proficiency, certified analysts must maintain their certification by testing a set of unknown samples prepared by a reference laboratory or by participating in a proficiency sample exchange program four times per year. A standardized protocol should be used to prepare and distribute samples. Parallel testing of a subset of each proficiency sample lot is conducted by the provider laboratory at the same time participating laboratories test their proficiency samples. Guidelines for the evaluation of proficiency sample results are based on expected method performance supported by scientifically derived data using the proficiency samples and the method used. Analysts successfully completing their proficiency samples maintain their certification. Failure may require re-testing of a second set of proficiency samples, de-certification, re-training or a combination thereof, and clearly defined rules for making these decision need to be established and documented in advance. This sample proficiency program can be adapted for use in implementing and/or validating a new method, by ring testing among a group of qualified laboratories.

Appendix C. Methods for the inactivation of *Trichinella spiralis* larvae in pork by heating

1. All parts of the pork muscle tissue shall be heated according to one of the time and temperature combinations in Table 1.
2. Time and temperature shall be monitored by a calibrated recording instrument.

Table 1

Minimum internal temperature		Minimum time (h)
(°F)	(°C)	
120	49.0	21 h
122	50.0	9.5 h
124	51.1	4.5 h
126	52.2	2 h
128	53.4	1 h
130	54.5	30 min
132	55.6	15 min
134	56.7	6 min
136	57.8	3 min
138	58.9	2 min
140	60.0	1 min
142	61.1	1 min
144	62.2	Instant

3. The time to raise product temperature from 60 to 120°F shall not exceed 2 h unless the product is cured or fermented.
4. Time, in combination with temperatures of 138–143°F, need not be monitored if the product's minimum thickness exceeds 2 in. (5.1 cm) and refrigeration of the product does not begin within 5 min of attaining 138°F (58.9°C).
5. The establishment shall use procedures which ensure the proper heating of all parts of the product. It is important that each piece of sausage, each ham, and other products treated by heating in water be kept entirely submerged throughout the heating period; and that the largest pieces in a lot, the innermost links of bunched sausage or other massed articles, and pieces placed in the coolest part of a heating cabinet or compartment or vat be included in the temperature tests.

Appendix D. Methods for the inactivation of *Trichinella spiralis* larvae in pork by freezing

At any stage of preparation and after preparatory chilling to a temperature of not above 40°F. or preparatory freezing, all parts of the muscle tissue of pork or product containing such tissue shall be subjected continuously to a temperature not higher than one of those specified in Table 2, the duration of such refrigeration at the specified temperature being dependent on the thickness of the meat or inside dimensions of the container.

1. Group 1 comprises product in separate pieces not exceeding 6 in. in thickness, or arranged on separate racks with the layers not exceeding 6 in. in depth, or stored in crates or boxes not exceeding 6 in. in depth or stored as solidly frozen blocks not exceeding 6 in. in thickness.
2. Group 2 comprises product in pieces, layers, or within containers, the thickness of which exceeds 6 in. but not 27 in., and product in containers including tierces, barrels, kegs, and cartons having a thickness not exceeding 27 in.

Table 2
Required period of freezing at temperature indicated

Temperature (°F/°C)	Group 1 (Days)	Group 2 (Days)
5/−15	20	30
−10/−23	10	20
−20/−29	6	12

Table 3
Alternate periods of freezing at temperatures indicated

Minimum internal temperature		Minimum time
(°F)	(°C)	
0	−17.8	106 h
−5	−20.6	82 h
−10	−23.3	63 h
−15	−26.1	48 h
−20	−28.9	35 h
−25	−31.7	22 min
−30	−34.5	8 h
−35	−37.2	30 min

3. The product undergoing such refrigeration or the containers thereof shall be so spaced while in the freezer as will insure a free circulation of air between the pieces of meat, layers, blocks, boxes, barrels, and tierces in order that the temperature of the meat throughout will be promptly reduced to not higher than 5, −10, or −20°F, as the case may be.
4. In lieu of the methods prescribed in Table 2, the treatment may consist of commercial freeze drying or controlled freezing, at the center of the meat pieces, in accordance with the times and temperatures specified in Table 3.
5. The rooms or compartments containing product undergoing freezing shall be equipped with accurate thermometers placed at or above the highest level at which the product undergoing treatment is stored and away from refrigerating coils.

Appendix E. Species/genotype identification of *Trichinella* larvae

Knowledge of the species or genotype of *Trichinella* found in domestic or wild animals is often useful for understanding the epidemiology or the parasite as well as the risk posed to humans. Knowledge of the species or genotype of *Trichinella* causing human disease is useful in determining treatment strategies. Speciation/genotyping is performed by the International *Trichinella* Reference Centre in Rome, Italy. Samples may be submitted for speciation/genotyping using the following guidelines:

E.1. Forwarding of fresh meat

Muscle sample(s) from domestic or game animals found to contain *Trichinella* larvae, or from human biopsies, should be packaged in several plastic bags, preferably under vacuum or in plastic screw cap vials sealed with parafilm. Samples should be packaged in styrofoam boxes for insulation and sent by an international carrier to the address listed below.

E.2. Forwarding of frozen meat

Frozen samples should be sent under dry ice in styrofoam containers to ensure that tissue does not thaw during shipment. Samples should be packaged in styrofoam boxes for insulation, and sent by an international carrier to the address listed below.

E.3. Forwarding of larvae in ethyl alcohol

Muscle larvae collected by artificial digestion should be washed three times in distilled water and preserved in absolute ethyl alcohol in small conical plastic vials. Only motile/viable muscle larvae should be preserved. Vials should be sealed in parafilm, packaged to avoid leakage or breakage, and sent by an international carrier to the address listed below.

Samples should be sent to:

International *Trichinella* Reference Centre, Laboratory of Parasitology, Istituto Superiore di Sanita viale Regina Elena 299, 00161 Rome, Italy. Tel.: +39-06-4990-2304; fax: +39-06-4938-7065; e.mail: pozio@iss.it.

An import permit is not necessary. Packages should be labeled “biological material without any commercial value”.

References

Code of Federal Regulations, 1990. Animals and Animal Products, Office of the Federal Register, Government Printing Office, Washington, DC. Vol. 9, pp. 212–220.

European Economic Community, 1977. Commission Directive 77/96/EEC. Off. J. Eur. Comm. 26, 67–77.

European Economic Community, 1984. Commission Directive 84/319/EEC. Off. J. Eur. Comm. 167, 34–43.

Forbes, L.B., Rajic, A., Gajadhar, A.A., 1998. Proficiency samples for quality assurance in *Trichinella* digestion tests. J. Food Prot. 61, 1396–1399.

Office Internationale des Epizooties, 1999. International Animal Health Code. 8th Edition, Office Internationale des Epizooties, Paris.

Gajadhar, A.A., Forbes, L.B., Rajic, A., 1996. The double separatory funnel procedure for the detection of *Trichinella* larvae in pork. Official Protocol, Agriculture and Agri-Food Canada, Version 1.0, 14 November 1996, 18 pp.

Gamble, H.R., 1998. Trichinellosis. In: OIE Manual of Standards for Diagnostic Tests and Vaccines, pp. 477–480 (Chapter 3.5.3).

Tret’Yakov, A.D. (Ed.), 1972. Veterinary Code of the USSR: Provisions, Guidelines, Instructions, Directions and Rules on Veterinary Matters, Vol. II, Kolos, Moscow, 1972.