

# **International Commission on Trichinellosis**

## ***Recommendations on Methods for the Control of Trichinella in Domestic and Wild Animals Intended for Human Consumption***

### ***ICT Standards for Control Guidelines Committee***

This document provides a uniform set of recommendations for the control of *Trichinella* at all levels (on the farm, at slaughter, and for processing meats). These recommendations are based on the best scientific information available and represent the official position of the International Commission on Trichinellosis (ICT) regarding acceptable control methods. These recommendations were first published in 2000 by an ICT Committee that included the following ICT members: A. S. Bessonov (Russia), K. Cuperlovic (Serbia), A. A. Gajadhar (Canada), H. R. Gamble (U.S.A.), F. van Knapen (Netherlands), K. Noeckler (Germany), H. Schenone (Chile), and X. Zhu (China). A revised version of these guidelines was prepared in 2006 based on new scientific information. This revision was prepared by P. Boireau (France), F. Bruschi (Italy), H. R. Gamble (U.S.A.), A.A. Gajadhar (Canada), and K. Noecker (Germany) and was approved by the ICT Executive Committee.

#### ***Part 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 sample digestion using a minimum of 1 gram per sample (for testing of pork products) are generally sufficient to detect infection levels which would cause clinical trichinellosis in humans. For products, which are eaten without cooking or other treatment to inactivate *Trichinella*, more intensive inspection is recommended to prevent human disease. Trichinoscopic examination fails to detect non-encapsulated *Trichinella* species infecting domestic and wild animals and therefore trichinoscopy and similar compression methods are not recommended for the routine examination of food animals and game meats intended for human consumption.

#### **1.1 - Slaughter Testing of Swine**

For routine examination of pork carcasses using pooled sample digestion testing, a minimum of 1 gram of muscle tissue and preferably 5 grams of muscle tissue is recommended for prevention of human disease. A description of the magnetic stirrer pooled sample digestion method can be found in Appendix I of this document. Other descriptions of pooled sample digestion methods can be found in the OIE Manual of Standards for Diagnostic Tests and Vaccines (Article 2.2.9) and European Union Commission Regulation No. 2075/2005 (Ch 1 of Annex 1). Any of the described methods for pooled sample digestion testing should be performed in combination with a Quality Assurance system as described in Parts 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 and the reliability of the results. 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 gram or greater size originate from the appropriate number of pigs and that samples are clearly identified back to the pig.
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, storage of the 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  $45 \pm 2^\circ \text{C}$ . Higher temperatures will result in the inactivation of pepsin, incomplete digestion, destruction of larvae, and poor recovery rates.
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 increasing digestion times, and, if this is not effective, verifying the quality of the pepsin.
5. Sedimentation procedures and times should be adjusted to maximize recovery of larvae. Existing methods employing sedimentation times of 30 minutes are sufficient. Shortening the recommended times will result in reduced recovery rates and the potential for false negative results. Recovery of sediment from separatory funnels must include complete opening of the stopcock to avoid larval retention.
6. The sediment of digested 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 containing the digest sediment. 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 X. In addition, regular microscope maintenance is required to assure the quality of the optical system.
8. Carcasses should not leave the premises until digests have been found to be negative for *Trichinella* larvae. This system is necessary to assure that positive carcasses are not distributed for human consumption.

9. Records should be kept which assure the accurate identification of samples and carcasses.

### **1.2 - Slaughter Testing of Horses**

Due to the habits of eating horsemeat without substantial cooking, and given the history of human trichinellosis resulting from eating horsemeat, additional testing requirements are indicated when testing horse carcasses for *Trichinella* infestation.

The ICT recommends that a minimum of 5 grams of tissue from the tongue or masseter of horses should be tested using a pooled sample digestion method. When horsemeat is to be consumed raw, testing of a 10 gram sample is preferred. Diaphragm (crus muscle or *Crura diaphragmatica*) is an alternative site for sampling if masseter or tongue is not available. The example of pooled sample digestion testing provided in Appendix I 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 horsemeat. Particular attention should be paid to clarification of digests and the interference of intact muscle fibers or debris in visualizing sediments, particularly if tongue is used in the digestion. Due to the history of horse meat related outbreaks of human trichinellosis, it is recommended that all countries which export horse meat for human consumption use adequate quality assurance measures within their slaughter inspection programs (see Parts 1.5 and 1.6).

### **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 (from carnivores or omnivores) 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 and all other game meats should include tongue.
- Samples used for testing crocodile should include masseter, pterygoid and intercostal muscles.

For testing the species described above, a minimum of 10 grams of muscle tissue should be tested by artificial digestion. 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 I). 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 Part 1.1)

#### **1.4 - Recommended Actions When a Positive Test Result is Obtained**

The following actions are recommended when detecting a positive sample at slaughter testing:

1. Maintain established procedures that will allow the positive carcass to be accurately identified. Verification should be performed using a larger amount of tissue for digestion. Parasites recovered from domestic swine, horses, or game animals should be submitted to a national reference laboratory or may be submitted to the International Trichinellosis Reference Centre, Rome, Italy<sup>1</sup> for species identification.
2. Positive carcasses should be rendered using an officially permitted procedure that is known to kill *Trichinella*.
3. For positive swine, a detailed plan (Standard Operating Procedure) 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 World Organization for Animal Health/Office Internationale des Epizooties (OIE).

#### **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 system for testing.

Quality assurance measures are necessary to ensure that testing processes are working properly and

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results are reliable. In order to do this, it is necessary to provide analysts with proficiency samples from which muscle larvae can be recovered. Proficiency samples should be administered regularly (2-4 times/year) and be under the supervision of an external authority, such as a national reference laboratory. Samples used in proficiency testing should be appropriate for the test method used (e.g., 1.0 gram samples for pooled sample digestion testing of pork). Proficiency testing evaluates the integrity of the inspection system and also the ability of technical personnel to accurately visualize *Trichinella* larvae. An example of a quality control system for digestion testing is given in Appendix II.

## 1.6 - Validation of Testing Systems

The ICT recommends that all post-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 food safety and trade.

New methods should be evaluated in a minimum of three (3) laboratories with experience in the methods of testing. Validation should include performance of 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)<sup>2</sup> or Vallee et al. (2007)<sup>3</sup>. Any test that is intended to protect consumers from human disease resulting from infection with *Trichinella* should detect samples containing as low as 1 larva per gram of muscle tissue at a 95% confidence interval.

## 1.7 - Alternative Testing Systems

Indirect (serological) testing methods are **not** recommended as a substitute for direct (pooled sample digestion) testing of individual carcasses at slaughter. Improvements in indirect testing methods should be evaluated against currently used digestion methods for sensitivity and specificity; the magnetic stirrer method is currently considered the “gold standard”.

PCR assay is also unacceptable as an alternative detection method because of the limitation of sample size; a small tissue sample from a positive pig may not contain larvae and thus the PCR would yield false-negative results.

The ICT has issued guidelines for the use of serological methods in epidemiological studies, surveillance, and certification/verification programs. These guidelines are published in the scientific literature (Gamble

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<sup>2</sup> Forbes, L.B., Rajic, A. & Gajadhar, A.A. 1998. Proficiency samples for quality assurance in *Trichinella* digestion tests. *Journal of Food Protection*, 61: 1396-1399.

<sup>3</sup> Vallee, I., Mace, P., Scandrett, B., Gajadhar, A. & Boireau, P. 2007. Use of proficiency samples to assess diagnostic laboratories in France performing a *Trichinella* digestion assay. *Journal of Food Protection*, 70: 1685-1690.

et al., 2004)<sup>4</sup> and are available on the ICT web site (<http://www.med.unipi.it/ict/Recomm.htm>). The OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals also contains guidelines for the preparation of reagents and performance of ELISA for detection of *Trichinella* in pigs.

## **Part 2 - Processing Methods to Control Trichinellosis**

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

The ICT recognizes three (3) acceptable means of treatment, which can be used to render meat safe, if not otherwise proven to be free from *Trichinella* infection. These treatment methods include cooking, freezing (for some host species), and irradiation.

### **2.1 - Cooking to Inactivate *Trichinella* larvae**

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 (Appendix III) 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 gray throughout, and a change in texture such that muscle fibers are easily separated from each other are indicators that meat has been adequately cooked to kill any *Trichinella* larvae present. (As a precautionary note, processing of meat under loosely controlled conditions creates opportunities for error. Color change is only a general indicator of safety.)

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<sup>4</sup>Gamble H.R., Pozio E., Bruschi F., Nöckler K., Kapel C.M.O. & Gajadhar A.A. 2004. International Commission on Trichinellosis: Recommendations on the use of serological tests for the detection of *Trichinella* infection in animals and man. Parasite, 11: 3-13.

## 2.2 - Freezing to Inactivate *Trichinella* larvae

When the proper equipment is available for accurately achieving and monitoring time and temperature combinations, the conditions set forth for commercial freezing methods in the United States Department of Agriculture's Code of Federal Regulations (9CFR318.10), Commission Regulation No. 2075/2005 of the European Union, and Appendix IV of this guideline are acceptable for treatment of pork to prevent human trichinellosis due to freeze-sensitive *Trichinella* spp. In the absence of proper temperature and time control and monitoring systems, freezing may not eliminate risk of *Trichinella* in pork, particularly in areas where pigs may be exposed to sylvatic species such as *T. britovi*.

Species of *Trichinella* occurring in game meats (e.g., bear, wild boar) and horses may be resistant to freezing using the time and temperature combinations described in Appendix IV. For this reason, freezing is not an acceptable method for control of *Trichinella* in these animals.

## 2.3 - Irradiation to Inactivate *Trichinella* larvae

The ICT considers irradiation, at levels proven to inactivate *Trichinella* larvae (0.3 kGy)<sup>5</sup>, 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* larvae

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* larvae, 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 *Trichinella*-free meats be used in the preparation of cured or smoked products.

## 2.5 - Consumer Education

In all areas where adequate methods of *Trichinella* control have not been fully implemented and regulated, 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

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<sup>5</sup> U. S. Code of Federal Regulations (1997). Irradiation in the Production, Processing and Handling of Food, 21 CFR Part 179.

reduce the public health risk, include:

- Cooking to an internal temperature of 71°C (160° F)
- Freezing solid (-15°C or less) for 20 days (cuts up to 15 cm in thickness), and freezing solid (-15°C or less) for 30 days (cuts up to 69 cm in thickness)<sup>6</sup>

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 the potential for the presence of freeze resistant *Trichinella* in game meats.

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

### **Part 3 - On-Farm Control**

The transmission of *Trichinella* to domestic livestock is limited to certain 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 qualified animals raised under these conditions could be eliminated. There are minimal requirements that need to be met for livestock to be considered *Trichinella* free, based on husbandry. These management requirements are summarized as follows:

#### **3.1.1 - Swine - Requirements for *Trichinella* free pig production**

##### Architectural and environmental barriers

- Pigs are reared exclusively in buildings constructed to prevent rodents, other mammals, and large carnivorous birds from entering.
- Openings to the building, such as those for air ventilation or water pipes are covered with wire (1 cm openings or less).
- Areas within 100 meters of pig buildings are free from debris and rodent harborage.

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<sup>6</sup> In areas where freeze resistant *Trichinella* are endemic, consumers should be informed that freezing is not recommended.

- A 2 meter perimeter consisting of gravel or vegetation mowed to a height of less than 10 cm is maintained around all pig buildings.

#### 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 (for example, see Regulation [EC] No 1831/2003 of the European Union).
- Waste food, containing meat products is cooked in accordance with waste food laws, and to inactivate *Trichinella* larvae.

#### Rodent control

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

#### Farm hygiene

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

#### New animals

- New animals originate from certified *Trichinella*-free farms, or unweaned piglets under 6 weeks of age.
- New animals, originating from farms that are not certified *Trichinella*-free, are held in quarantine and are analyzed serologically after four weeks to assure the absence of antibodies to *Trichinella*. Serological testing should be performed using an ICT recommended method as described in Part 1.7

#### Animal identification

- Pigs are identified in such a way that they can be traced to their origin.

### 3.1.2 - Certification of *Trichinella* free pig production

Programs, which allow certification of pigs as free from *Trichinella*, based on good management practices that 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 such as on-site audits of *Trichinella*-free production practices that addresses all of the points raised in Part 3.1.1 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, and/or conduct periodic risk-based monitoring programs of wildlife. Examples of certification programs may be found in Commission Regulation (EC) No 2075/2005 of the European Union and the National Trichinae Certification program Standards of the U.S. Department of Agriculture ([www.aphis.usda.gov/vs/trichinae/](http://www.aphis.usda.gov/vs/trichinae/)).

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

### 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, particularly in areas where *Trichinella* is highly endemic. Horses slaughtered for human consumption should be subjected to individual carcass testing in accordance with methods described in Part 1.2.

### Part 4 - Regional Freedom from *Trichinella* Infection in Pigs

The ICT does not endorse any programs (e.g., OIE Terrestrial Animal Health Code, Article 2.2.9.3) 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 derogation from individual carcass inspection for *Trichinella*.

## Part 5 - Legislative Recommendations

The ICT recommends that all countries establish and actively enforce legislation that makes it illegal to distribute *Trichinella* contaminated meat, including pig meat, horse meat and game meats. Commercial distributors should be required to perform parasitological control on these species that are intended for human consumption.

Hunters should be educated, and made responsible, for the safety of game meats which they distribute to others. Game meats that are transported across borders should be subject to control at customs to prevent transport of *Trichinella* infected meat.

The ICT recommends that *Trichinella* infection in food animals and game meats should be reportable, by country, to national/federal veterinary and public health organizations, which in turn report occurrence to the OIE.

The ICT recommends 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 designated as food animals 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. Special attention should be paid to the possibility of *Trichinella* infection in livestock and game animals that are transported across borders.

**Appendices**

**Appendix I** – Pooled sample digestion method for *Trichinella* testing.

**Appendix II** - Components of a quality assurance program.

**Appendix III** - Methods for the inactivation of *Trichinella spiralis* in pork by heating.

**Appendix IV** - Methods for the inactivation of *Trichinella* in pork by commercial freezing.

## Appendix I. Pooled Sample Digestion Method for *Trichinella* Testing

### 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 bibliography at the end of this appendix). The following discussion outlines steps for a generic protocol, which may be used to detect *Trichinella* infection in meat. Any method 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 II).

### 2. Sample collection

Muscle samples should be collected from sites of predilection for the species being tested (Table 1). If *Trichinella* predilection sites are not known for the species to be tested, tongue or diaphragm are recommended.

**Table 1.** Elective muscle sample according to the host species tested.

Host species	Main elective muscles	Weight to be tested	References
Pig	Diaphragm, masseter, tongue	1 to 2 g	Olsen et al, 1964
Horse	Diaphragm, masseter, tongue	at least 5g	Soule et al., 1989
Wild boar	Diaphragm, masseter, tongue, intercostal	5g	Kapel, 2001
Wild carnivores	Leg muscles	10g	Kapel, 1993
Bear	Diaphragm, masseter, tongue,	10g	
Marine mammals (seal, walrus)	diaphragm, intercostal and rear flipper muscles	10g	Kapel et al., 2003
Crocodile	Masseter, pterygoid, intercostal	10g	Pozio et al., 2004

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 grams of tissue. The sensitivity of testing has been reported as follows: a 1 gram sample will detect infections  $\geq 3$  larvae per gram of tissue; a 3 gram sample will detect infections  $\geq 1.5$  larvae per gram of tissue; a 5 gram sample will detect infections  $\geq 1$  larva per gram of tissue. For public health purposes, testing a 1 gram 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 greater amounts of meat is recommended.

### 3. Sample preparation

Samples should be trimmed free from all fat and fascia since these tissues are undigestible 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 grams of tissue is mixed with an equal volume of tap water and subjected to several short (5-10 second) bursts in a Waring or similar blender (e.g., Retsch Grindomix GM 200). 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.

### 4. Artificial digestion

Each 100 g of tissue should be digested in a total volume of 2-3 liters of an acidified pepsin solution using a validated method. A 1:30 ratio of meat to digest solution 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 liter beaker. Pre-warmed ( $45 \pm 2^\circ$  C) acidified (0.5 - 1.0% HCl or 0.06N) 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 bring the volume to 2-3 liters.

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 liter with acidified tap water as described above.

For digestion, the sample/acidified pepsin mixture, 2-3 liter volume contained in a 3-4 liter 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 minutes (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.

## 5. Recovery of larvae

At the conclusion of the digestion process, the entire mixture is poured from the beaker through a sieve (180 - 355  $\mu\text{m}$  mesh) screen into a 2-4 liter 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 minutes. 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 minutes, after which all but 10 ml are 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 sediment 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. 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 minutes, 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.

## 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 - 40X 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 in order to identify the infected animal(s).

## 7. Bibliography

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## Appendix II. Quality Assurance Program for *Trichinella* Testing

### 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. A comprehensive quality assurance program should be in place when testing for microbial hazards to ensure public health and to facilitate equivalency for international trade. The ISO/IEC 17025 standards specify details which can be used in the development of a quality assurance program for *Trichinella* testing (see Gajadhar and Forbes, 2002)<sup>7</sup>. ISO/IEC 17025 was used to develop the following guidelines for a quality assurance program for laboratories testing meat for *Trichinella*:

### 2. Quality Assurance Manual

A quality assurance system based on ISO/IEC 17025, or a similar internationally accepted quality standard, is required to document that properly trained and certified 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.

### 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 (BSL-2). 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).

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<sup>7</sup> Gajadhar, A.A. & Forbes L.B. 2002. An internationally recognized quality assurance system for diagnostic parasitology in animal health and food safety, with example data on trichinellosis. *Veterinary Parasitology* 103: 133-140.

#### **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.

#### **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.

#### **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, safety, and impact on public health. The training should be provided by qualified persons in a laboratory with adequate facilities (e.g., a national reference laboratory), 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.

#### **7. Proficiency Sample Program for Certified Analysts/Laboratories**

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 at least 2 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 method being evaluated.

Analysts/laboratories successfully completing their proficiency samples maintain their certification. Failure may require retesting of a second set of proficiency samples, de-certification, retraining or a

combination thereof, and clearly defined rules for making this 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 III.** Methods for the inactivation of *Trichinella spiralis* in pork by commercial heating

(i) All parts of the pork muscle tissue shall be heated according to one of the time and temperature combinations in the following table:

Minimum internal temperature		Minimum time
Degrees Fahrenheit	Degrees Centigrade	
120	49.0	21 hours
122	50.0	9.5 hours
124	51.1	4.5 hours
126	52.2	2 hours
128	53.4	1 hour
130	54.5	30 minutes
132	55.6	15 minutes
134	56.7	6 minutes
136	57.8	3 minutes
138	58.9	2 minutes
140	60.0	1 minute
142	61.1	1 minute
144	62.2	Instant

(ii) Time and temperature shall be monitored by a calibrated recording instrument.

(iii) The time to raise product temperature from 60° F to 120° F shall not exceed 2 hours unless the product is cured or fermented.

(iv) Time, in combination with temperatures of 138° F to 143° F, need not be monitored if the product's minimum thickness exceeds 2 inches (5.1 cm) and refrigeration of the product does not begin within 5 minutes of attaining 138° F (58.9° C).

(v) The establishment shall use procedures, which insure the proper heating of all parts of the product. It is important that each piece of sausage, each ham, and other product 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 IV.** Methods for the inactivation of *Trichinella* in pork by commercial freezing<sup>8</sup>

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 1, the duration of such refrigeration at the specified temperature being dependent on the thickness of the meat or inside dimensions of the container.

**TABLE 1-REQUIRED PERIOD OF FREEZING AT TEMPERATURE INDICATED**

Temperature °F (°C)	Group 1 (Days)	Group 2 (Days)
5 (-15.0)	20	30
-10 (-23.3)	10	20
-20 (-28.9)	6	12

(i) Group 1 comprises product in separate pieces not exceeding 6 inches (15 cm) in thickness, or arranged on separate racks with the layers not exceeding 6 inches (15 cm) in depth, or stored in crates or boxes not exceeding 6 inches (15 cm) in depth or stored as solidly frozen blocks not exceeding 6 inches (15 cm) in thickness.

(ii) Group 2 comprises product in pieces, layers, or within containers, the thickness of which exceeds 6 inches (15 cm) but not 27 inches (69 cm), and product in containers including tierces, barrels, kegs, and cartons having a thickness not exceeding 27 inches (69 cm).

(iii) 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° F., - 10° F, or - 20° F, as the case may be.

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<sup>8</sup> Commercial freezing of pork products is not suitable for inactivation of *Trichinella* species such as *T. nativa* that are cold tolerant. However, cold tolerant species and genotypes of *Trichinella* have low infectivity for domestic pigs and therefore pose little risk to humans (Pozio, E., Kapel, C.M.O., Gajadhar, A.A., Boireau, P., Dupouy-Camet, J. & Gamble, H.R. 2006. *Trichinella* in pork: current knowledge on the suitability of freezing as a public health measure. Eurosurveillance, 11: 277-278).

**TABLE 2-ALTERNATE PERIODS OF FREEZING AT TEMPERATURES INDICATED**

Minimum internal temperature		Minimum time
Degrees Fahrenheit	Degrees Centigrade	
0	-17.8	106 hours
-5	-20.6	82 hours
-10	-23.3	63 hours
-15	-26.1	48 hours
-20	-28.9	35 hours
-25	-31.7	22 hours
-30	-34.5	8 hours
-35	-37.2	1 / 2 hour

(iv) In lieu of the methods prescribed in Table 1, 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 2.

(v) 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.