International Commission on Trichinellosis (ICT)

Recommendations for Quality Assurance in Digestion Testing Programs for Trichinella

ICT Quality Assurance Committee (see Appendix 1)

Part 4

Recommendations for training and qualifying analysts to perform the *Trichinella* digestion assay

Contents

A. Training to Qualify Analysts for Testing

All personnel performing any aspect of the artificial digestion assay for *Trichinella* for regulatory or food safety purposes should be trained to meet a set of minimum requirements. Training should take into account all quality assurance measures in regulatory testing as

described in Part 1 of these recommendations, and should be performed in a laboratory which meets the quality assurance standards as described in Part 5 of this series. The requirements for training as described here should follow the quality assurance recommendations of test performance as described in Part 2, and incorporate the use of proficiency samples and periodic proficiency testing as described in Part 3. Training should be provided by qualified personnel and should be conducted in a bio-secure facility, as determined appropriate by the competent authority.

The following minimal elements and necessary resources are required for training laboratory analysts to perform the digestion assay for *Trichinella*. Actual training should be consistent with requirements of national legislation and the national competent authority.

A1 Training Elements

1. An introduction given to analysts should include the historical setting and objectives of training including the rationale for using the technology (pooled sample digestion testing) and the importance of the results of testing as they affect public health, trade and the economy. Relevant legislation, policies, guidelines and recommendations should be discussed. The potential consequences of a false negative result should be reviewed in light of public health consequences and the impact on the producers, packers and competent authorities. See Table 1 for additional details.

2. Analysts should be provided with an overview of *Trichinella* including biology, epidemiology, control measures and public health implications of *Trichinella* infection in food animals and game meats. Emphasis should be placed on factors that will motivate analysts to understand the importance of their testing work. This includes: a description of the disease resulting from exposure to *Trichinella*, citing recent human cases; the social and economic impact (cost of testing, cost of outbreaks) of testing; and, the potential consequences of missing a positive carcass. See Table 1 for additional details. The following are useful references for this part of the training:

   Murrell, K.D., and Pozio, E. 2011. Worldwide occurrence and impact of human trichinellosis, 1986-2009. Emerging Infectious Diseases 17:2194-202.

   Gamble, H.R., Boireau, P., Noeckler, K. and Kapel, C.M.O. 2007. Prevention of *Trichinella* infection in the domestic pig. In, (Dupouy-Camet, J and Murrell, K.D. eds.), FAO/WHO/OIE Guidelines for the Surveillance, Management, Prevention and Control of Trichinellosis, Paris, pp. 99-108.

   Gottstein, B., Pozio, E. and Nöckler, K. 2009. Epidemiology, Diagnosis, Treatment, and Control of Trichinellosis. Clinical Microbiology Reviews, Jan. 2009, p. 127–145.

   Pozio, E. 2007. Taxonomy, biology and epidemiology of *Trichinella* parasites. In, (Dupouy-Camet, J and Murrell, K.D. eds.), FAO/WHO/OIE Guidelines for the Surveillance, Management, Prevention and Control of Trichinellosis, Paris, pp. 1-35

   Vet Parasitology (Special Issue) 2000. Trichinella and Trichinellosis. Vol. 93: 181-412.

3. Analysts should be given an overview of control programs and processes to prevent human exposure to *Trichinella* in meat from pigs and other food animals. In this section analysts should be provided with the theory of testing for *Trichinella*, focusing on the digestion

method. Analysts should be informed of the requirements of qualifying and retaining qualification to test for purposes of food safety. See Table 1 for additional details. The following are useful references for this part of the training:

Gajadhar, A.A., Pozio, E., Gamble, H.R., Nöckler, K., Maddox-Hyttel, C. Forbes, L.B., Vallée, I., Rossi, P., Marinculić, A. and Boireau, P. 2009. *Trichinella* diagnostics and control: Mandatory and best practices for ensuring food safety. *Veterinary Parasitology*, 159: 197-2O5.

Nöckler, K. and C.M.O. Kapel. 2007. Detection and surveillance for Trichinella: Meat inspection, hygiene and legislation. In, (Dupouy-Camet, J and Murrell, K.D. eds.), FAO/WHO/OIE Guidelines for the Surveillance, Management, Prevention and Control of Trichinellosis, Rome, pp. 69-974
World Organization for Animal Health (OIE), 2011. Trichinellosis. *In*, Terrestrial Animal Health Code Ch 8.13. Paris, France

Alban L, Pozio E, Boes J, Boireau P, Boué F, Claes M, Cook AJ, Dorny P, Enemark HL, van der Giessen J, Hunt KR, Howell M, Kirjusina M, Nöckler K, Rossi P, Smith GC, Snow L, Taylor MA, Theodoropoulos G, Vallée I, Viera-Pinto MM, Zimmer IA. 2011. Towards a standardised surveillance for Trichinella in the European Union. Preventive Veterinary Medicine, 99: 148-160.

4. Analysts should receive training on good laboratory practices. QA information on the components of a testing laboratory's Quality Management System should be covered, including essential components of the digestion assay and its critical control points and minimum standards for test performance as described in Part 2 of this series of recommendations. The importance of documentation and proper record keeping should be stressed. The training should also include a demonstration of the practical aspects of maintenance of all equipment and reagents used in the artificial digestion method.

5. The trainer(s) should provide a practical demonstration and detailed discussion of the artificial digestion method with special attention to critical control points as described in Part 2 of these recommendations. The trainer should emphasize the importance of having an approved standard operating procedure (SOP) and other reference documents, such as a training manual and figures available during routine testing. Various pre- and post-training materials, such as the International Commission for Trichinellosis (ICT) guidelines, review articles and reference CD-ROMs may be useful aids for the trainer and analysts. The following are useful references for this part of the training:

European Community. 2005. Regulation (EC) No 2075/2005 of the European Parliament and of the Council of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat. *Official Journal of the European Commission*, L 338, 60-82.

Gamble, H.R., Bessonov, A., Cuperlovic, K., Gajadhar, A.A., van Knapen, F., Nöckler, K., Schenone, H. and Zhu, X. 2000. Recommendations on methods for the control of *Trichinella* in domestic and wild animals intended for human consumption. *Veterinary. Parasitology*, 93: 393-408.

World Organization for Animal Health (OIE), 2011. Manual of Diagnostic Tests and Vaccines, Terrestrial Animal Health Code Ch 2.1.16 pp 344-351. Paris, France

6. The trainer(s) should provide an overview and demonstration on the operation and maintenance of the stereomicroscope and provide ample time for in depth practical identification of *Trichinella* and other parasite larvae recovered from digestions. This part

of the training should include a demonstration of sources of false positives, including other nematode larvae and artifacts. Photos should have a scale so as to familiarize analysts with the relative size of *Trichinella* larvae along with the characteristic spiral shape and movement of active larvae.

7. Analysts should perform adequately supervised practice of the artificial digestion/magnetic stirrer method using *Trichinella* spiked samples until they are able to consistently recover and identify larvae.

8. The successful independent performance of a series of digestion tests is necessary for an analyst to be deemed qualified to test for the purposes of meeting regulatory requirements or food safety standards. For a trainee to be deemed a qualified analyst, at the time of training, a complete set of samples from a proficiency testing panel (PTP) must be successfully tested as outlined in Part 3. The specific criteria for qualifying an analyst should be set by the PTP provider, testing laboratory or Competent Authority and available as an SOP or documented in the Quality Manual or elsewhere in the lab.

   The following is provided as <u>example</u> outcome(s) that may occur from this proficiency testing:
   - analyst completes testing of the PTP in a satisfactory manner and is qualified
   - results on initial an PTP are unsatisfactory; trouble shooting and additional training is conducted and an additional PTP is tested with satisfactory results
   - trainee repeatedly fails to properly perform the test using PTP and cannot be qualified as an analyst.

9. Discussion should be held with all qualified analysts regarding reporting requirements and other obligations as required by the testing laboratory and the competent authority. Topics discussed should include procedures to be followed in the event of the identification of a positive sample. An outline of such procedures and other requirements should be included in an SOP and available at the testing Laboratory.

10. Training should culminate in a short written examination or other form of evaluation to assess and document the knowledge acquired by the newly qualified analyst. This final session should include a review of the training, expectations regarding proficiency testing by the qualified analyst at the certified laboratory, and any feedback to the trainers for improvement of the training program.

Table 1. – Recommended topics for inclusion in training.

Topic	Objectives	Key points
History	Demonstrate the importance of human cases both historically and at the present time	*Trichinella* is a public health hazard, worldwide. Analysts must be attentive to the possibility of finding *Trichinella* in any sample.
Life cycle of *Trichinella*	Describe the basic life history of the parasite, reproductive capacity index	The entire life cycle occurs in one host; infective larvae are found in Nurse cells, modified muscle cells. Digestion frees the larvae from the capsule and that is what is observed in the test.
Phylogeny of *Trichinella*	Describe the species and genotypes relative to differences such as freeze resistance	It is not possible to differentiate species in the digestion test. If larvae are recovered, they need to be preserved for genotyping and trace back.
***Trichinella* morphology**	Provide a detailed description of the anatomical structure of the parasite including the stichosome and the cuticle	This section must be sufficiently detailed so as analysts are able to accurately identify *Trichinella* from artifacts and other nematode larvae. Discuss the use of microscopes – what is visible at the appropriate magnification – and the size of *Trichinella* relative to other nematodes. Photos of larvae in various shapes (coiled, moving, dead) should be used and the details from this part of the training should be reinforced during the practical sessions.
Epidemiology	Describe the domestic and wildlife cycles and the species and hosts involved; geographical distribution	Focus should be on at risk species in the area to be covered by the testing. Differences should be described among pigs reared in biosecure (*Trichinella*-free) housing versus backyard and free-ranging pigs (at risk for *Trichinella* infection)
Clinical disease	Describe the clinical disease resulting from human exposure to infected meat and susceptibility to all genotypes of *Trichinella*	This section should include a discussion of the enteral and parenteral phases, the most common symptoms, diagnostic methods, and the treatment and outcome of infections. Some details of outbreaks should be given.
Detection in animals	Describe direct and indirect tests to detect infection – benefits and drawbacks to use; predilection sites in host species; note that *Trichinella*-infected animals do not show any signs of	The only tests currently suitable for protecting public health are direct tests – artificial digestion methods. Indirect tests may be used for surveillance. Provide some theory of the digestion method, including why *Trichinella* resist digestion when alive and the problems with

	disease	recovering larvae following digestion if larvae are dead.
Prevention	Describe current programs to prevent infection in domestic pigs	Analysts should be made aware of national programs that are designed to prevent infection in mammals. Also, they should understand that free-range and outdoor pigs as well as wildlife are at risk and are much more likely to yield positive results.
The training Prophylaxis	Describe processes (cooking, freezing, curing) used to killed *Trichinella* in prepared meats	Specific guidelines are available for the commercial cooking, freezing and curing of pork products, when meat has not been otherwise proven free from *Trichinella*. Home cooking guidelines are also available. Curing may be insufficient to kill larvae if not done properly.
Laboratory Safety	Describe requirements for biocontainment when working with infectious material	Biocontainment applies specifically to handling of the PTP, but trainees should be informed that any piece of meat might harbor Trichinella larvae until tested and determined free.

B. Initial Proficiency Qualification, Re-Qualification, Disqualification and Re-Training

Following training, and prior to performing testing for regulatory or food safety purposes, analysts should be required to demonstrate competency when performing the artificial digestion method in their laboratory. The specific requirements and criteria for analyst qualification, re-qualification, disqualification and retraining should be specified by the testing laboratory or Competent Authority, and appropriately documented at the laboratory. The criteria should incorporate the minimum recommendations for pass/fail as outlined in Part 3. Only a reasonable number of repeat testing should be allowed when failure on PT panels occurs, and the use of trouble-shooting and repeat training should be considered as corrective actions as appropriate. The following sections are examples for use in "on-site" qualification process and additional monitoring and training requirements.

B1 On-Site Qualification – Initial Proficiency Samples

Following successful training, a newly qualified analyst should perform on-site proficiency testing at the testing laboratory as soon as possible, but no longer than three months from the initial training, and prior to conducting testing for regulatory or food safety purposes. Proficiency samples should be prepared and distributed by an agent of the competent authority using PTP as described in Part 3 of this series. Proficiency samples tested on site must be performed using the facilities and equipment which analyst will use on a regular basis.

Analysts must conduct PT independently without any assistance or interference from other persons.

Initial proficiency panels should include, at minimum, the number of samples described in Part 3 of these recommendations.

In performing this initial PTP, the following outcomes may occur:

- the analyst completes testing in a satisfactory manner and is qualified to test for regulatory or food safety purposes
- the analyst fails the initial on-site PTP at the certified laboratory; an additional panel is tested. If passed, the analyst is qualified to test for regulatory or food safety purposes.
- the second on-site proficiency panel at the certified laboratory is failed, and non-technical sources of error have been ruled out; the competent authority will determine if additional testing, training or analyst disqualification is appropriate.

B2 Re-Qualification

Analysts must be re-qualified at least once each year. Re-qualification is granted pending acceptable analysis of a PTP as outlined in Part 3. In the event that PTP results are not in compliance with the requirements of the competent authority, a re-test may be granted.

Failure of an analyst to accurately analyze a second PTP should result in disqualification and the individual should undergo re-training in accordance with the training plan outlined here.

B3 Re-Training

The competent authority should determine a need for periodic updating of training for qualified analysts and specific training requirements for analysts who are disqualified based on failure of PTP testing.

C. Recommended Content of a Training Manual

C1 Introduction

The introduction should discuss the history and relevance of regulatory testing for *Trichinella* in food animal species and game meats intended for human consumption. It should include references to relevant legislation and outbreaks of *Trichinella*, which emphasize the importance of testing. It should describe the roles of qualified analysts performing the training and the roles of others in the testing chain, including the competent authority.

C2 Information about the parasite *Trichinella*

This section should include general information about Trichinella that will provide the qualified analyst with basic knowledge and an understanding of the importance of the testing which they perform. Recommended topics include the following:

Trichinella life cycle and biology

- *Trichinella* morphology

Geographical distribution of species

Epidemiology in food animal species

C3 Information about the disease

This section should include general information about the disease in humans – trichinellosis - that will provide the qualified analyst with basic knowledge and an understanding of the importance of the testing which they perform. Recommended topics include the following:

Routes of exposure (outbreak examples)

Clinical features in humans

Diagnosis and treatment

C4 Control methods

This section should provide basic information on how *Trichinella* is controlled – on the farm or production unit – and the options for preventing exposure of humans to infected meat (slaughter testing and post-slaughter processing). Recommended topics should include any of the following:

- Pre-harvest control – describe bio-secure production systems that reduce risk of exposure to *Trichinella* in domestic pigs
- Slaughter Inspection – describe regulations governing slaughter inspection including the use of the pooled sample digestion method
- Processing – discuss further processing to inactivate *Trichinella* in ready-to-eat products
    - Cooking
    - Freezing
    - Curing

IRRADIATION

CONSUMER PREPARATION – DISCUSS HOME PREPARATION OF MEATS WHICH HAVE A RISK OF CONTAMINATION WITH *TRICHINELLA* LARVAE

The following resources can be used to obtain information on these subjects:

Regional freedom from infection

European Community (2005). Regulation (EC) No 2075/2005 of the European Parliament and of the Council of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat. *Official Journal of the European Community*, L 338, 60-82.

Office Internationale des Epizooties. 2010. Trichinellosis. *In*, Terrestrial Animal Health Code, Chapter 8.13.1., Paris, France.

Herd certification

European Community (2005). Regulation (EC) No 2075/2005 of the European Parliament and of the Council of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat. *Official Journal of the European Community*, L 338, 60-82.

U.S. Code of Federal Regulations. (2010) Title 9, Part 149: Voluntary Trichinae Certification Program. http://www.gpo.gov/fdsys/pkg/CFR-2010-title9-vol1/xml/CFR-2010-title9-vol1-part149.xml.

Post-slaughter processing

European Community (2005). Regulation (EC) No 2075/2005 of the European Parliament and of the Council of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat. *Official Journal of the European Community*, L 338, 60-82.

U.S. Code of Federal Regulations. (1990). - *Animals and Animal Products*, Office of the Federal Register, Government Printing Office, Washington, D.C. 9, 212-220.

C5 Test methodologies

This section should include a detailed description of the methods to be used in testing for Trichinella in food animals and game meats intended for human consumption.
The following resources can be used for this purpose:

European Community (2005). Regulation (EC) No 2075/2005 of the European Parliament and of the Council of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat. *Official Journal of the European Community*, L 338, 60-82.

Office Internationale des Epizooties. 2010. Trichinellosis. *In*, Terrestrial Animal Health Code, Chapter 8.13.1., Paris, France.

Other resources may include local or national legislation which is provided by the competent authority.

This section should refer to the quality assurance recommendations as described in Part 2.

C6 Quality assurance

This section should describe standard operating procedures (SOP's), a quality manual, and record-keeping and reporting requirements, along with any other essential components of a laboratory quality management system (QMS). A plan for recording and retaining results of all testing should be described which is consistent with recommendations found in Parts 2 and 5 of these documents and as required by the competent authority.

C7 Morphological identification of the parasite

This section should provide extensive and detailed information on the accurate identification of *Trichinella* in the pooled sample digestion test. Both written descriptions and photographs should be used, including a scale of size. Side by side comparisons of *Trichinella* larvae with other parasite larvae are instructive. Subjects to be covered in this section include the following:

- Photos of *Trichinella* – live/coiled, live uncoiled and motile, dead/C-shaped
- Other parasite larvae that may be found in tissue digests
- Artifacts – pollen, bubbles, hairs, etc.
- Resources for these images may be found in a variety of places, including the internet.

C8 Proficiency testing

The quality of analyst performance requires periodic assessment, which is accomplished, in part, by the use of proficiency panels (see OIE - http://www.oie.int/our-scientific-expertise/reference-laboratories/proficiency-testing/).

A description of the requirements and procedures for performance of proficiency panels for *Trichinella* should be included in this section. This section should refer to the quality assurance recommendations and other guidance on proficiency panels as described in Section 3.

C9 Establishing a *Trichinella* testing laboratory

Testing for *Trichinella* in food animals and game meats intended for human consumption should be performed in a certified laboratory to ensure adequate quality assurance. A

description of the minimum requirements for laboratory certification and re-certification are provided in Section 5 of these recommendations.

C10 Laboratory safety

An adequate description of the hazards, precautions and mitigations for working with *Trichinella* infected meat should be described, including the use of Material Safety Data Sheets (MSDS's) as appropriate. This section should also describe the safe handling of reagents used in digestion testing including HCl and pepsin.

C11 ADDITIONAL REFERENCES

APPENDIX 1 ICT Quality Assurance Committee Members

Alvin Gajadhar	Canada	Committee Chair

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Sub-Committee on QA for Proficiency Panels

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