International Commission on Trichinellosis (ICT)

Recommendations for Quality Assurance in Digestion Testing Programs for Trichinella

ICT Quality Assurance Committee (Appendix 1)

Part 3

Recommendations for Quality Assurance in Proficiency Testing

Contents

A. Minimum requirements for production of proficiency samples

Proficiency samples enable the accurate assessment of test performance. Therefore, a reliable method to prepare proficiency samples containing known numbers of *Trichinella* larvae is an important component of a quality assurance system for *Trichinella* digestion assays. To date, several methods for proficiency samples have been developed (1, 2, 3, 4).

A1 Muscle tissue used in preparing proficiency samples

- Source of muscle tissue: Muscle from the same host species and anatomical site routinely tested should be used (e.g. a lab which routinely tests pig diaphragm should use proficiency samples composed of tissue from pig diaphragm). Muscle with a low level of fat and fascia, or trimmed of such tissue, should be used for preparation of proficiency panels.
- Weight and composition of muscle sample to be spiked with *Trichinella* larvae: Muscle should be ground to facilitate preparation of "meatballs" into which either encapsulated larvae (larvae still in the muscle capsule) or free larvae (larvae not in the muscle capsule) are inserted. For spiked samples using encapsulated larvae on agar plugs or using free larvae suspended in water, a minimum of 10 g of ground meat should be used to ensure that the spike is fully contained within the sample.
- Additional muscle tissue required for use in completing the pool for digestion: Up to 100 g of additional muscle should be provided, free from contamination with *Trichinella* larvae, and meeting the same requirements as for the spiked sample.

A2 *Trichinella* larvae used in proficiency samples

- Source of larvae: Laboratory animals such as mice, rats or guinea pigs may be used to propagate *Trichinella* sp. for proficiency samples. If samples are prepared by homogenizing muscle tissue with larvae *in situ*, a host species capable of harboring a large number of *Trichinella* larvae is required.
- *Trichinella* species/genotype: *Trichinella* larvae from either encapsulating species that form capsules in the host (T1-T3, T5-T9, and T12) or non-encapsulating species that do not form capsules in the host (T4, T10, T11) may be used for the preparation of proficiency samples. However, larvae from non-encapsulating species recovered after digestion show survival times less than that of encapsulating species. Therefore, encapsulating species are strongly recommended when available.
- Use of encapsulated and free larvae: Encapsulated larvae or free larvae freshly released from capsules are used in proficiency samples. The use of encapsulated larvae is more labor intensive with respect to preparation and individual capsules must be assessed to ensure they contain only a single larva. However, encapsulated larvae are preferred as they are more resistant to environmental conditions and their use in proficiency testing (PT) helps to evaluate the ability of the digestion process used to release larvae from capsules for subsequent recovery and detection.
- Methods to recover larvae from muscle for use in proficiency samples: The recovery of free larvae can be accomplished by the standard method of artificial digestion as described in Part 2. For the harvesting of intact encapsulated *Trichinella* larvae two procedures have been described.

    1. The original method is based on filtration of blended infected rat muscle tissue (2). The blended muscle is mixed with phosphate-buffered saline and filtered through a double layer of tulle or gauze to yield a suspension of encysted larvae and fine muscle debris.

2. A modified method generates larger numbers of encysted larvae for large-scale preparation of proficiency samples (3). The method incorporates an incomplete artificial digestion of muscle tissue from infected mice, followed by neutralisation of pepsin and HCl.

- Condition of larvae/capsules to be spiked: It is recommended that live *Trichinella* larvae be used for the preparation of proficiency panels. Death and degradation of larvae affects both their characteristic morphology and sedimentation in the funnel, and may cause false negative test results.

In *Trichinella* free areas, risks of environmental or routine test sample contamination associated with the use of live larvae can be mitigated by following packaging and shipping procedures in accordance with international guidelines, and appropriate procedures for handling and containment of hazardous organisms. Additional mitigation measures include use of *Trichinella* species that have low or no infectivity for pigs.

Optimal storage conditions and shelf life for *Trichinella* larvae in proficiency samples should be determined for use in setting minimum recommendations. The rate of degradation of larvae in intact capsules and larvae previously released from capsules may vary markedly, and should be determined separately for use in setting recommendations for storage conditions and shelf life.

A3 Preparation of proficiency samples

After recovery from muscles of an infected animal, encapsulated or free *Trichinella* larvae should be collected, counted and embedded ('spiked') into each sample. Specific methods to reliably prepare these samples have been described (2, 3). Any method used should be validated to assure it meets the requirements for its intended purpose, including the minimum standards described in these recommendations

A4 Storage and transport of proficiency samples

- Packaging (with/without vacuum-pack): Appropriate packaging must ensure no leakage of sample, including cysts or larvae. Vacuum packing can be used to prolong freshness of samples and larval survival.
- Labeling: Sample labels must not contain the number of larvae in the spike. As a minimum, each sample should be labeled with a unique code that can be cross-indexed to a confidential master database maintained by the proficiency sample provider. The master database should contain details of sample production, including dates, spike numbers and intended recipients.
- Storage conditions for samples: Proficiency samples should be stored at 5°C±3°C and shelf life limitations should be determined prior to distribution for testing. Samples made with either encapsulated or free larvae should not be frozen.
- Transportation of proficiency samples: Samples containing live larvae should be shipped/transported under bio-secure conditions for infectious material (UN 3373) (5) and under appropriate temperature conditions (5°C±3°C). Ideally a probe to record the temperature during transport will facilitate monitoring of these conditions. Transport times should be minimised; receipt of samples within 48 h is recommended.

A5 Verification of proficiency sample integrity

Representative batch testing should be performed by the proficiency sample provider after samples have been prepared. Ideally, this should include initial testing of a representative group of samples prior to releasing the batch and final testing following completion of the last sample

in the field laboratory, or at the expiry date of the batch, whichever occurs first. Verification should include assessing the viability of larvae and the number of larvae contained in samples.

B. Proficiency testing panels (PTP) for *Trichinella* digestion testing

B1 Number of negative and positive samples in a panel

The number of samples in a PTP should be large enough to allow for variations in panel composition over time to ensure that panel composition is not predictable. It should include at least one negative sample and a minimum of two positive samples in order to evaluate the proficiency of a single analyst. PTP's consisting of a large number of samples should be avoided in routine use, as they do not provide significant additional information and may cause workflow interruptions in testing laboratories. However, each PTP should consist of a minimum of three samples containing positives and negative samples as recommended above.

B2 Number of larvae contained in positive samples

- Considering the sensitivity of the digestion assay (see Part 2), and the requirement to assess the technical ability of analysts to detect low numbers of larvae and the possibility of individual larvae being lost or damaged during sample production, it is recommended that positive samples within the proficiency panel should be spiked with 3-5 larvae, and at least one of these samples in a PTP contain 3 larvae; numbers of larvae in samples should be verified as described in section A5.
- Spiked samples containing higher numbers of larvae can be useful for training, corrective actions, validation of digestion method, and may also be used as proficiency samples at the discretion of the PTP provider or the certifying body.

B3 Frequency of proficiency testing panels

Each analyst should successfully complete at least one PTP per year. Factors that may require increasing the frequency include:

- Unsatisfactory results - a PTP should be repeated immediately or other corrective actions taken.
- Requirements of a national accreditation body - ISO 17025 generally requires a minimum of one PTP per year (7).
- *Ad hoc* local or national requirements – may be imposed by a competent authority for purposes such as ensuring that expertise is maintained in non-endemic regions.

C. Evaluation of proficiency testing results and implications for analyst and laboratory qualification for testing

C1 Evaluation of proficiency testing results

Proficiency samples are used to demonstrate test performance at an adequate level of sensitivity and for training and troubleshooting.

- Successful identification of samples containing a low number of larvae (3-5) indicates acceptable technical competence and an adequate analytical procedure. These samples represent low level infections which may be encountered in field testing.
- Samples containing a higher number of larvae may be useful for training and for investigating problems within the testing system. High spike samples help to identify deviations from critical control points during recovery of larvae, problems in reading gridded plates (microscopic detection), and generally contain a wider variety of larval configurations than is usually seen with low spike samples.
- Pass/fail criteria should be established to objectively measure the competence of an analyst in the performance of a digestion assay. Preparation, distribution and use of PTP should be followed as recommended in other sections of this document. These criteria include stringently controlled production and distribution systems for PTP to ensure that proficiency samples are not a source of error in evaluating technical proficiency.

C1.1 Acceptable recoveries of larvae from proficiency samples:

Published data from PT programs in use in several countries indicate that recoveries of > 75% of larvae in spiked samples are consistently achievable (1, 3, 8). These data also show that laboratories with poor results improve rapidly when participating in a PT program, and that laboratories with good results generally employ more QA components in their testing systems, including use of a standardized test method. Although it is recommended that the correct identification of positive and negative samples in a PTP be used for evaluating analyst performance, the reporting of results should include the number of larvae recovered from each sample. Such additional information facilitates documentation for continuous improvement and any future trouble shooting that may be required.

C1.1.1 Acceptable results for positive samples:

The recommended spike level for positive samples in a PTP is 3-5 larvae, and at least 1 larva should be recovered from each spiked sample within the panel.

- Pass: Recovery of **≥ 1** larva
- Fail: No larvae recovered*

*****Reporting of additional larvae beyond the expected numbers may indicate false positive results and analyst competence should be investigated by review of performance data records and/or retest.

C1.1.2 Acceptable results for negative samples:

One or more negative samples should be included in each PTP and should be varied to ensure that panel composition is not predictable. For example, a proficiency testing panel could contain either one or two negative samples.

- Pass: Correct identification of a sample that does not contain larvae.
- Fail*: Report of one or more larvae in a negative sample (false positive result).

* Failure of analyst in this case should be supported by confirmation of results, historical performance data or results of an immediate retest as appropriate.

C1.1.3 Acceptable results for high spiked samples:
Samples spiked with high numbers of larvae are useful for training and troubleshooting. PTP containing sample(s) with high spikes should also contain at least one low spiked sample, and all samples with 3-5 larvae should have acceptable recoveries to pass the panel. The actual number of larvae in high spiked samples and the evaluation of results should be determined according to the intended purpose, the competent authority or designate (e.g. reference laboratory).

C2 Proficiency requirements for qualification of analyst and laboratory

C2.1 Initial qualification of analyst as competent to conduct digestion testing

For an analyst to be deemed qualified, proficiency testing panels should be passed as part of training exercises and on-site at the testing laboratory. If problems occur, the reference laboratory or PTP provider can assist with troubleshooting to rule out non-technical causes, and may recommend retraining, retesting or other actions as required (see Part 4).

- Follow-up actions can vary according to results, qualification status of the analyst, unforeseen factors affecting results and resources of the reference and testing laboratory. The comprehensive activities required for initial qualification are described in Part 4 of these QA recommendations.

C2.2 Ongoing qualification of analysts

The purpose of ongoing maintenance is to demonstrate that qualified analysts in a testing laboratory continue to be competent in performing the assay.

- A qualified analyst must test at least one external PTP at least once per year and meet the pass criteria as set out in these recommendations.

The reference laboratory may provide solicited advice for troubleshooting problems and may provide non-scheduled proficiency samples under special arrangements. Any follow-up actions will similarly depend on factors indicated above.

C2.3 Initial and ongoing capacity of certified testing laboratories

The goal of PT is to assess, qualify and re-qualify individual analyst for performance of the artificial digestion method for *Trichinella*. A testing laboratory should have at least one qualified analyst as described in C2.1 and C2.2 (above) in order to achieve and maintain acceptable capacity for *Trichinella* digestion testing.

C3 Timelines for testing and reporting

Determination of testing schedules and reporting time-lines is the responsibility of the PTP provider and should take into account the shelf life of the proficiency samples.

C3.1 Testing laboratory

- The testing laboratory should analyze proficiency samples and report back to the PTP provider as stipulated by the competent authority.

- The testing laboratory should immediately inform the competent authority and/or PTP provider if analysts fail a PT, and take appropriate remedial actions.

C3.2 Proficiency testing panel provider

- An official report on PT results of each analyst should be provided to testing laboratories in a timely manner following receipt of results.
- A summary report (not identifying performance of individuals), which includes statistical analysis/comparison/trending of performance amongst all participating testing laboratories, would be useful for all such laboratories in a timely manner following receipt of the last set of test results.

C3.3 Competent authority

- In case of failure in PT, the competent authority or designate (e.g. NRL) should evaluate and approve, or reject if not appropriate, the proposed corrective actions, and verify their timely implementation.

C3.4 Reference Laboratory Reports

- The reference laboratory or other authorised PTP provider should provide reports to meet the requirements of different organizational levels. The report recipients and the content of the reports are determined by the competent authority. All results are confidential and each laboratory and analyst should be identified by a code when referred to in reports. A variety of reports may be required including reports to the testing laboratory, individual analyst, responsible regulatory body (competent authority), and ad hoc summary reports for various management and regulatory purposes.

C3.4.1 Record keeping

Requirements for record keeping are determined by the competent authority or designate. It is recommended that record keeping comply with internationally recognized QA standards such as ISO series documents.

C3.4.2 Testing laboratory

It is recommended that the record keeping activities of the testing laboratory associated with *Trichinella* digestion testing be based on the principles of ISO 17025. Formal accreditation to ISO 17025 is preferable but not essential.

C3.4.3 Proficiency testing provider

The record keeping system of the PT provider should be based on ISO 17025 requirements and ideally should also comply with ISO 17043 (7, 9). Records of statistical analyses should follow ISO 13528 guidelines as appropriate (10). An adequate documentation system should include records of proficiency sample preparation, panel configuration, verification testing, sample distribution, reporting and follow-up activities.

A copy of each report generated by the PTP provider should reside in the testing laboratory and with the PTP provider.

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