ICT recommendations for quality assurance in digestion testing programs for Trichinella

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History of recommendations

- recommendations were elaborated by a quality assurance committee (QAC) over a 3 years period
- workshops: Calgary, Canada (2009)
  - Paris, France (2010)
  - Changchun, China (2011)
  - Rome, Italy (2011)
  - last workshop supported by OECD

ICT Quality Assurance Committee (QAC)

Committee Chair: Alvin Gajadhar
QA digestion testing: Karsten Nöckler, Christian Kapel
QA proficiency panels: Pascal Boireau, Marleen Claes, Patrizia Rossi, Sandrine Lacour, Fritz Franssen, Lorry Forbes, Edoardo Pozio, Isabelle Valiée
QA lab certification: Brad Scandrett, Clive Pigott, Edoardo Pozio
QA technician training: Ray Gamble, Bruo Gottstein
Other QAC members: Francisco Bolas, Jean Dupouy-Camet, Caroline Frey, Teresa Garate, Joke van der Giessen, Albert Marinculic, Liu Mingyuan, Juan Olmedo, Ljiljana Sofronic

Publication of recommendations

www.trichinellosis.org
ICT Recommendations: main content

Part 1 Quality assurance in regulatory testing for Trichinella
Part 2 Essential quality assurance standards for Trichinella digestion assays
Part 3 Quality assurance in proficiency testing
Part 4 Training and qualifying analysts to perform the Trichinella digestion assay
Part 5 Essential components and minimum requirements for a Trichinella testing laboratory certification program

ICT Recommendations

Part 1 Quality assurance in regulatory testing for Trichinella

ICT Recommendations: Part 1

Basics

- recommendations are based on the best scientific information currently available
- they follow the principles of ISO/IEC documents (e.g. 17025) and guidelines set by international organizations such as WHO, OIE and CODEX

ICT Recommendations: Part 1

Quality assurance system for Trichinella testing: main requirements

- quality manual
- validated test method with identified critical control points
- training program for analysts
- proficiency testing to confirm technical capability of analysts
- suitable equipment, calibration and maintenance
- documentation and reporting
- regular internal and third party audits
ICT Recommendations: Part 1

Rationale for quality assurance system (QAS)

The competent authority is ultimately responsible for determining minimum quality standards for *Trichinella* testing; implementation, maintenance, enforcement of QAS will result in additional costs compared to the impact of trichinellosis outbreaks on public health and trade, the rationale for meeting QAS needs is compelling.

QA terms and definitions (Annex I)

- "Accredited Laboratory"
- "Harmonization"
- "Validation"

ICT Recommendations: Part 1

Progress and development

"ICT will update these recommendations as necessary to address relevant advances in science and technology".
ICT Recommendations: Part 2

General aspects on digestion assays

- for food safety and trade, digestion assays are the only reliable procedures for detection of Trichinella larvae in meat
- effectiveness of digestion testing depends on the application of proper QA standards
- standards include scientifically derived validation data
- need for monitoring and documentation of critical control points (CCP's)
- magnetic stirrer method = internationally accepted standard method

ICT Recommendations: Part 2

Main components for digestion assays

- digestion assays do not include internal controls to monitor their effectiveness – need for QA standards which should address the following components:
  1. Muscle sample collection and preparation
  2. Equipment and consumables
  3. Performance of the digestion assay
  4. Verification of findings
  5. Documentation

**Objective:** To ensure a test sensitivity which allows detection of the lowest number of larvae that may cause clinical symptoms in humans

ICT Recommendations: Part 2

1. Muscle sample collection and preparation

**Sample size**

- appropriate size of muscle sample should be collected from a predilection muscle of the respective animal species
- sample size should be at least twice the weight required for examination (for trimming of non-digestible tissues)
- sample weight to be tested should be determined by the competent authority
- sample weight = detection limit:
  - 1 g: ≥ 3.0 larvae per g
  - 3 g: ≥ 1.5 larvae per g
  - 5 g: ≥ 1.0 larvae per g

ICT Recommendations: Part 2

1. Muscle sample collection and preparation

<table>
<thead>
<tr>
<th>Predilection muscle</th>
<th>Animal species</th>
<th>Predilection muscles</th>
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<tr>
<td>Domestic pig</td>
<td>Diaphragm, masseter</td>
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<td>Horse</td>
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<td>Fox</td>
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<tr>
<td>Raccoon dog</td>
<td>Diaphragm, foreleg tongue</td>
<td></td>
</tr>
</tbody>
</table>
ICT Recommendations: Part 2

1. Muscle sample collection and preparation

Other minimum requirements
- samples should be free from non-digestible fat, tendons, fascia; removal of indigestible tissue for tongue
- sample should be labelled upon collection (tracing) and tested as soon as possible
- storage of sample at 2-8°C
- extended storage of samples by freezing (wildlife monitoring) is possible but can result in decreased recovery of larvae
- rejection of samples which are not in a good condition (e.g. insufficient weight, decay, no labelling)

ICT Recommendations: Part 2

2. Equipment and consumables

Minimum requirements
- all equipment must be properly cleaned prior to testing in order to avoid cross contamination
- plasticware or teflonware should not be used for beakers, funnels, or separatory funnels since a rough surface and electrostatic charge may contribute to larval adherence to the inner surface of the equipment
- calibration should be performed at least once a year for all instruments used for measurements (scale, thermometers and pipettes)

ICT Recommendations: Part 2

2. Equipment and consumables – overview on list

- labeled collection tins or plastic bags for samples
- knives, scissors and forces for cutting samples and removing non-digestible tissue
- blender
- 1 l wide-mouth beaker (metal or plastic)
- 1 l high-speed blender
- hand-held homogenizer
- small and large metal dish (e.g. 100-200 ml)
- lissoclinum striatus for 24-28°C
- meat samples: 100 g
- lissoclinum striatus 40 mg
- 50 ml Erlenmeyer flask
- 100 ml Erlenmeyer flasks
- 1 l Erlenmeyer flask
- ice (for meat)
- weighing scale (minimum 25 kg)

ICT Recommendations: Part 2

3. Performance of the digestion assay

Critical control points
1) blending of muscle sample
2) preparation of digest fluid
3) digestion of chopped meat
4) filtration of the digest fluid
5) sedimentation in sep. funnel
6) primary/secondary sediment
7) microscopic examination
ICT Recommendations: Part 2

3. Performance of the digestion assay

1. Blending of muscle sample
   - muscle samples are chopped to increase the surface for enzymatic degradation
   - no visible pieces of meat should remain after blending (usually 5-10 s at max. speed)
   - too little blending may result in incomplete digestion; too much blending may damage muscle larvae
   - rinse chopping blade and blender bowl with digest fluid to remove adhering muscle tissue and avoid larval loss
   - blender bowl should be made of acid resistant material to avoid etching

2. Preparation of digest fluid
   - use of pre-heated tap water (2 l, 46-48°C)
   - correct sequence of 1) water, 2) hydrochloric acid (final concentration 0.2%) and 3) pepsin (final concentration 0.5%)
   - use of pepsin with appropriate activity (declared in units)
   - shelf life of the pepsin should be displayed on the label
   - granular or liquid pepsin may reduce the risk of aerosolisation and possible allergic reaction
   - maximum muscle weight in a pool is 115 g for 2 l of digest fluid

3. Digestion of chopped meat
   - the maximum ratio of meat to digest fluid is 1:20
   - a constant temperature of 44-46°C should be monitored the whole digestion which lasts 30 min; time may be increased to 60 min for less digestible muscles
   - too low temperature or shortened digestion time may lead to incomplete digestion; too high temperature or prolonged digestion time could result in inactivation of pepsin or destruction of larvae
   - digest fluid must be stirred (deep vortex without splashing)

4. Filtration of the digest fluid
   - carefully pour the digest fluid through a clean sieve into a separatory funnel to retain undigested debris
   - rinse the glass beaker and sieve with tap water (min. 100 ml) to carry adhering larvae into the separatory funnel
   - debris remaining on the sieve should consist of undigestible non-muscle tissue; if visible muscle tissue remains on the sieve, the whole procedure must be repeated
ICT Recommendations: Part 2

3. Performance of the digestion assay

5. Sedimentation in the separatory funnel
   • digest fluid should remain undisturbed in the funnel for a minimum of 30 min; if time is less than 30 min not all larvae may have settled to the bottom
   • gentle tapping of the funnel wall may facilitate the larvae settling to the bottom
   • sedimentation speed for uncoiled (dead) larvae is lower; therefore sedimentation time should be extended for up to 60 min for frozen muscle samples

ICT Recommendations: Part 2

3. Performance of the digestion assay

6. Collection of the primary/secondary sediment
   • 40 ml of digest fluid (primary sediment) should be quickly dispensed into a tube (stopcock fully opened)
   • if the volume of the primary sediment is too small, larvae may remain in the digest fluid in the separatory funnel; if the volume is too high, there may be more debris
   • after sedimentation for 10 min, supernatant should be carefully withdrawn by aspiration from the top, leaving a volume of 10 ml (secondary sediment)
   • pour the sediment into a Petri dish and rinse the tube with 10 ml tap water to carry sticking larvae

ICT Recommendations: Part 2

3. Performance of the digestion assay

7. Microscopic examination
   • the secondary sediment should stand in Petri dish for 1 min
   • focus the microscope to ensure that gridlines of the Petri dish are easily visualised
   • the sediment must be transparent to visualise any larvae; if not transparent, the sediment must be washed (see 3.6.)
   • the sediment is systematically examined grid by grid
   • suspect or positive Trichinella findings must be traced from the pool to the individual carcass

ICT Recommendations: Part 2

4. Verification of findings
   • analysts must know basic morphological characteristics of Trichinella larvae (stage 1)
     - nematode (round worm)
     - approx. 0.7-1.1 mm in length and 0.03 mm in width
     - shape: coiled or motile (live), c-shaped (dead)
   • detailed morphology (stichosome) by compound microscopy
   • larvae should be transferred to a small vial (70-90% ethyl alcohol) for subsequent molecular identification (PCR)
ICT Recommendations: Part 2

5. Documentation

- need of documentation to demonstrate that Trichinella testing was correctly performed according to QA standards
- laboratory worksheet should be used by analysts to record data for test reports such as:
  - sample tracking information
  - correct performance by qualified analysts
  - documentation of problems and irregularities
  - written record of results
- worksheets should be stored according to the requirements of competent authority

ICT Recommendations: Part 3

1. Production of proficiency samples

Muscle tissue

- for larval recovery, predilection muscle of good quality should be used (see Part 2)
- “meatballs” are made of blended muscle tissue (as used for testing) and should have a minimum weight of 10g
- additional muscle tissue required for completing the pool weight (up to 100 g) shall be free from Trichinella larvae and should have the same quality
ICT Recommendations: Part 3

1. Production of proficiency samples

**Trichinella larvae**

- laboratory animals with a high susceptibility (e.g. mice, rats, guinea pigs) should be used for harvesting larvae
- larvae from encapsulated *Trichinella* species (T1-3, T5-T9 and T12) should be used due to higher survival times
- live *Trichinella* larvae shall be used for the preparation of proficiency panels (death and degradation will affect morphology and sedimentation behavior)
- in *Trichinella* free areas, species with low or no infectivity for pigs can be used (e.g. T2)

ICT Recommendations: Part 3

2. Storage and transport of proficiency samples

- packaging must ensure no leakage of proficiency sample
- vacuum packing can prolong freshness of samples and larval survival
- each sample should be labeled with a unique code be cross-indexed to a database
- proficiency samples should be stored at 5°C±3°C and shelf life limitations should be determined prior to distribution
- samples should be shipped/transported (max. time 48 h) under bio-secure conditions for infectious material (UN 3373)
ICT Recommendations: Part 3

3. Proficiency testing panels (PTP)

Composition of the PTP

- PTP should comprise of at least three samples (two negative and one positive samples)
- low spiked positive samples should contain 3-5 larvae and one sample should contain 3 larvae
- samples containing higher numbers of larvae can be useful for training, corrective actions, validation (identify deviations from critical control points during recovery of larvae) and proficiency testing

Frequency for testing PTP

- each analyst should successfully complete at least one PTP per year
- frequency of testing may change due to:
  - unsatisfactory results of analyst/laboratory and obligations for corrective actions
  - specific requirements of a national accreditation body
  - ad hoc local or national requirements – may be imposed by a competent authority

ICT Recommendations: Part 3

4. Requirements, timelines for testing and reporting

- PT provider should work according to ISO 17025
- laboratory should analyze proficiency samples according to instruction and report results to the PTP provider
- an official report on PT results of each analyst should be provided to laboratory in a timely manner
- a summary report giving an overview on performance amongst all participating laboratories would be useful (results for labs must be presented in anonymous way)
- additional reports may be required such as ad hoc reports for various management and regulatory purposes

ICT Recommendations: Part 3

5. Evaluation of proficiency testing results

- controlled systems for PTP are required to ensure that proficiency samples are not a source of error in evaluating the performance of analysts
- pass/fail criteria should be established to objectively measure the performance of an analyst:
  - a) low spiked positive samples
    - pass: recovery of ≥ 1 larva
    - fail: no larvae recovered (false negative result)
  - b) negative samples
    - pass: a sample does not contain larvae
    - fail: ≥ 1 larva were detected (false positive result)
- criteria for high spiked samples have to be determined
ICT Recommendations: Part 3

6. Corrective actions

- if analysts fail a PT the competent authority and/or PTP provider should immediately be informed
- corrective actions proposed by laboratory should be approved by the competent authority or designate (NRL)
- actions should be monitored and verified in their timely implementation

ICT Recommendations

Part 4 Training and qualifying analysts to perform the *Trichinella* digestion assay

ICT Recommendations: Part 4

All personnel performing for regulatory or food safety purposes

Goal: meet a set of minimum requirements

Should include:

- All QA measures (part 1)
- In a lab that meets QA standards (part 5)
- QA recommendations of test performance (part 2)
- Use and periodic evaluation by proficiency samples (Part 3)
- Provided by qualified personnel, in a bio-secure facility

ICT Recommendations: Part 4

A. Minimal elements (actual training: consistent with National legislation and competent authority)

1. Introduction
2. Overview of *Trichinella*
3. Overview of control programs and processes
4. Good Laboratory Practices
5. Practical demo on the method
6. Stereomicroscope
7. Perform test samples (supervised)
8. Qualification: independent performance of a set of PTP
9. Reporting: procedures in case of a positive sample
10. Evaluation
ICT Recommendations: Part 4

1. Introduction
   Historical setting, objectives and rationale for pooled sample digestion test, importance of results (public health, trade and economy)
   Relevant legislation, policies, guidelines and recommendations
   Potential consequences of a false negative result

2. Overview of Trichinella
   Biology, epidemiology, control measures, public health implications of infection
   Emphasis on motivating analysts to understand the importance of their work (ie description disease, recent human cases, social and economic impact of testing, consequences of missing a positive carcass)

ICT Recommendations: Part 4

3. Overview of control programs and processes to prevent human exposure
   Theory of testing (focus digestion), requirements of qualifying and retraining

4. Good Laboratory Practices
   Components of a Quality Management System, with essential components of the digestion assay and its CCP's
   Documentation and proper record keeping
   Demo of the maintenance of all equipment and reagents used

5. Practical demo on the method
   Special attention to the CCP's, SOP and other reference documents

ICT Recommendations: Part 4

6. Stereomicroscope
   Operation and maintenance, practical identification of Trichinella and other larvae, sources of false positives, photo's with a scale, characteristic spiral shape and movement of live larvae

7. Perform test samples (supervised)

8. Qualification: independent performance of a set of PTP

9. Reporting: procedures to follow in case of a positive sample

10. Evaluation: to assess the knowledge acquired. Review of the training, expectations on PT testing and feed back to trainers for improvement

ICT Recommendations: Part 4

B. Following training:
1. On-site Qualification:
   demonstrate competency in own lab
   Criteria specified by the testing lab or Competent authority
   Example: on site performance of a PTP, within 3 months of training

2. Re-Qualification: at least once/year (ie. acceptable analysis of a PTP)

3. Re-training: periodic updating all analysts, and specific training for analysts who disqualified a PTP.
ICT Recommendations: Part 4

C. Content of a training Manual:

1. Introduction
2. Info on the parasite
3. Info about the disease
4. Control methods
5. Test methodologies
6. QA
7. Morphological identification
8. Proficiency testing
9. Establishing a Trichinella testing lab
10. Lab safety

ICT Recommendations: Part 5

Lab certification program

1. Quality management system
2. Regulatory oversight
3. Structure of program
4. Standardized and validated assay
5. Laboratory facilities/equipment
6. Sample collection and handling
7. Traceability
8. Training
9. Proficiency assessment
10. Audits

1. Quality management system

= foundation
= All policies, procedures and associated documentation to ensure that testing is reliable and fit for purpose (Quality Manual)

Certifying body (CB): accredited in accordance with ISO 17025 + Trichinella digestion test in its scope

Testing lab: minimal: management and technical requirements of ISO 17025 + quality management system approved by CB

CB and testing lab: quality manual, SOPs and records/reports to document fulfillment of respective responsibilities
ICT Recommendations: Part 5

2. Regulatory oversight
CB: legal empowered to oversee/govern the certification process
Typically: Competent authority, Veterinary Authority or similar

3. Structure of program
Documented description of certification program provided by CB.
Key info on the program can be compiled into an info package to
candidate labs, incl. checklist of milestones, time frames and
responsibilities

4. Standardized and validated assay
Magnetic stirrer method for pooled sample digestion (ICT, OIE,
EU)

ICT Recommendations: Part 5

5. Laboratory facilities/equipment
Whenever possible Biosafety level 2
Equipment: see part 2

6. Sample collection and handling
CB to approve all requirements for sampling
~ purpose of testing, species tested, client demands
Specific recommendations: see part 2
Detailed prescriptive SOP for sample collection and handling.

7. Traceability
CB to approve identification and traceability procedures and
documentation to link samples, test results and carcasses, and
procedures when positive results are obtained

8. Training
See part 4
Digestion test, basic lab procedures and equipment, QMS and
safety policies and procedures

9. Proficiency assessment
See part 3

10. Audits
To identify and document deficiencies in the QMS, also
opportunity to foresee potential technical needs and problems
and implement continual improvements
Both internal (yearly) and external (biannually)
Inform CB of findings
Standard audit checklist

Thank you
for your attention