

## Guidelines on minimum recommendations for official laboratory appointed for the detection of *Trichinella* in meat

### Introduction

These guidelines provide a set of minimum recommendations to recognize the competence of a laboratory for *Trichinella* testing. The Commission Implementing Regulation (EU) 2015/1375 of 10 August 2015 [1] laying down specific rules on official controls for *Trichinella* in meat, (*Chapter II - Article 2 (1)(b)*) states that all samples collected from those animals to be examined for *Trichinella* shall be tested “.... **in a laboratory designated by the competent authority (official laboratory)**, using one of the following methods of detection: (a) the reference method of detection set out in Chapter I of Annex I; or (b) an equivalent method of detection set out in *Chapter II – Equivalent Methods of Annex I*”. The minimum requirements for all official laboratories are laid down in Regulation (EU) 2017/625 [2] (official control Regulation). Such requirements include that in principle the official laboratory operates in accordance with the standard ISO/IEC 17025 and is accredited in accordance with that standard.

In order to avoid a disproportionate burden, official laboratories only carrying out *Trichinella* controls can be derogated from such accreditation under certain conditions. The conditions laid down in Regulation (EU) 2017/625 include:

- Sole activity is the detection of *Trichinella* in meat
- Only use of methods laid down in Regulation (EU) 2015/1375
- Supervision by competent authorities or accredited official laboratory
- Regular participation and satisfactory performance in inter-laboratory comparative tests or proficiency tests organized by the national reference laboratories
- Compliance with all other obligations for official laboratories (Articles 34 to 42 (and in particular 38) of Regulation (EU) 2017/625.

The Competent Authorities (CA) has the legally empowered authority to appoint *Trichinella* testing laboratories by approving their policies and procedures, to monitor their performance over time and to revoke their designation in case of significant failures.

The CA should provide to the testing laboratory a documented description of all requirements that should be met by the testing laboratory to achieve and maintain the designation status.

The aim of these guidelines is to provide to the MSs CA a reference document on minimum quality assurance requirements to be fulfilled by the official laboratory appointed by their CA to perform controls for the detection of *Trichinella* larvae in meat in case no other certification system (e.g. ISO 17025 accreditation) is in place, while still obliged to fulfill the other requirements/conditions for official laboratories such as those in Article 38 of Regulation (EU) 2017/625..

Laboratories conducting *Trichinella* testing should implement a quality management system (QMS) with policies and procedures including quality control, analyst competence, suitable facilities, validated method(s), and sample identification and traceability.

The laboratory should apply for granting the designation as *Trichinella* testing laboratory, according to the relevant procedures established by CA of the MS, which will carry out (or delegate other Institutions, e.g. the National Reference Laboratory for Parasites) on- site assessments to evaluate the laboratory QMS and its technical competence in performing *Trichinella* testing.

The World Organization for Animal Health (OIE) [3], the International Commission on Trichinellosis (ICT) [4], The Codex

Alimentarius [5] and the International Organization for Standardization (ISO) [6] provide extensive recommendations and standards, respectively, for quality management in testing laboratories for *Trichinella*. Based on the same principles and guidelines, the following essential components are recommended for designation of a *Trichinella* testing laboratory:

1. **Quality management system**
2. **Personnel**
3. **Test methods**
4. **Laboratory facilities**
5. **Equipment**
6. **Sample handling**
7. **Traceability**
8. **Training of personnel**
9. **Proficiency testing**

A checklist for auditing laboratory appointed of official controls for the detection of *Trichinella* in meat is also included in the document as an annex (Annex 1).

## **Components and Requirements**

### 1. Quality Management System (QMS)

The Testing Laboratory should put in place all standard operating procedures (SOPs), instructions and associated documents to ensure that *Trichinella* testing is reliable and fits for the purpose.

The testing laboratory should have a quality management system approved by the CA.

### 2. Personnel

The Testing Laboratory should ensure the competence of personnel, managerial and technical, involved in *Trichinella* testing activities. Minimum recommendations include:

- a. supervisor or head of the laboratory, in charge of the test report issue, should have knowledge of the epidemiology, biology and diagnosis of nematodes of the *Trichinella* genus, of regulatory requirements, and experience on the detection of *Trichinella* larvae in meat;
- b. analysts should have basic knowledge on *Trichinella* parasites and their morphology, and proven experience on performing tests on the detection of *Trichinella* larvae in meat according to the Regulation (EU) 2015/1375.
- c. Laboratory staff complement should be adequate to cope with the volume of testing.

### 3. Test methods

The laboratory should apply the magnetic stirrer method for pooled sample digestion, indicated as the reference method for the detection of *Trichinella* larvae in meat (ISO 18743:2015), or equivalent methods as described in ANNEX I, Chapter 2 of the Regulation (EU) 2015/1375 [1].

The laboratory shall confirm that it can properly apply method/s (see Annex 1), in order to ensure achieving its/their performance characteristics, using reference material and participating to a proficiency test (Regulation (EU) 2017/625 Art. 40, a) (iv)), the results must be made available to the CA or another authority (e.g. NRL) designated by CA prior to its designation. The laboratory can be supported by the National Reference Laboratory or by the European Reference Laboratory ([www.iss.it/crlp/](http://www.iss.it/crlp/)) for reference material and expertise.

The test result should at least be qualitative, i.e. presence or absence of *Trichinella* muscle larvae (MSL) in tested samples.

#### 4. Laboratory facilities

Laboratory premises should be adequate for the testing and assure safety of the personnel.

The competent authority should provide the minimum requirements for the laboratory setting where testing activity is carried out, including premises, environmental monitoring and hygiene.

Laboratory facilities shall be properly distinct from the slaughterhouse when it is in the same compound..

Whenever possible, biosafety Level 2 guidelines should be followed, as those provided in the *Laboratory biosafety manual* of the World Health Organization (WHO) [7], and in the Eurachem guidelines *Accreditation for Microbiological Laboratories* [8], which give further guidance for laboratories carrying out microbiological testing.

#### 5. Equipment

The number of each type of apparatus should be related to the number of samples which have to be tested per day.

At least one apparatus for each type should be available for emergency situation. It is recommended that also this supply should be kept in working order with periodical maintenance. According to the amount of tests carried out by the laboratory, the laboratory should establish the wear time of materials (e.g. knife, scissors, tweezers, blender, meat chopper, magnetic stirrer, sieves, glass containers) to be periodically renewed, and a stock should be always available. Consumable materials (both disposable and chemical) should be stored in appropriate cabinets and a suitable stock should be available, taking into account the time between ordering and delivery of materials. The expiration date of chemicals should be periodically checked at storage conditions defined by the manufacturer.

The laboratory should have a list of qualified suppliers for materials and apparatuses and for their maintenance and assistance (e.g., microscope, scale, magnetic stirrer).

#### 6. Sample handling

The laboratory should describe in a prescriptive quality system (QS) document the sample handling, including acceptability criteria, identification, storage, decontamination and disposal.

The acceptability criteria should fulfill the requirements of the Regulation (EU) 2015/1375 (*Chapter II, article 2. Sampling of carcasses*, and in *Annex III. Examination of animals other than swine*) [1].

#### 7. Traceability

The head of the laboratory should clearly establish with the official veterinarian of the slaughterhouse:

- a) the responsibility of the sample traceability from the carcass to the laboratory;
- b) describe how the sample traceability is assured in the laboratory, from its arrival at the laboratory to the test result management;

- c) provide evidences for identification, collection, indexing, access, filing, storage, maintenance and disposal of technical records;
- d) retain records of original observations, staff records and a copy of each test report for a defined period. The records for each test should include the identity of the person in charge for the performance of the test and result checking;
- e) under the CA supervision and responsibility should provide evidence of a reliable document linking carcasses, sample collection, testing and results, as well as the procedures for the management of positive results according to Chapter II, Article 7. Contingency plans of Regulation (EU) 2015/1375 [1].

#### 8. Training of personnel

The head of the laboratory should ensure that the personnel involved in the sample testing to detect *Trichinella* MSL, shall be trained by participating in: a) a quality control program of the tests used to detect *Trichinella* MSL; and (b) a regular assessment of the testing, recording and analysis procedures used in the laboratory, as stated in *Chapter II, Article 5. Training* of Regulation (EU) 2015/1375.

The CA or another delegated authority should establish an adequate **training program for analysts, including** *Trichinella* biology and epidemiology, the test methods, pre- and post-testing requirements, reporting, and safety procedures. The training should be provided by qualified persons, and the acquired competence should be demonstrated by successful participation to Proficiency Testing (PT). Detailed requirements on training to qualify analysts for *Trichinella* testing are given in Recommendations for Quality Assurance in Digestion Testing Programs for *Trichinella* - Part 4. Recommendations for training and qualifying analysts to perform the *Trichinella* digestion assay of the International Commission on Trichinellosis (ICT) [4].

#### 9. Proficiency Testing

The laboratory personnel performing the test on the detection of *Trichinella* MSL in meat should participate regularly and have satisfactory performance to the Proficiency Testing (PT) organized by the National Reference Laboratory of each MS. The frequency of participation can be based on the laboratory performance during prior proficiency tests, in accordance with the opinion of the CA. The PT panel should be composed by at least 3 samples, and has to be considered successfully passed if all positive samples are detected positive and negative samples are properly identified as negative. The PT results should be provided to CA by the delegate authority (e.g. NRL) upon request. In case of failure, the laboratory should analyze the causes of such failure, implement adequate corrective actions, and repeat the test on further samples provided by the NRL. If the laboratory fails again, the CA should suspend the designation to the laboratory until the evidence of positive PT results is provided.

#### References

1. Commission Implementing Regulation (EU) 2015/1375 of 10 August 2015 laying down specific rules on official controls for *Trichinella* in meat (2015) (OJ L 212, 11.8.2015, p. 7–34).
2. Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No

999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation) (2017) (OJ L 95/1, 7.4.2017, p. 1-95).

3. World Organisation for Animal Health (OIE), Chapter 3.1.20 — "Trichinellosis (infection with *Trichinella* spp.)", Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 2018.  
[https://www.oie.int/fileadmin/Home/eng/Health\\_standards/tahm/3.01.20\\_TRICHINELLOSIS.pdf](https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.20_TRICHINELLOSIS.pdf).
4. Gajadhar AA, Noeckler K, Boireau P, Rossi P, Scandrett B, Gamble HR. International Commission on Trichinellosis: Recommendations for quality assurance in digestion testing programs for *Trichinella*. Food Waterborne Parasitol. 2019 Jun 5;16:e00059. doi: 10.1016/j.fawpar.2019.e00059.
5. Codex Alimentarius (2015) Guidelines for the control of *Trichinella* spp. in meat of suidae. CAC/GL 86-2015.
6. International Organization for Standardization (2015) ISO 18743: Microbiology of the food chain - Detection of *Trichinella* larvae in meat by artificial digestion method. Geneva, Switzerland.
7. World Health Organization, 2020. Laboratory biosafety manual, fourth edition.  
<https://www.who.int/publications/i/item/9789240011311>.
8. Eurachem. 2013. Accreditation for Microbiological Laboratories, second edition. Eurachem Guide.  
([https://eurachem.org/images/stories/Guides/pdf/Eurachem\\_Guide\\_AML\\_2013.P2.pdf](https://eurachem.org/images/stories/Guides/pdf/Eurachem_Guide_AML_2013.P2.pdf)).

**Annex 1. Checklist for auditing laboratory appointed of official controls for the detection of *Trichinella* in meat.**

| CHECK                                                                                                                                                                                                                                                               | EVIDENCE |    | COMMENTS |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|----|----------|
|                                                                                                                                                                                                                                                                     | YES      | NO |          |
| <b>1. Quality management system</b>                                                                                                                                                                                                                                 |          |    |          |
| 1.1. Does the testing laboratory have a quality management system?                                                                                                                                                                                                  |          |    |          |
| 1.2. Standard operating procedures (SOPs)                                                                                                                                                                                                                           |          |    |          |
| 1.3. Instructions                                                                                                                                                                                                                                                   |          |    |          |
| 1.4. Associated documents (forms, registration documents, etc.)                                                                                                                                                                                                     |          |    |          |
| 1.5. Has the laboratory a person in charge of administrative procedures, including chemicals, consumable and apparatus purchasing?                                                                                                                                  |          |    |          |
| 1.6. Has the laboratory a person in charge to inform the CA on detection of a positive sample?                                                                                                                                                                      |          |    |          |
| 1.7. Are the CA and NRL contacts (e.g., telephone, email) available?                                                                                                                                                                                                |          |    |          |
| <b>2. Personnel</b>                                                                                                                                                                                                                                                 |          |    |          |
| 2.1. Does the laboratory have a supervisor or head of the laboratory?                                                                                                                                                                                               |          |    |          |
| 2.2. Does the supervisor or head of the laboratory:                                                                                                                                                                                                                 |          |    |          |
| <ul style="list-style-type: none"> <li>have the authority to manage the activities carried out by the laboratory?</li> </ul>                                                                                                                                        |          |    |          |
| <ul style="list-style-type: none"> <li>have the authority to sign the test report?</li> </ul>                                                                                                                                                                       |          |    |          |
| <ul style="list-style-type: none"> <li>have knowledge of the morphology, epidemiology, biology and diagnosis of nematodes of <i>Trichinella</i> genus, of regulatory requirements, and experience on the detection of <i>Trichinella</i> larvae in meat?</li> </ul> |          |    |          |
| <ul style="list-style-type: none"> <li>have the experience in the field of direct diagnosis of <i>Trichinella</i> infections in susceptible animals?</li> </ul>                                                                                                     |          |    |          |
| 2.3. Does the technical personnel have basic knowledge on <i>Trichinella</i> parasites and their morphology?                                                                                                                                                        |          |    |          |
| 2.4. Does the technical personnel have proven experience on performing tests on the detection of <i>Trichinella</i> larvae in meat according to the Regulation (EU) 2015/1375?                                                                                      |          |    |          |
| <b>3. Test method/s</b>                                                                                                                                                                                                                                             |          |    |          |
| 3.1. Does the laboratory apply ISO 18743:2015 or one or more of the methods as described in ANNEX I, Chapter 2 of the Regulation (EU) 2015/1375?                                                                                                                    |          |    |          |
| 3.2. Did the laboratory verify that it can properly apply this/these method/s ?                                                                                                                                                                                     |          |    |          |

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| 3.3. Are the documents of the verification process available?                                                                                                                   |  |  |  |
| 3.3.1. Do they include:                                                                                                                                                         |  |  |  |
| • Number of performed tests                                                                                                                                                     |  |  |  |
| • Sample origin (e.g., NRL, EURLP)                                                                                                                                              |  |  |  |
| • Statistical analysis                                                                                                                                                          |  |  |  |
| • Result evaluation                                                                                                                                                             |  |  |  |
| 3.4. Does the lab have reference material ( <i>Trichinella</i> larvae stored in appropriate preservatives, pictures of different larva shapes)?                                 |  |  |  |
| <b>4. Laboratory facilities</b>                                                                                                                                                 |  |  |  |
| 4.1. Are the biosafety Level 2 guidelines followed by the laboratory?                                                                                                           |  |  |  |
| 4.2. If not, are the laboratory premises adequate for testing and for assuring personnel safety?                                                                                |  |  |  |
| 4.3. Is a system for the appropriate disposal of waste, digestion fluid, and meat scraps in place?                                                                              |  |  |  |
| <b>5. Equipment</b>                                                                                                                                                             |  |  |  |
| 5.1. Is the number of each type of apparatus related to the number of samples, which have to be tested per day/week?                                                            |  |  |  |
| 5.2. Is at least one apparatus for each type available for emergency situation?                                                                                                 |  |  |  |
| 5.2.1. Is this supply kept in working order with periodical maintenance?                                                                                                        |  |  |  |
| 5.3. Has the laboratory established the wear time of materials (e.g. knife, scissors, tweezers, blender, meat chopper, magnetic stirrer, sieves, glass containers)?             |  |  |  |
| 5.3.1. Are these materials periodically renewed, and a stock always available?                                                                                                  |  |  |  |
| 5.4. Are consumable materials (both disposable and chemical) stored in appropriate cabinets?                                                                                    |  |  |  |
| 5.4.1. Is a suitable stock available, taking into account the time between ordering and material delivery?                                                                      |  |  |  |
| 5.5. Is the expiration date of chemicals periodically checked?                                                                                                                  |  |  |  |
| 5.6. Are the storage conditions of pepsin and hydrochloric acid as established by the manufacturer followed?                                                                    |  |  |  |
| 5.7. Does the laboratory have a list of qualified suppliers for materials and apparatuses and for their maintenance and assistance (e.g., microscope, scale, magnetic stirrer)? |  |  |  |
| <b>6. Sample handling</b>                                                                                                                                                       |  |  |  |
| 6.1. Does the laboratory have a prescriptive QS document describing                                                                                                             |  |  |  |

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| sample handling, acceptability criteria, identification, storage, decontamination and disposal?                                                                                                                                                                                                                                                                                                                                                                                                       |  |  |  |
| 6.1.1. Does the laboratory manage unsuitable samples (e.g., low amount of muscle tissue, lack of identification code)? How?                                                                                                                                                                                                                                                                                                                                                                           |  |  |  |
| 6.2. Do the acceptability criteria fulfill the requirements of the Regulation (EU) 2015/1375 ( <i>Chapter II, article 2. Sampling of carcasses, and in Annex III. Examination of animals other than swine</i> )?                                                                                                                                                                                                                                                                                      |  |  |  |
| 6.3. Is the laboratory access under control?                                                                                                                                                                                                                                                                                                                                                                                                                                                          |  |  |  |
| <b>7. Traceability</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |  |  |  |
| 7.1. Does the laboratory have a prescriptive QS document on sample traceability from the carcass to the test report?                                                                                                                                                                                                                                                                                                                                                                                  |  |  |  |
| 7.2. Are samples univocally identified?                                                                                                                                                                                                                                                                                                                                                                                                                                                               |  |  |  |
| 7.3. Is the correlation between samples, test reports and customer assured?                                                                                                                                                                                                                                                                                                                                                                                                                           |  |  |  |
| 7.4. Are there evidences for identification, collection, indexing, access, filing, storage, maintenance and disposal of technical records?                                                                                                                                                                                                                                                                                                                                                            |  |  |  |
| 7.5. Are the records of original observations, personnel qualification and test reports retained?<br>By whom?<br>For how long?                                                                                                                                                                                                                                                                                                                                                                        |  |  |  |
| 7.6. Does the test report include:<br><ul style="list-style-type: none"> <li>• Animal species</li> <li>• Sample code</li> <li>• Test method performed</li> <li>• The amount of digested muscle per animal</li> <li>• The amount of undigested meat</li> <li>• Test results</li> <li>• The name of the analyst performing the test</li> <li>• Date and time of the test execution</li> <li>• Signature of the analyst</li> <li>• Signature of the supervisor</li> <li>• Comment/observation</li> </ul> |  |  |  |
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| 7.7. In case of positive results, does the prescriptive document (procedure) include:                                                                                                                                                                                                                                                                                                                                                                                                                 |  |  |  |
| <ul style="list-style-type: none"> <li>• Identification of positive sample/s</li> <li>• Full traceability of the positive sample up to the carcass</li> </ul>                                                                                                                                                                                                                                                                                                                                         |  |  |  |
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| <ul style="list-style-type: none"> <li>Collection, preservation of larvae in 90% ethyl alcohol and check the real presence of larvae in the vial</li> <li>Forwarding of the vial containing the larvae to the NRL or EURLP for species identification</li> </ul> |  |  |  |
| 7.7.1. Does the laboratory follow the ICT or OIE guidelines for the handling of positive samples and result management?                                                                                                                                          |  |  |  |
| <b>8. Test methods: Critical Control Points</b>                                                                                                                                                                                                                  |  |  |  |
| <i>8.1. Apparatus</i>                                                                                                                                                                                                                                            |  |  |  |
| 8.1.1. Is the chopping blade of the blender regularly inspected and/or changed?                                                                                                                                                                                  |  |  |  |
| 8.1.2. Is the sieve made of brass or stainless steel and of 180µm mesh size?                                                                                                                                                                                     |  |  |  |
| <ul style="list-style-type: none"> <li>Is it regularly and properly cleaned?</li> </ul>                                                                                                                                                                          |  |  |  |
| 8.1.3. Is the stereomicroscope, with a sub-stage transmitted adjustable light source, good enough to allow recognizing <i>Trichinella</i> larvae?                                                                                                                |  |  |  |
| <i>8.2. Consumables</i>                                                                                                                                                                                                                                          |  |  |  |
| 8.2.1. Are there appropriate pipettes for larva collection?                                                                                                                                                                                                      |  |  |  |
| 8.2.2. Are there small conical vials (1-1.5 ml) for storing larvae?                                                                                                                                                                                              |  |  |  |
| <i>8.3. Reagents</i>                                                                                                                                                                                                                                             |  |  |  |
| 8.3.1. Is the hydrochloric acid of the appropriate molar concentration?                                                                                                                                                                                          |  |  |  |
| 8.3.2. Pepsin: <ul style="list-style-type: none"> <li>Is the activity appropriate?</li> <li>Properly stored?</li> <li>The expiration date clearly reported?</li> </ul>                                                                                           |  |  |  |
| 8.3.3. Is there 90 % ethyl alcohol for larvae preservation?                                                                                                                                                                                                      |  |  |  |
| <i>8.4. Preparation of the digest fluid</i>                                                                                                                                                                                                                      |  |  |  |
| 8.4.1. Is the sequence of adding the components of the digest fluid respected? 1. water, 2. hydrochloric acid, and 3. Pepsin                                                                                                                                     |  |  |  |
| <i>8.5. Collection of the primary and secondary sediment</i>                                                                                                                                                                                                     |  |  |  |
| 8.5.1. Is the sedimentation time appropriate?                                                                                                                                                                                                                    |  |  |  |
| 8.5.2. Is the analyst able to evaluate if the digest fluid is not clear enough to be examined, requesting to perform additional washing steps?                                                                                                                   |  |  |  |
| <i>8.6. Microscopic examination</i>                                                                                                                                                                                                                              |  |  |  |
| 8.6.1. Were analysts trained to: <ul style="list-style-type: none"> <li>Recognize larvae in the counting basin or Petri dish?</li> <li>Immediately collect the larvae and preserve them in the conical vial under 90% ethyl alcohol?</li> </ul>                  |  |  |  |
| <i>8.7. Assuring the quality of test results</i>                                                                                                                                                                                                                 |  |  |  |

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| 8.7.1. Does the laboratory have a prescriptive QS document for the internal quality control of the materials and the critical points of the tests? |  |  |  |
| <b>9. Training of personnel</b>                                                                                                                    |  |  |  |
| 9.1 Was the personnel involved in the sample testing trained by participating in:                                                                  |  |  |  |
| • a quality control program of the tests used to detect <i>Trichinella</i> larvae?                                                                 |  |  |  |
| • a regular assessment of testing, recording and analysis procedures used in the laboratory?                                                       |  |  |  |
| <b>10. Proficiency testing</b>                                                                                                                     |  |  |  |
| 10.1. Did the analysts performing the test participate regularly to PT organized by the NRL?                                                       |  |  |  |
| 10.2. In case of a PT failure, the laboratory :                                                                                                    |  |  |  |
| • analyzes the causes of such failure?                                                                                                             |  |  |  |
| • implements adequate corrective actions?                                                                                                          |  |  |  |
| • repeats the test on further samples provided by the NRL or EURL?                                                                                 |  |  |  |