Guidance Document
for the organisation of Proficiency Tests by NRLs for national networks, including partial outsourcing

Drafted by EURLs *Campylobacter,* Coagulase Positive Staphylococci, *Listeria monocytogenes*, *Salmonella*, STEC

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DISCLAIMER: The organisation of proficiency tests by an NRL for the national network of official laboratories, including possible sub-contracting of certain PT steps, shall be approved by the National Competent Authority of its (EU) Member State.

0. Foreword

One of the duties of a National Reference Laboratory (NRL) is to organise where appropriate proficiency tests (PTs) for the national network of designated official laboratories (OLs) it coordinates, according to Regulation (EU) 2017/625 (article 101) [1].

For different reasons (too small or too large size of the network, economic aspects, availability of human resources etc.), some NRLs currently outsource (parts of) these PTs.

The five EU Reference Laboratories (EURLs) on *Campylobacter*, Coagulase Positive Staphylococci (CPS), *Listeria monocytogenes (Lm)*, *Salmonella* and Shiga-toxin-producing *Escherichia coli* (STEC), in agreement with their respective NRL networks, identified the need to draft a common guidance document, defining:

- criteria for NRLs to organize PTs in their respective scope (see clauses 2, 4);
- if PTs are outsourced (see clause 3):
    - criteria to outsource (parts of) PTs organised by NRLs for national networks, including PT steps that can be outsourced or not;
    - criteria to select PT providers.

This document gives only guidance, since organisation of PTs at national level is a Member State's responsibility.

This document is based upon an EURL *Listeria monocytogenes* document on the same topic.

1. Scope

This document gives guidance on the choice of technical criteria for NRLs to organise PTs on the following analyses, depending on the NRL mandate:

- Detection of *Campylobacter, Lm, Salmonella* and STEC in the food chain;
- Enumeration of *Campylobacter,* CPS and *Lm* in the food chain;
- (Sero)Typing of *Campylobacter*, *Salmonella*, *Lm*, CPS and STEC.

The content of this guidance document is based upon EN ISO 22117 [2] .

For several reasons (see Foreword), an NRL may decide, with the approval of its national Competent Authority (CA), to outsource some steps of the PTs it organises for the national network of official laboratories it coordinates.

The steps of PTs organised/supervised by an NRL that may be outsourced include:

- the steps related to test samples: preparation, homogeneity/stability studies, dispatch;
- collection and treatment of the results of the participants: calculation of individual performance results, preparation of PT report.

The follow-up of the results of the participants cannot be outsourced , including follow-up of deviating results:

- identification of corrective actions by the laboratories concerned;
- assessment of the corrective actions by the NRL.

In addition to PTs organised by NRLs, OLs may participate in PTs organised by other bodies (i.e. commercial PT schemes). A list of the PT schemes organised at an international level can be found for information at the website of the European PT Information System (EPTIS) database: https://www.eptis.bam.de/en/index.htm

In some cases, an NRL may request its OLs network to participate in a commercial PT scheme identified by the NRL which meets the criteria defined in this document.

2. General criteria for NRL PTs

2.1 Frequency

The NRL should define an annual plan for the PTs it organises/supervises. The PT frequency should preferably be at least 1/year per target microorganism.

2.2 Methods

The NRL should define the method(s) to be used in the NRL PT, according to the rules defined at national level by the CA and either of the alternatives:

- only a reference method, in particular defined in EC Regulation 2073/2005 [3];
- a choice between the reference method and alternative (including proprietary) methods, if validated/certified according to the relevant part of EN ISO 16140 series [4];
- both the reference method and an alternative method meeting the validation/certification conditions defined in the second indent, when the laboratory has the two methods in use;
- a method developed and/or recommended by the EURL/NRL.

3. General criteria for the selection of a PT provider

3.1 Quality management

The PT provider being outsourced by the NRL should preferably be accredited according to EN ISO 17043 [5] for the organisation of PTs in the relevant working field, if possible on the microorganism targeted by the PT.

If the PT provider is accredited, PTs should be conducted under accreditation.

3.2 Data to be provided to NRL

The PT provider should send to the NRL in a given time, for each PT and with the agreement of the concerned OLs:

- the results of each participating OL, with its identity;
- the value of individual performance parameters for each participating OL (see 4.3).

As an alternative, OLs (or the PT provider with OLs' agreement), can provide the NRL with the lab codes, so that the NRL can review the results of all OLs after receiving the complete table(s) of results from the PT provider.

When the PT includes participants other than OLs, the PT provider should also provide the NRL with a complete anonymous report of all participants.

4. Technical criteria for NRL PTs

In general, the prescriptions of EN ISO 22117 should be respected.

4.1 Homogeneity & stability studies

The requirements of EN ISO 22117 (clauses 6.2-6.5) should be followed for conducting homogeneity & stability studies of the material used to prepare the PT samples.

4.2 Contamination levels

4.2.1 For qualitative PTs (*Campylobacter, Listeria monocytogenes, Salmonella*, STEC)

- EN ISO 22117 recommends using 18 samples: 6 replicates at each of 3 levels (negative, low and high). Alternatively to this optimal design, the design below, derived from EN ISO 22117 but with 10 samples, can be used when the NRL organizes only one PT per year, for a given target microorganism. When the annual frequency of NRL PTs is higher, the total number of samples per PT may be reduced while maintaining the total number of samples per year (10 to 18).
- Design derived from EN ISO 22117 with 10 samples:
    - 3 levels: negative, low and high.
    - Negative level:
        - to verify the absence of false positive results, possibly due to cross-contamination at a participating laboratory. The target bacterium has to be not detected, other background flora can be present.
        - 2 replicates (2 samples) per participant.
    - Low level of the target microorganism:
        - Goal: to approach the regulatory limit, that is 1 cfu/25 g for *Salmonella, Listeria monocytogenes* and STEC. If there is no regulatory limit specified for the target microorganism or the matrix (e.g. *Campylobacter*), the goal should be to approach the expected LOD_{50} of the method and matrix. If this is not known, the value should be assumed to be equal to or lower than 1 cfu/test portion (the theoretical level of detection).
            - At such low level, microorganisms are distributed according to Poisson distribution, and a part of test portions may not be contaminated.
            - EN ISO 22117 provides an approach to deal with low level samples, which can be implemented for this purpose. A table provides a way to interpret the results: comparison of the proportion of positive replicates to the expected proportion according to the binomial distribution.
            - This low level may be difficult to achieve in practice. If it is not possible to reach the target level (regulatory limit or LOD_{50}), a level of up to 10 times this level would be acceptable (e.g. 1-10 cfu/test portion if the goal level is 1 cfu/test portion).
            - Certain existing PT schemes have multiple target microorganisms per sample, so that a level of 1 cfu/25 g cannot be reached for each microorganism.
        - 6 replicates (6 samples) per participant as a minimum

- High level of the target microorganism:
  - Level giving 100% positive results for all replicates.
  - Choose a level of approx. 5-10 times the low level (e.g. 50-100 cfu/test portion if the low level is 10 cfu/test portion).
  - 2 replicates (2 samples) per participant.

4.2.2 For quantitative PTs (*Campylobacter, Listeria monocytogenes, Coagulase Positive Staphylococci)*

- Design:
  - 4 levels: negative, low, medium and high.
    - May be reduced to 3 levels (see below).
  - 1 replicate/level.
- Negative level: see 4.2.1
- Low level: close to the enumeration limit of the method, but avoid that participants may obtain a count on a plate of <10 cfu, corresponding to an estimated number according to EN ISO 7218 [6].
- Medium level (optional).
- High level.
- The choice of the low, medium and high levels should take into account the regulatory limit(s) for the target microorganism and matrix.

4.3 Artificial contamination of samples

PT samples should preferably be contaminated by the PT provider, in order to better mimic conditions of natural contamination of food matrices. Alternatively, PT samples may be contaminated by each OL participant with stabilized target cells (e.g.: lyophilized cells) supplied by the PT provider and of unknown content. This second option may be required in certain cases, e.g. in case the viability of the target microorganism is not stable in the matrix during transportation.

In addition to the contamination with the target microorganism (4.2), the samples may also be contaminated at all levels with cross-reacting microorganisms, which may give false positive results at isolation stage with the method used.

Examples for the Standard methods EN ISO 11290-1&2 on *Listeria monocytogenes (Lm)* [7, 8]:

- *L. ivanovii* and *B. cereus*, which cannot be distinguished from *Lm* on isolation LOA agar and which require the confirmation step to be distinguished from *Lm*;
- *L. innocua* and *L. welshimeri*, which are often present in food and which may hinder *Lm* recognition on LOA agar.

4.4 Matrices for PT samples

- Preferable use of a 'real', relevant matrix, representing a matrix routinely analysed by the majority of the OLs participating in the PT scheme.
- Use of one matrix per PT trial.
- Matrices may be fresh, dried, lyophilised or frozen.
- To mimic 'real' samples, the matrix samples should preferably contain (i) background flora likely to be naturally present in the matrix considered and (ii) a flora competing with the target microorganism, which may hinder the target microorganism confirmation with the method used.

- The target microorganism should not be detected in the flora naturally present in the matrix.
- Artificial contamination of the matrix preferably with a stressed culture, representing the stress encountered in routine samples.
- Preferable to change the matrix regularly in successive PTs on the same target microorganism.

4.5 PTs for (sero)typing of microorganisms (*Salmonella*, *Lm*, CPS, *Campylobacter*, STEC)

For (sero)typing studies, EN ISO 22117 does not give any recommendation. Therefore, some guidance is given below.

The samples may be pure cultures (on a culture (transport) medium, lyophilized or frozen), or purified DNA of target strains. Choose well characterized strains and preferably (sero)types frequently found in routine samples, as well as closely related types.

The number of samples may vary per type of method. For example, for serotyping based on serology, the number of samples may be 10–20 per PT. For whole genome sequence (WGS)-based typing, this number may be reduced.

4.6 Criteria to assess individual performance

The following criteria are suggested to assess the individual performance of laboratories:

- Detection of target microorganism: percentage of expected results (negative or positive results) per participant.
- Enumeration of target microorganism:
  - $\log_{10}$ transformation of participants' results;
  - calculation of performance parameters as defined in EN ISO 22117 (z-scores and others).
- Typing of target microorganism: expected (sero)type.
- Trend analysis of results of successive PTs for the same target microorganism.

4.7 Transportation of samples

Biological samples containing microorganisms of risk class 2 shall be packed and sent according to IATA Regulations as Biological Substance Category B (UN 3373). This type of samples cannot be sent with regular post, but qualified courier services shall be used.

Higher risk class microorganism shall be sent as dangerous goods as Biological Substance Category A (UN 2814). Use a qualified organization/courier to send samples containing these high-risk class microorganisms.

References

[1] 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. Official Journal of the European Union L95: 7 April 2017. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0625&rid=3 (access date 26-02-2019).

[2] EN ISO 22117:2019 Microbiology of the food chain- Specific requirements and guidance for proficiency testing by interlaboratory comparison.

[3] Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Official Journal of the European Union L338: 22 December 2005. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R2073&qid=1518448728272&from=EN (access date 26-02-2019).

[4] EN ISO 16140 series. Microbiology of the food chain — Method validation, all parts.

[5] EN ISO 17043:2010. Conformity assessment - General requirements for proficiency testing.

[6] EN ISO 7218:2007. Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations.

[7] EN ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method.

[8] EN ISO 11290-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method.