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EPPO STANDARD - DIAGNOSTICS

PM 7/147 (1) Guidelines for the production of biological reference material

Specific scope: These guidelines include the steps that should be considered for the production of biological reference material and descriptors for such material.

These guidelines do not cover the management and maintenance of collections nor the production of certified reference material (the latter is covered in ISO 17034:2016). This Standard should be used in conjunction with PM 7/76 *Use of EPPO diagnostic protocols*[1].

Specific approval and amendment: Approved in 2021–09. Authors and contributors are given in the Acknowledgements section

1 | INTRODUCTION

Well-defined and characterized reference material is essential for internal and external quality checks (PM 7/98; EPPO, 2019), validation and verification of tests (including morphological identification) (PM 7/98; EPPO, 2019) and interlaboratory comparisons (PM 7/122; EPPO, 2014). In plant health, the commercial availability of reference material or certified reference material is very limited and consequently reference material often needs to be produced by individual diagnostic laboratories.

The aim of this Standard is to support laboratories in describing and producing biological reference material in a consistent manner. This Standard is based on the work performed in the framework of the VALITEST project. In this project, guidelines have been developed for the production of biological reference material to be used as panel samples in test performance studies (TPS). The approach developed in this framework was considered valid to produce other biological reference material such as positive and negative controls for routine testing and samples for proficiency testing or in validation/verification studies. Although non-biological materials (e.g. images of a diagnostic quality or sequence data) are important, they were not considered in VALITEST and are not covered by this Standard.

Reference material is defined in PM 7/76 (EPPO, 2018b) and biological reference material is described in PM 7/98 (EPPO, 2019). In the present Standard, the properties of biological reference material are further described by intended use, type of material/commutability, identity, traceability, purity, homogeneity, stability and assigned values. These descriptors are detailed in Section 4. The level of characterization required for each of the descriptors depends on the intended use of the material, which should be clearly defined beforehand. Standardization of the description and characterization of biological reference material aim to improve traceability and comparability of results obtained through its use.

The process for the production of biological reference material is described in Section 5. Briefly, the production process can be divided into the following steps:

- Describing the biological reference material and its required properties based on its intended use
- Planning of production
- Sourcing the candidate biological reference material
- Processing the candidate biological reference material
- Characterizing and performing final quality control of the candidate biological reference material
- If the material is to be shared with other laboratories, preparing a document summarizing the properties of the biological reference material in relation to its intended use and the processing steps.

Reference material should be produced in appropriate facilities. Specific guidance on handling quarantine organisms has been developed (see Table 1 in EPPO Standard PM 3/64 *Intentional import of live organisms that are plant pests or potential plant pests*) and specific regulations may apply in countries, e.g. Regulation (EU) 2016/2031[2] (EU, 2016).

2 | DEFINITIONS

Terms used in this Standard are defined in PM 7/76 *Use of EPPO diagnostic protocols* (EPPO, 2018b). However, further information on some terms is also given in PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* (EPPO, 2019) and PM 7/122 *Guidelines for the organization of*

[1]This Standard will be revised in 2023 based on experience following its use in laboratories until this date.

[2]The relevant articles in this Regulation are 60 to 64.

interlaboratory comparisons by plant pest diagnostic laboratories (EPPO, 2014).

3 | BASIC QUALITY MANAGEMENT REQUIREMENTS NEEDED TO PRODUCE BIOLOGICAL REFERENCE MATERIAL

The basic quality management requirements to produce biological reference material are described in EPPO Standard PM 7/84 *Basic requirements for quality management in plant pest diagnostic laboratories* (EPPO, 2018a).

4 | DESCRIPTORS OF BIOLOGICAL REFERENCE MATERIAL

The properties of biological reference material should be described. In Sections 4.1 to 4.8, information is given on how each descriptor should be characterized and documented. Descriptors may not be relevant for all types of reference material (e.g. pinned insects) or the relevance may depend on the intended use.[3] Thus, not all types of descriptors are always required. The level of characterization for each descriptor should be determined at the beginning of the production process based on the intended use of the biological reference material in order to plan the different production steps (see Table 1 and Section 5.2). The descriptors should be documented and if the material is shared with another laboratory, they should be made available in a document (e.g. technical sheet) (see Table 2 and Section 5.6).

4.1 | Intended use of biological reference material

The intended use of the biological reference material should be described and documented by detailing:

- The type of activities it will be used for (e.g. for internal and external quality checks, validation and verification, and inter-laboratory studies)
- The target organism
- Any limitations of its use (e.g. suitability for a specific method and/or test and matrix, when relevant).

Depending on the intended use of the biological reference material, different properties may be required. For example, the properties required for a positive amplification control for a real-time PCR test differ from the properties required for material used to determine the analytical sensitivity of a test that includes a DNA extraction step. Thus, the properties of the biological reference material for each of the descriptors listed in Section 4.2 to 4.8 should be defined based on its intended use.

[3]A table on the applicability of descriptors for different types of biological reference material will be prepared for the next revision of this Standard.

4.2 | Type of material/commutability

Commutability is defined by the level of agreement between the test results obtained with the biological reference material and the ones obtained with an authentic sample. In the plant health field commutability is described as how similar the biological reference material is to an authentic/naturally infested sample.

In general, commutability correlates positively with the complexity of the biological reference material (Fig. 1). Materials generally increase in commutability from (a) synthetic materials (e.g. synthetic nucleic acids or proteins), (b) extracts prepared from purified target organisms or individual pests/pure culture isolates, (c) spiked samples, (d) artificially inoculated plants and, finally, (e) naturally infested material. Spiked samples prepared by mixing the target organism (or parts thereof) with plant extracts are, for example, used to determine the analytical sensitivity of tests in bacteriology. Although they have a lower level of commutability than naturally infested material, spiked samples have the advantage that the concentrations of the target organisms in spiked samples can be precisely controlled.

While high commutability is often preferred it is not always necessary. For example, synthetic nucleic acids may be sufficient for a routine positive amplification control for molecular tests. Synthetic nucleic acids can be a good alternative for preparing biological reference material for inter-laboratory tests when the target organism or its nucleic acid is not available.

4.3 | Identity

The biological reference material should be identified unambiguously at the relevant taxonomic level based on appropriate methodologies and up-to-date taxonomy. If possible, two tests should be used that are based on different biological principles (e.g. molecular, serological, morphological etc.) and published in international, regional or national standards.

The identity should be documented as the name at a given taxonomic level together with the list of tests used for identification.

In cases where the biological reference material is prepared by mixing several components, the identity of each individual component should be determined and documented.

4.4 | Traceability

The traceability of the biological reference material covers information on the source of the material. If the material is obtained from a collection, it should be traceable to a specific specimen, isolate or strain in that collection. If possible, information is gathered on the history of maintenance and handling in that collection (e.g. lot number).

If material is not obtained from a collection, specific metadata should be documented whenever possible, i.e. when, where and by whom the material was collected, from which plant species and plant part, and whether or not the plant was showing symptoms (and what those symptoms were).

When the biological reference material is prepared by mixing several components, traceability should be documented for each component.

Preferably, the source of the biological reference material should be available over time and to a wider community to allow for a repeated production of similar biological reference material.

Depending on the source of the material, its use may be governed by the conditions under which it was obtained, e.g. covered under the Nagoya protocol (CBD, 2011) and material transfer agreements. These conditions should be met before the material can be further processed.

4.5 | Purity

Purity describes the components (including non-target organisms and, when relevant, components of the matrix) in the biological reference material that could interfere with the results of a test (e.g. leading to false-positive results).

Information on the purity of the biological reference material can be qualitative and defined based on the nature of its known components. When relevant, purity can be determined by testing a number of aliquots of the material using specific or generic tests. It is worth noting that often assessment of purity cannot be absolute.

When biological reference material is prepared by spiking targets into plant material or other matrices (e.g. soil), the prior absence of the target(s) from the plant material should be confirmed through testing.

In cases where plant material is used, it should be documented whether symptoms of any other organisms are present and what those symptoms are.

Purity is documented in a descriptive manner, e.g. the material contains only target organisms, the material contains material causing false-positive results in a specific test, etc. When purity is evaluated, information on the tests performed and the results should be documented.

4.6 | Homogeneity

Homogeneity of the biological reference material should be fit for its intended use. Material which lacks homogeneity may introduce measurement uncertainty, which may be inappropriately attributed to the test (in validation and TPS) or the lack of proficiency of the participant (in proficiency tests).

Depending on the use of the biological reference material, homogeneity can be determined qualitatively (e.g. positive controls should give positive results) or quantitatively (e.g. positive test results should be within a predefined range). Homogeneity should be determined experimentally with the test(s) for which the biological reference material is intended to be used. In general, it is assumed that homogeneity data are transferrable among tests targeting a specific organism and using a specific method (e.g. a nucleic acid extract determined homogeneous with a specific PCR test is considered homogeneous for all other molecular tests targeting the same organism). Preferably, homogeneity is determined after aliquoting. EPPO Standard PM 7/122 provides information on the assessment of homogeneity for different types of reference material used for inter-laboratory comparisons.

When relevant, homogeneity and how it was determined (e.g. number of aliquots, tests) should be documented with the associated measurement uncertainty.

4.7 | Stability

Biological reference material should be demonstrated to be sufficiently stable for its intended use to ensure that it will not undergo any significant change throughout its period of use, including storage and transport. Consequently, stability should be determined experimentally at different points in time or under different conditions, either with a test for which the biological reference material is intended to be used or a test which will provide information on the general stability of the biological reference material. The stability data obtained with a specific test is not always transferable to other tests using the same method. For example, as DNA often gets fragmented over time, DNA reference material will be stable for a longer period of time for a PCR test targeting a 200 bp fragment compared to a test targeting a 2000 bp fragment. Thus, the stability of the material used to assess different tests may be determined within the 'worst-case scenario' approach, using the PCR test targeting the longest DNA fragments. Preferably, stability is determined after aliquoting the biological reference material. Further information regarding the assessment of stability for different types of biological material used for inter-laboratory comparison is provided in EPPO Standard PM 7/122.

Stability should be documented together with the conditions and tests used to determine it. When relevant, the stability of the biological reference material should be documented together with the associated measurement uncertainty.

4.8 | Assigned values

Assigned values and their levels are defined in EPPO Standard PM 7/122. Assigned values can be qualitative (positive/negative) or quantitative (number of individuals, concentration, etc.).

They are usually determined experimentally and should be documented together with the approach and tests used and the number of aliquots tested. Where relevant, the assigned values should be documented together with the associated measurement uncertainty.

While it may not be always feasible or necessary, it is possible to provide information on the quantity of the target in the biological reference material. When producing biological reference material, the biologically relevant concentrations that can be found in naturally infested samples should be considered if known.

5 | DESCRIPTION OF THE PROCESS FOR THE PRODUCTION OF BIOLOGICAL REFERENCE MATERIAL

This section describes the process for the production of different types of biological reference material in plant health. The production process can be divided into the steps detailed in Sections 5.1 to 5.6. The flow chart in Fig. 2 aims to provide an overview of the necessary steps for production of biological reference material. The specific sequence of steps, as well as the number of quality control (QC) points, depends on the biological material and should be determined during the planning procedure. In addition, suitable tests used to characterize the biological reference material itself will vary with the nature of the biological reference material and the process described in this section is not exhaustive.

5.1 | Describe the biological reference material and its properties

At the start of the process, the list of descriptors is used to describe the biological reference material to be prepared, together with its intended use and the associated required properties (target values) (see Table 1). Note that the table provides a general guideline and not all descriptors are always necessary.

Whenever tests are needed it may be possible to determine different descriptors in a single test. To optimize the use of resources this should be taken into account during the planning.

5.2 | Plan the production

The production should be planned taking the biological reference material description and its required properties into account. It includes defining the source of the candidate biological reference material, a sequence of processing steps, a sequence of experiments to determine the properties of the biological reference material and a risk analysis to define critical points in the process at which quality control (QC) checks may be required or useful. The specific sequence of steps, as well as the number of QC points, depends on the biological reference material and on the source of the material. As identity is a very important property of a biological reference material, it is usually checked several times during the production process of the biological reference material.

TABLE 1 Use of the descriptors to plan the production process

Descriptor	Description of the biological reference material and planning
Intended use (see Section 4.1)	Describe the target organism, the type of study and limitations (e.g. method, test, matrix).
Type/commutability (see Section 4.2)	Describe the type of biological reference material to be produced, reflecting its commutability.
Identity (see Section 4.3)	Plan tests to identify the target organism at the appropriate taxonomic level.
Traceability (see Section 4.4)	Plan where the material to be used in production of the biological reference material will be sourced from.
Purity (see Section 4.5)	Plan the required level of purity for the reference material and the tests to be performed to determine purity.
Homogeneity (see Section 4.6)	Plan the tests to be used to determine homogeneity and the number of aliquots to be tested.
Stability (see Section 4.7)	Plan the tests to be used to determine stability and the number of aliquots to be tested.
Assigned values (see Section 4.8)	Plan the approach to determine the assigned values and the relevant experiments.

5.3 | Source of the components for the candidate biological reference material

The components of the biological reference material can come from various sources (e.g. collections, field material, sequence data; see Fig. 3). Each component of the material should be clearly identified and information on its traceability and identity should be documented. At this stage, purity is usually checked experimentally, if required.

5.4 | Process the candidate biological reference material

The source material(s) usually needs to be processed before being considered as candidate biological reference material.

All processing steps should be documented, including information on homogenization, comminuting in buffer, preparation of defined solutions (e.g. bacterial suspensions), extraction and purification of nucleic acids, mixing of components and slide mounting protocol, including the process of digestion of non-sclerified parts, possible staining steps, sample dehydration steps, fixation steps and slide mounting medium (Figure 3). If insufficient material is available for future use or if a different type of biological material is needed, the source material may need to go through one or more multiplication steps. This may include multiplication of target organisms in or on plants or on specific substrates or amplification of specific target genes.

5.5 | Characterize the candidate biological reference material and perform the final QC

During characterization, values of critical descriptors (e.g. identity, assigned values, homogeneity, purity, stability; Fig. 2) are determined and results are documented.

During the final quality control of the candidate biological reference material, all data on relevant descriptors collected in the previous steps should be cross-checked with the starting description and the required properties of the biological reference material. This allows an evidence-based decision to be taken on whether the produced material is fit for the intended use as defined in the first step.

If all required properties are met, the candidate biological reference material can be regarded as biological reference material.

If the candidate biological reference material does not meet all requirements, it should be rejected. It can, however, be repurposed. For example, if a material is not sufficiently homogenous with respect to the target concentration, it may be suitable for use as a positive isolation control. However, the repurposed material needs to include a revised definition of its intended use and a risk analysis of the available data to judge its suitability for the new intended use.

5.6 | Reporting information on the biological reference material

The description of the properties of the biological reference material for all descriptors (Table 2), a list of the processing steps and a declaration that the biological material is fit for the intended use should be made available to the laboratories

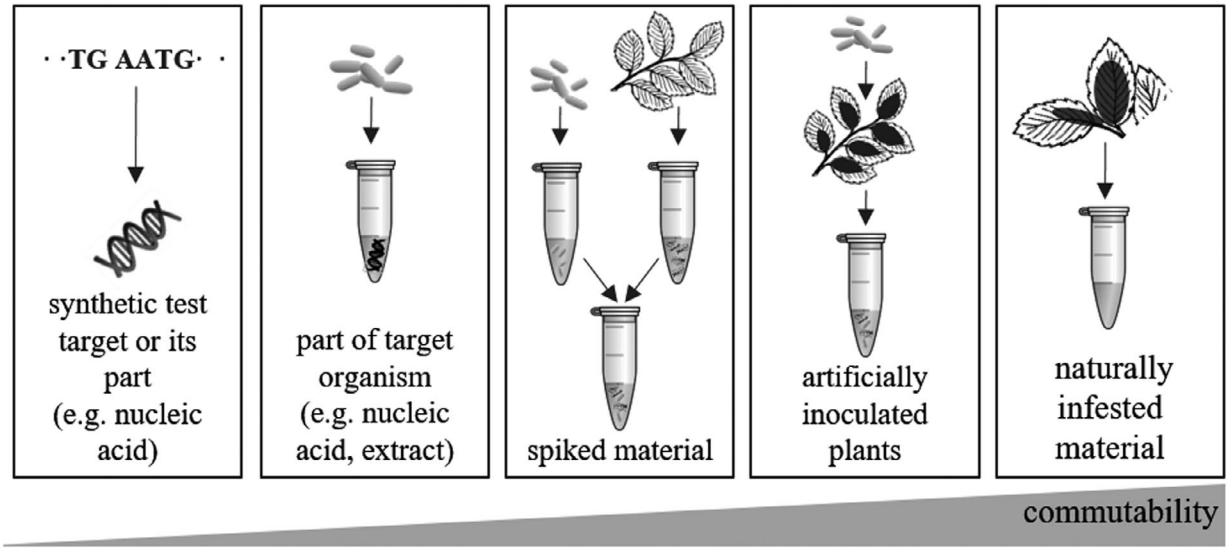

FIGURE 1 Biological reference material can vary in its commutability.
Note: the figure is illustrative rather than exhaustive and depicts some of the commonly used types of biological reference material

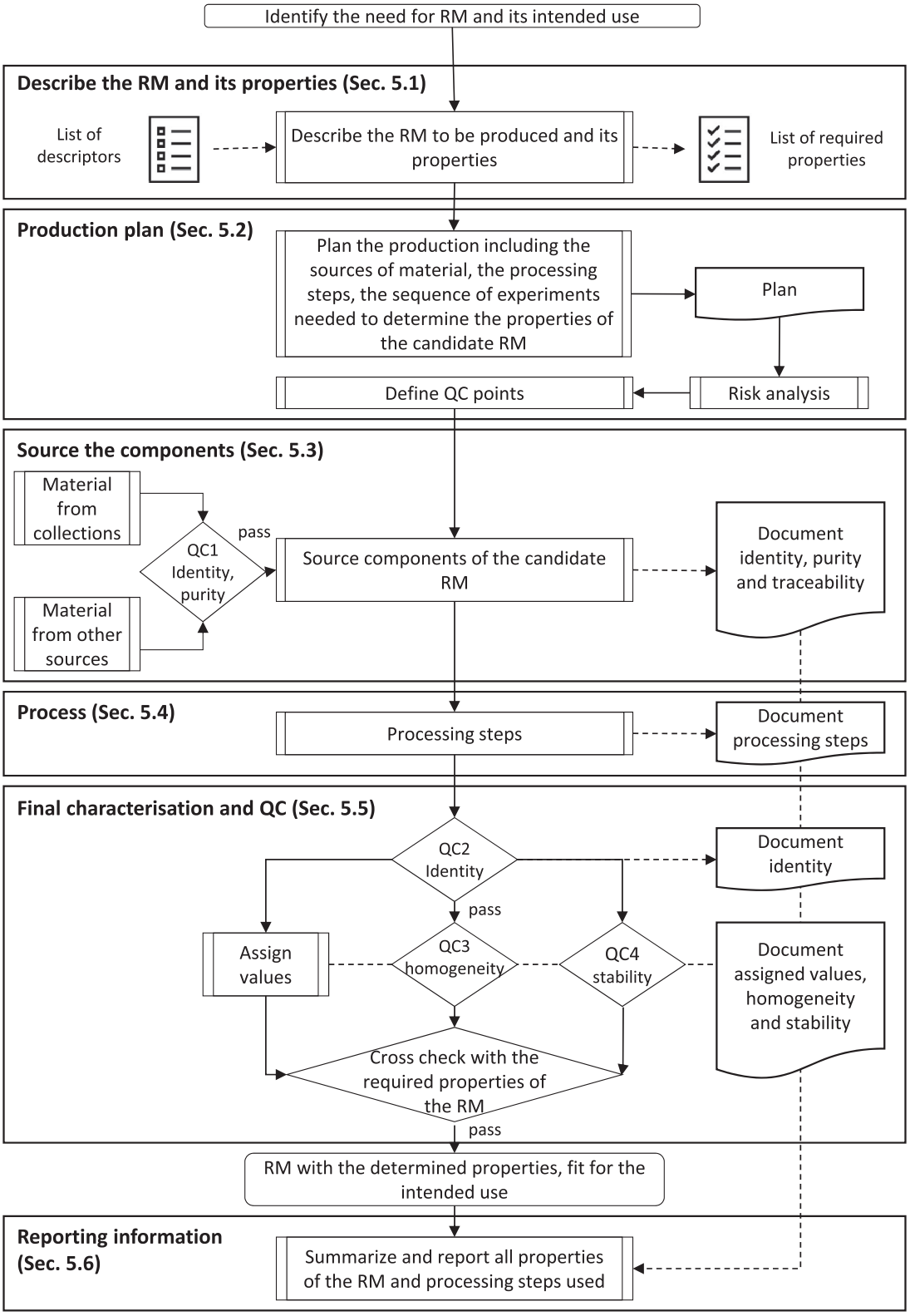

FIGURE 2 Graphical representation of the process for the production of biological reference material (RM). Additional information for the different blocks can be found in Sections 5.1 to 5.6. The specific sequence of steps, as well as the number of quality control (QC) points, depends on the biological reference material and its sources and should be determined during the planning procedure

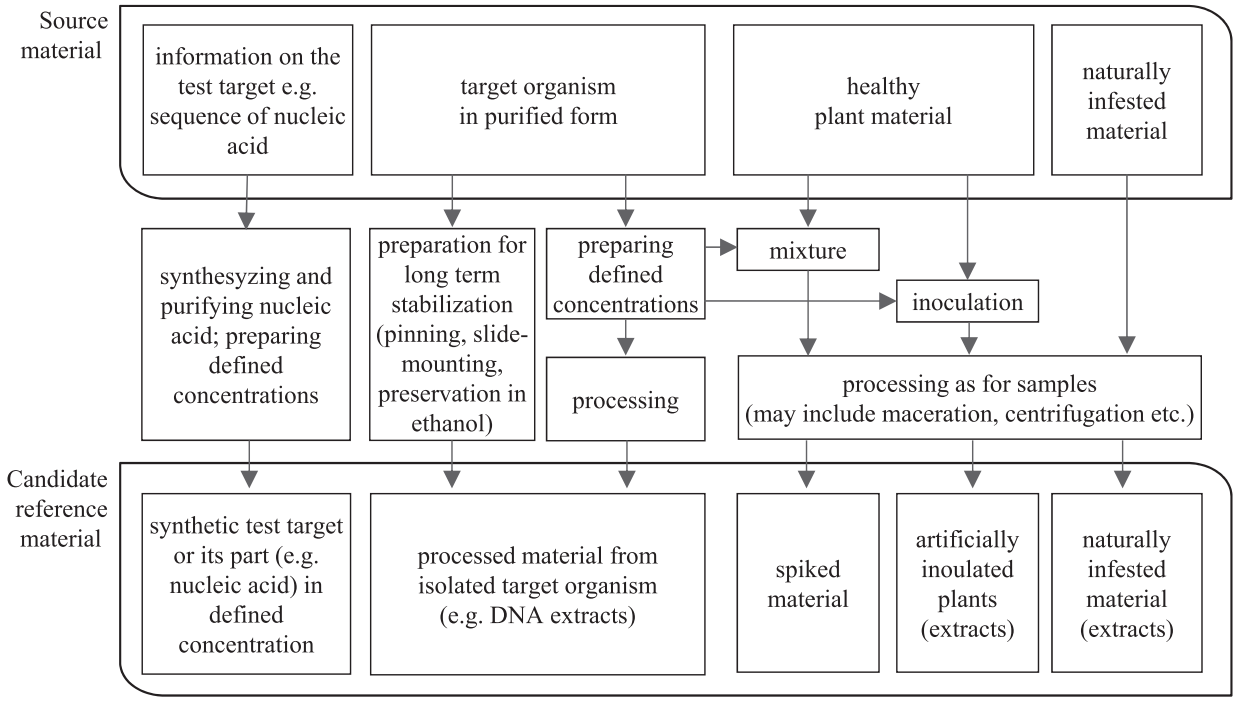

FIGURE 3 Possible sources and processing steps for different biological reference materials

TABLE 2 Summary of how the relevant descriptors of the biological reference material should be reported to the laboratories to which the material is supplied

Descriptor	Details to be included in the technical sheet
Intended use (see Section 4.1)	Amend the description if necessary.
Type/commutability (see Section 4.2)	Describe the type of biological reference material, reflecting its commutability.
Identity (see Section 4.3)	Report the preferred name and authority of the target organism contained in or used to produce the biological reference material. List tests used for identification.
Traceability (see Section 4.4)	Provide traceability to specific specimens, isolates or strains from a collection. For material not obtained from a collection, report relevant information which may include what, when, where and by whom the material was collected.
Purity (see Section 4.5)	Report the level of purity for the reference material, including the presence of cross-reacting material and the tests used to determine purity.
Homogeneity (see Section 4.6)	List the test(s) used to determine homogeneity and the results, including the associated measurement uncertainty, if available.
Stability (see Section 4.7)	List the test(s) used to determine stability and the results, including the associated measurement uncertainty, if available.
Assigned values (see Section 4.8)	Report the assigned values together with the associated measurement uncertainty, where relevant.

to which the biological reference material is supplied. Any limitations should also be included, e.g. expiry date. This can be done in the format of a technical sheet that accompanies the material. For biological reference material supplied during interlaboratory comparisons, this information is typically provided in the final report.

6 | FEEDBACK ON THIS STANDARD

If you have any feedback concerning this Diagnostic Standard, please contact diagnostics@eppo.int.

7 | PROTOCOL REVISION

An annual review process is in place to identify the need for revision of Diagnostic Standards. Standards identified as needing revision are marked as such on the EPPO website.

When errata and corrigenda are in press, this will also be marked on the website.

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