Introduction of the webinar and training activities

Test Performance Studies organisation

Videos	What is a TPS?	On the week 02/15
Videos	VALITEST TPS: selection of the pests and of the TPS organizers	On the week 02/15
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Videos	Reporting TPS results	To be confirmed/announced

VALITEST webinar series and training activities

Production of Reference Material for TPS

March 10, 2021

René van der Vlugt

Wageningen University and Research

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773139

This webinar

- What is Biological Reference Material and why do we need it?
- What different types of Biological Reference Material are there?
- What are the criteria for Biological Reference Material?
- How to produce Biological Reference Material?

- Questions in between (Polls)

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Question #1

- Do you use Reference material ?

    1. Yes
    2. No

Question #2

- What lab are you from?
    1. EU/National reference laboratorium
    2. Official diagnostic laboratorium
    3. Private/Commercial laboratorium
    4. Other

Why Reference Material?

- Reference Material (RM) provides essential traceability in (plant health) testing
- Internal and external quality checks (EPPO PM7/98)
- Validation and verification of tests (EPPO PM7/98)
- Interlaboratory comparisons (EPPO PM7/122)

Some background

- VALITEST: EU project on the validation of diagnostic tests for plant health (2018 – 2021, coordinated by ANSES).

- VALITEST WP3: Quality assurance of reference materials for validation purposes.
- Two main tasks in WP3:
  - Criteria for reference materials production for validation
  - Standard Operating Procedures (SOP) for the production of reference materials

What is Reference Material?

- Reference material (RM), as defined by the ISO is any material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process (ISO/Guide 30:2015).

- The term ‘reference material’ is a generic term, of which properties can be quantitative or qualitative.

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What is Reference Material?

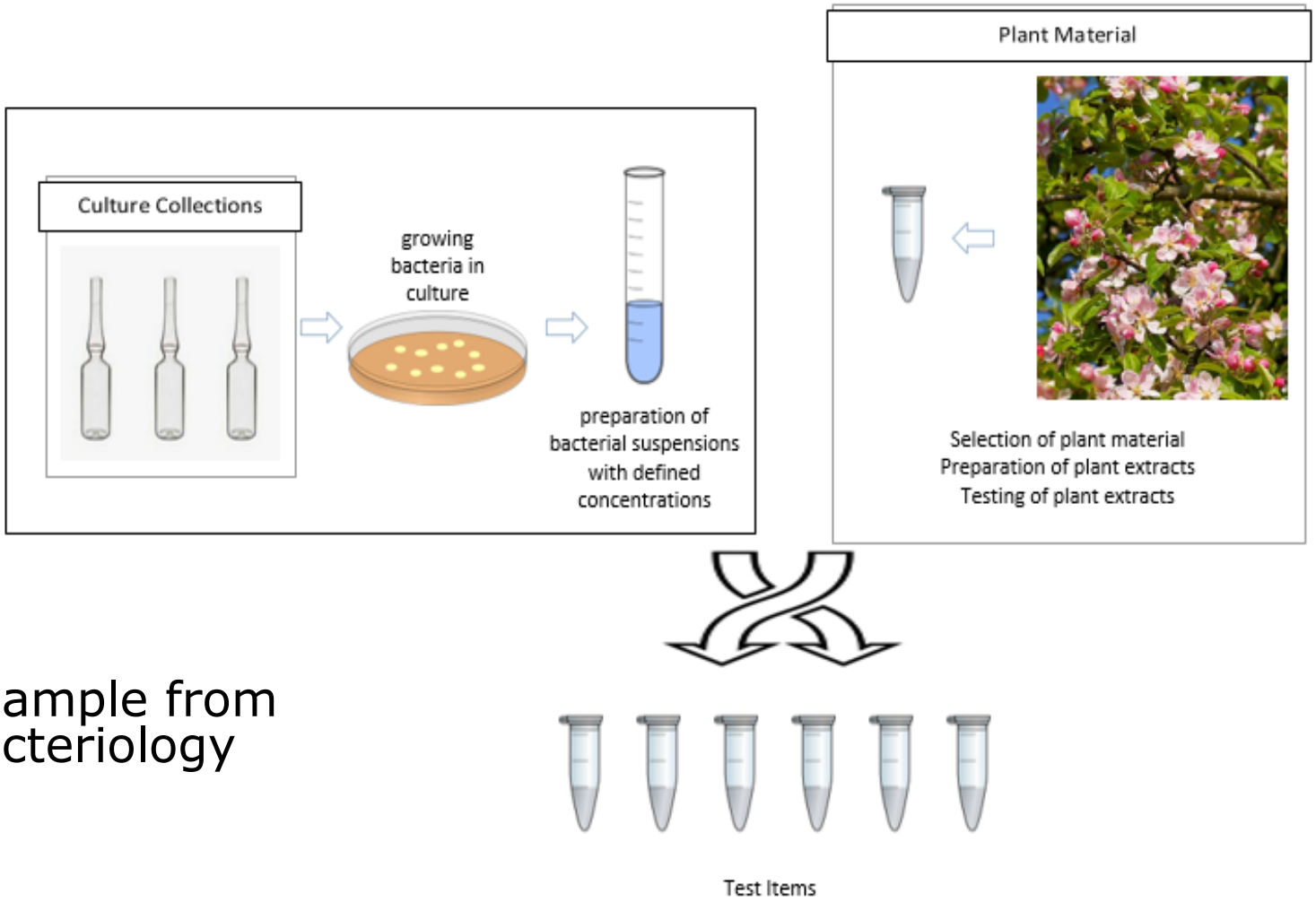
- ***EPPO PM 7/76 (5)****:* material appropriate to the test and diagnosis being performed
  - *live cultures, infested plant material, DNA/RNA preparations, images of a diagnostic quality or mounted specimens. The reference material used should be documented. It should be ensured that the material used is producing the features for which it was selected, for example expressing a desired antigen for use in serological diagnosis, or display specific physical features (e.g. sporulation) if used for morphological diagnosis.*

Where does RM come from?

- Diverse sources
  - Reference material is more reliable when sourced from an entity which certifies the authenticity and quality of the material.
- EU-project Q-collect:
  - Limited number of quality EU (reference) collections of quarantine plant pests and invasive plants.

- VALITEST WP3: the term RM is considered to encompass also the subsequent steps of preparing material.
  - Often culture collection material is mixed with a matrix to produce test items

Where does RM come from?



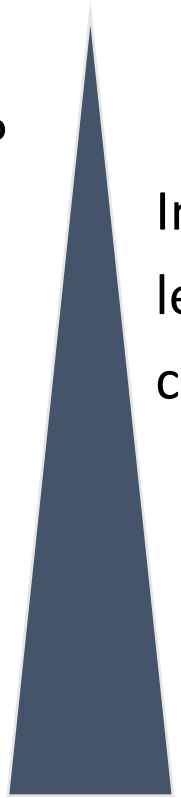
Example from bacteriology

Different types of RM

- Majority of pests not available in pure or cultured form.
- Different types of (candidate) biological RM defined:
  - naturally infested plant material
  - artificially infested plant material
  - spiked plant material
  - purified or isolated organisms
  - total nucleic acids from a sample (target organism in background)
  - purified nucleic acids
  - synthetic nucleic acids

Possible sources of (candidate) RM

- Field material
  - Naturally infested material. Identity? Purity? Co-infections? Concentration?
- Working collection material
  - Purity, Co-infections, Concentration?
- Reference Material
  - Materials from established collections, generally well characterized
- Certified Reference Material
  - Complies with ISO 17034:2016



Increasing levels of characterization

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Question #3

- Where do you get your RM from?
    1. Field material
    2. Working collection(s)
    3. Established collection(s)
    4. Reference collection (ISO17034:2016)

Is my reference material suitable?

- Determine and define its intended use
  - Clear define beforehand how and in what test (format) the RM is going to be used.
  - Describe and document
    - Type of study it is aimed for
    - Target pest or organism
    - Limitations of the specified use

- Desired properties depend on its intended use !

Identity of the Reference Material

- The biological reference material should be identified unambiguously at the relevant taxonomic level based on appropriate methodologies and up to date taxonomy.
- Use preferably 2 independent tests (different biological principles: e.g. PCR and ELISA).
- Identity should be documented (taxonomic level) together with list of tests used for identification.
- In case it is prepared by mixing, the identity of each component should be determined and documented.

Criteria for biological RM

- A defined set of descriptors is a basis for efficient collecting and comparison of data.
- Contributes to data (results) being **F**indable, **A**ccessible, **I**nteroperable and **R**e-usable (FAIR).
- Since criteria are inherently linked to the intended use of the reference material and may be test-specific, the criteria may be different for different uses and should be defined by the producer.

Criteria for biological RM

- Valitest WP3 defined a set of descriptors/criteria (D3.1)
  - Scope/intended use
  - Identity
  - Traceability
  - Commutability
  - Purity
  - Homogenity
  - Stability
  - Assigned values

Not all descriptors/criteria need to apply to a particular RM

www.valitest.eu/work_packages ➡ WP3

Scope/intended use

- Scope/intended use: a vital part of defining criteria for the reference materials. Should be defined prior to its preparation.
  - Why do you need the data?
  - What pest/pathogen are you going test for?
  - What is the testmethod you are going to use?
    - Morphology, serology, molecular?
  - Are there any specific limitations to its use?
    - Method, preparation, matrix?

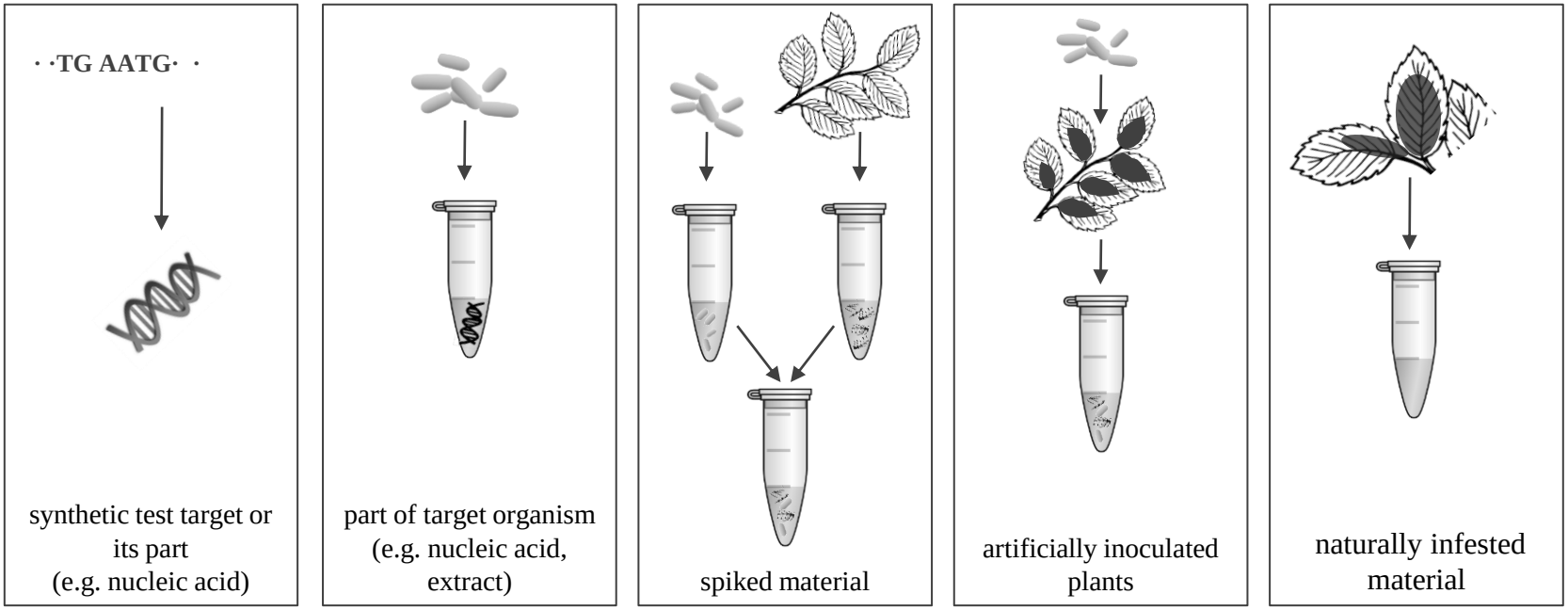
Traceability of RM

- Traceability of the biological reference material covers (documented) information on the source of the material.
  - Collection material: traceable to a specific specimen, isolate or strain. If possible, information on history of maintenance and handling in that collection (e.g. lot number).
  - For non-collection material, specific metadata should be documented whenever possible i.e. when, where and by whom the material was collected, from what plant species and part and whether or not it was showing which symptoms.
- Additional restriction? (Nagoya, MTAs etc.)

Commutability of RM

- Commutability: how similar is the biological reference material to an authentic sample.
  - To what extend does your RM resembles a ‘real-world sample’?
- Commutability is defined by the level of agreement between the test results obtained with a biological reference material and the ones obtained with an authentic sample.
  - Do the results of your ‘real-world sample’ match with the reference material?

Commutability of RM



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Different levels of commutability of Reference Material

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Purity of RM

- Describes the presence (or absence) of components (including non-target organisms and when relevant components of the matrix) that could interfere with the results of a test (false positive results)
- Document how purity was determined
  - Often assessment can not be absolute
  - Documented in a descriptive manner
    - Contains only target organism
    - Also contains material causing possible false-positives

Homogeneity of RM

- Biological reference material should be homogeneous
  - If not it may introduce measurement uncertainty and affect interpretation of test results (validation or TPS) or proficiency (in proficiency tests)
- May be determined
  - Qualitatively: Pos. Control should be positive
  - Quantitatively: Pos. test result must be within pre-defined range
- Must be determined with the same test (see also EPPO PM7/122)

Stability of RM

- Sufficiently stable: Not undergo any significant change throughout its period of use, including storage and transport.
- Should be determined experimentally at different points in time or under different conditions, either with a test:
  - intended to be used
  - which will provide information on the general stability
- Should be documented together with the conditions and tests used

Assigned values of RM

- EPPO PM7/122 (Guidelines interlabor. comparisons, pgr. 3.6)
- In the plant health field, assigned values correspond to the expected result of the test (pest present or absent, concentration of the pest, morphological characteristics of the specimen, etc.).
  - Known value: Present/absent, high/low, …
- Need to be assigned in a previously described procedure and uncertainty needs to be defined

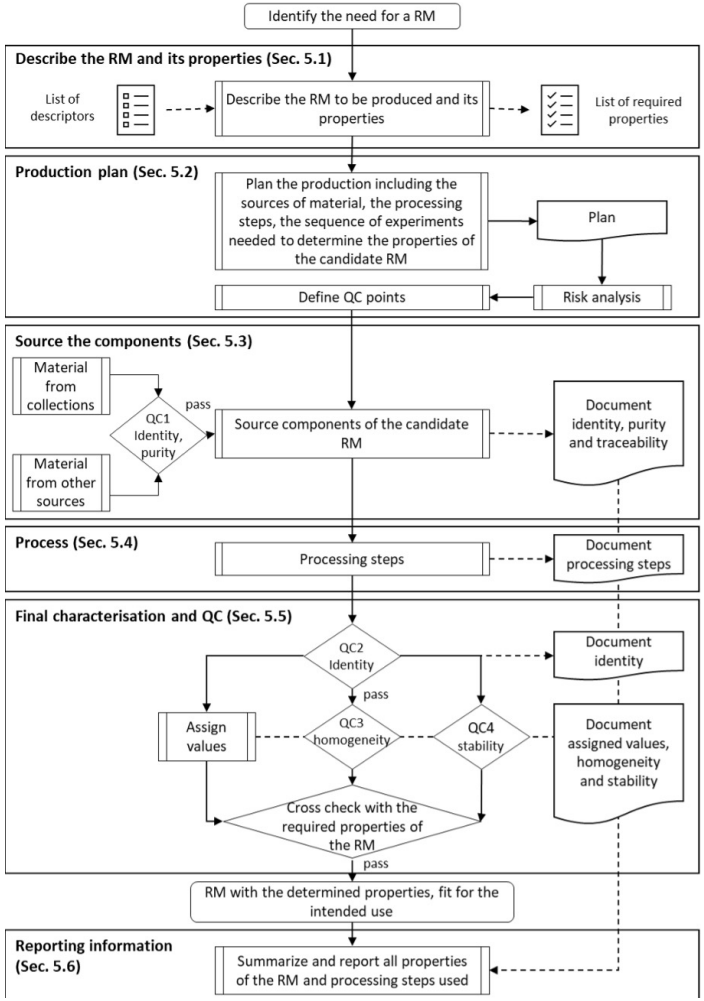
Question #4

- Do you produce your own reference material?

    1. YES
    2. NO

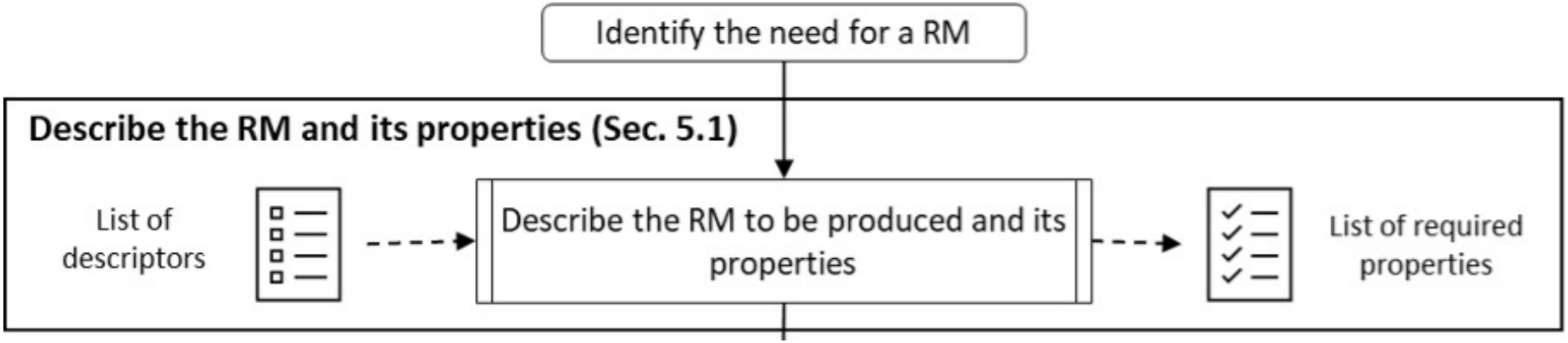
Process for production of RM

- Flow chart of different steps
  - Based on SOP from Valitest WP3 (D3.3; www.valitest.eu)
  - Specific sequence and quality control (QC) of steps depends on the biological reference material and its source
  - Should be determined in planning phase



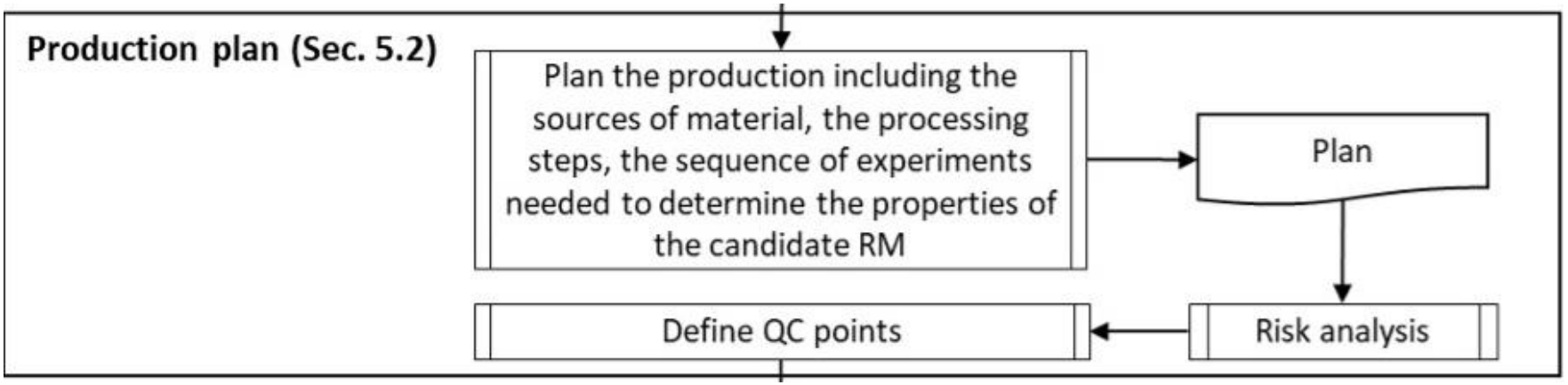
Process for production of RM

- Different steps
    1. Identify the need for the RM
    2. Describe the RM and its (desired) properties



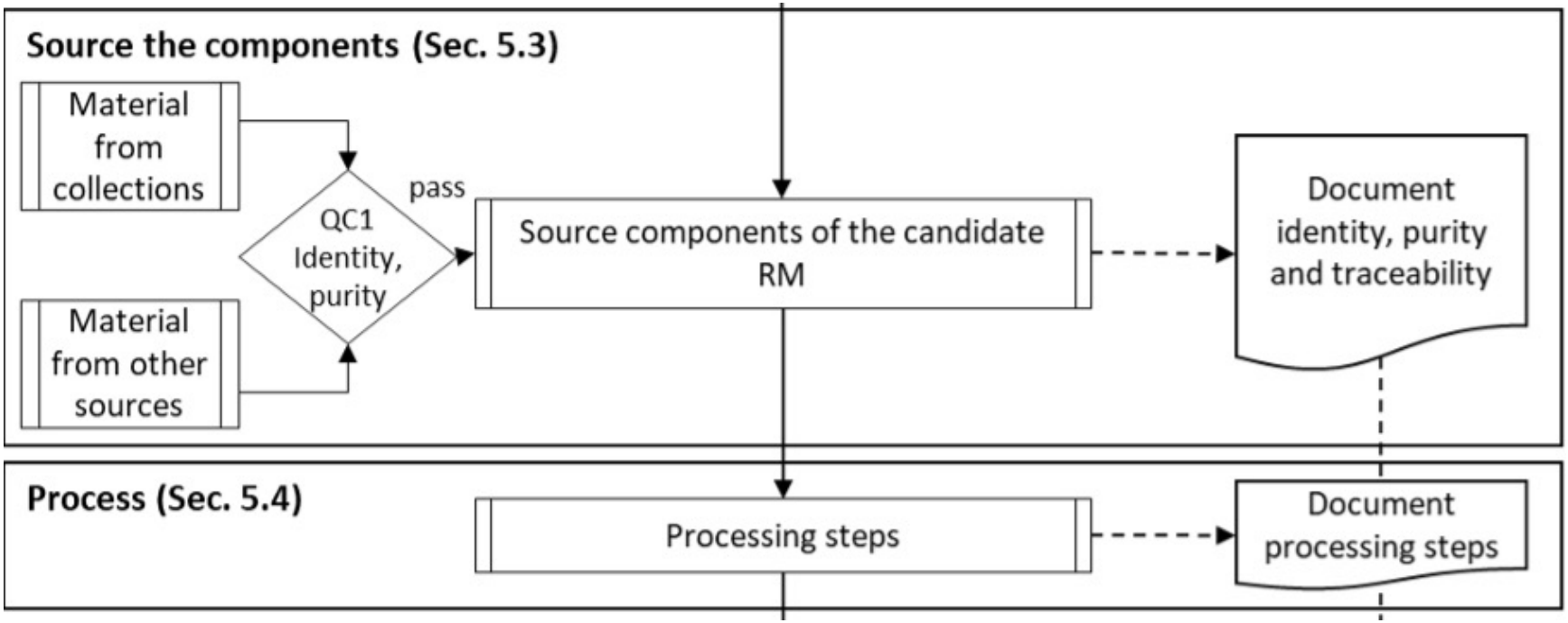
Process for production of RM

3. Plan production (include risk analysis and pre-define your QC points in production phase)
    - Identity is important property



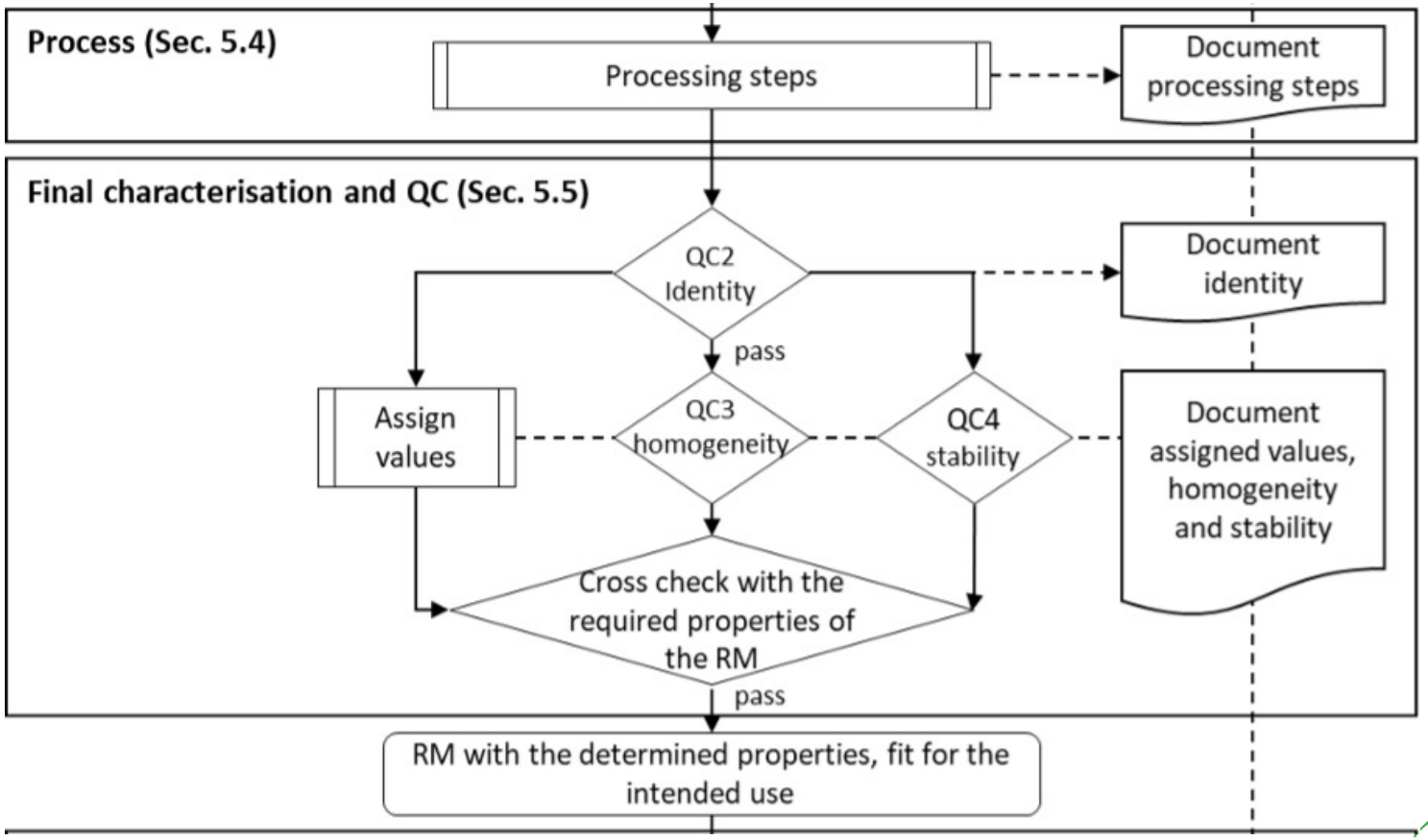
Process for production of RM

4. Source the components of the RM and process (e.g multiplication)



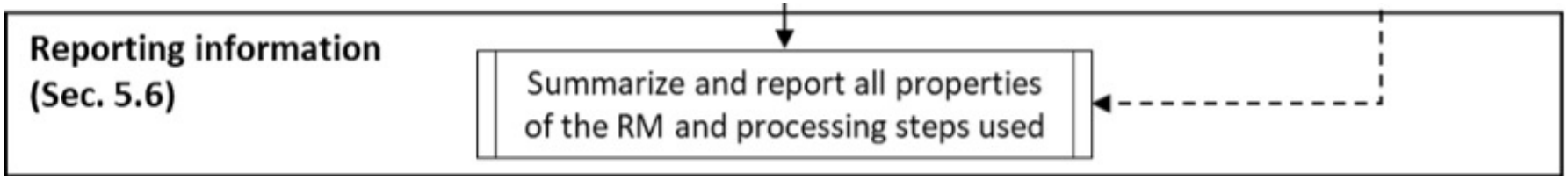
Process for production of RM

5. Characterisation of descriptors/criteria including QC



Process for production of RM

6. Report properties of RM and the processing steps, including any limitations (e.g. date of use…….). Can be reported through technical sheet.



In conclusion

- Biological RM may consist of different types of material and can come from different sources
- Its intended use should be clearly defined as it determines its required properties
- Different descriptors/criteria are used to describe its properties
- Production should follow specific steps (SOP)
- Documentation of materials and process is important

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Thank you for your attention!

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