

Guidance for the production of RMs with qualitative properties

An introduction to ISO 33406

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ISO 33406:2024

Approaches for the production of reference materials with qualitative properties



ISO/TC334 WG13*

-  **77** Working group members
- 13** Standardisation bodies
- 5** Liaison representations (incl. Eurachem, EC-JRC)

* Status at ISO 33406 finalisation

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<https://www.iso.org>



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RM production - ISO 17034, ISO 33405, ISO 33406

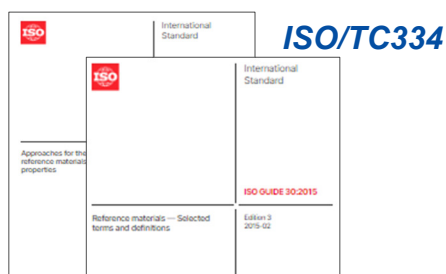
<p>RMs with quantitative property value(s)</p>	<p>ISO 17034:2016 <i>General requirements for the competence of RMPs*</i> :</p>	<p>ISO 33405:2024 <i>Approaches for characterization and assessment of homogeneity and stability:</i></p> <ul style="list-style-type: none"> • guidance, valid approaches 	
<p>RMs with qualitative property value(s)</p>	<ul style="list-style-type: none"> • requirements, that must be met • use of 'shall' 	<ul style="list-style-type: none"> • General technical details, e.g. characterization study, documentary traceability 	<p>ISO 33406:2024 <i>Approaches for the production of RMs with qualitative properties:</i></p> <ul style="list-style-type: none"> • guidance • use of 'should'

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* reference material producer (RMP)

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Different terminology



ISO Guide 30:2015
(in revision as ISO 33400)

*Reference materials -
Selected terms and definitions*



ISO/IEC Guide 99:2007
JCGM 200:2012 (VIM3)
(VIM4 under development)

*International vocabulary of
metrology — Basic and
general concepts and
associated terms (VIM)*

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Terminology & focus of ISO 33406*

‘**Qualitative property**’ as super-ordinate for ‘**nominal property**’ and ‘**ordinal property**’

‘**Categorical property**’ being a synonym for ‘qualitative property’

- ISO 33406 has a focus on nominal properties.
- ‘Examination’ and ‘determination’ used for qualitative analysis.

*‘A **nominal property** has values that divide the set of materials that have it into classes ... the **only comparison** that can be made between values of the property is of whether they are **identical or different**.
... **ordinal property** ... the comparisons that can be made between two values of the property are of relative rank order, i.e. whether **one is lower, equal to or higher than the other.**’*

ISO 33406, Section 4

* *Terms are not defined but explained in the introduction.*

6 *Terms are intended to be defined in ISO 33400 (successor of ISO Guide 30).*



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Approaches in ISO 33406

Section 5: Meeting technical and production requirements:

- Characterization, incl. traceability and confidence in qualitative values
- Assessment of homogeneity, stability, commutability



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ISO 33406 characterisation approaches include ... (Section 5.1.1.3)

... using **qualitative determination(s)** • *can depend on comparison with reference data or another value-assigned RM (5.2.3.1)*

... based on **provenance*** (5.1.2) • *knowledge of the origin of the material (5.1.2.1)*
• *confirmed by additional evidence (5.1.2.3)*

... using **measurements** of quantitative properties (5.1.3) • *measurement results from methods to characterize the identity (5.1.3.1)*
• *as different substances can share the same measurement response, info about the source of the raw material is vital (5.1.3.1)*

.... by a **combination of methods*** (5.1.4) • *suitable for chemical substances of small to medium molecular mass (5.1.4.1)*
• *methods should probe different properties (5.1.4.1, e.g. pharmacopoeia criteria for identification)*

* ISO 33406, Annex A: Examples of DNA sequence and protein RMs

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Metrological traceability applies

... when a **quantitative measurement** is carried out.

- ISO 33406 notes that for quantitative measurements, measurement uncertainty and metrological traceability are relevant.
- ISO/IEC 17025 applies to the determination of quantitative and qualitative value assignments if they are based on measurement of a quantity.



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similarly

Traceability applies

(a) when a qualitative property value is established by **comparison with a reference**.

- ISO 33406 recommends to state the reference used for the comparison.

'The reference will not be a unit of the International System of Units (SI) but some reference recognized by the relevant scientific community ... This can be an artefact ... or authoritative data ...'

ISO 33406, Section 5.2.1



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and similarly

Traceability applies

(b) ... when a qualitative property value depends on provenance.

*'An RM may be characterized based on ... provenance of the material. ... (the) RMP should obtain **documentary or other evidence of the origin** of the material that shows an unbroken chain of evidence from origin to final packaging.'*

ISO 33406, Section 5.1.2.1

*'... qualitative properties can be **assigned through the provenance** of the material.'*

ISO 33406, Section 5.2.1

*'Documentation of provenance ... is sometimes referred to as **trackability** ...'*

ISO 33406, Section 5.2.1



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Measurement uncertainty applies

... when a **quantitative measurement** is carried out.

- ISO 33406 notes that for quantitative measurements, measurement uncertainty and metrological traceability are relevant.
- ISO 33406 reminds that the measurement uncertainties need to be sufficiently small to have no significant impact on the confidence in the assigned qualitative value.



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similarly

Confidence ...

*'... refers to the **degree of belief** one has in the value assigned to a qualitative property.'*

ISO 33406, Section 5.3.1.2

- no methodology for assigning an uncertainty to qualitative property values exist and uncertainty statements remain **optional**
- expressions of confidence are **helpful** for RM user
- ISO 33406 strongly recommends to certify only if a **sufficiently high confidence** for the intended use is given

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*'Every assignment of value to a property is surrounded by **uncertainty**.'*

ISO 33406, Section 5.3.1.1

*'In particular, where assigned qualitative values are **certified** the RMP should clearly **state** the **justification** for their **confidence** in the value.'*

ISO 33406, Section 5.3.1.2



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Expressing confidence in a qualitative property

- qualitatively, using an ordinal scale

- **MOST CONFIDENT** (have very well-supported and well-resolved phylogeny and/or multiple diagnostic nucleotides differentiating species from closest relatives; have data from multiple samples of both an inclusivity and exclusivity panel; data from multiple independent gene regions agree).
- **VERY CONFIDENT**
- **CONFIDENT**
- **AMBIGUOUS**

ISO 33406, Annex B: Example of *Ginkgo biloba* (leaves), NIST CRM
<https://tsapps.nist.gov/srmext/certificates/3246.pdf>

- or, quantitatively, e.g. as a likelihood ratio or as a probability distribution

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General statistical considerations* (Section 5.4.2)

- Methods returning **quantitative** information should be **preferred** (more information contained in the test result).
- Methods with a **high reliability** should be chosen (test failure vs. defect RM difficult to distinguish).
- Methods should be chosen to **accommodate low or zero proportions of discordant responses**.

‘... few statistical approaches have been widely applied to the characterization of qualitative properties.’

ISO 33406, Section 5.4.1



15 * Examples of statistical approaches are given in ISO 33406, Annex C



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Assessment of homogeneity* (Section 5.5)

Mandatory for all assigned values (ISO 17034), test methods that return a qualitative result (ISO 33406, 5.5.2.2):

- **Increased number of RM units** for a narrow confidence interval for the estimated proportion of defective units.
- Test methods with **negligible false response rate do not require replicate** testing.

Confidence interval for proportion of non-compliant RM units, procedures for low proportions (5.5.3.2)

Inspection by attributes* (ISO 2859-1 and ISO 2859-4) for RMs produced in frequent repeated batches (5.5.3.3)

Two-way contingency table analysis* for testing significant differences in the probability of a negative response (5.5.3.4)

Categorical analysis of variance* (CATANOVA) as test for a significant between-unit difference (5.5.3.5)

‘... should be assessed as response to test methods directly targeting the assigned nominal property value(s) and to any other methods listed in the intended use ...’

ISO 33406, Section 5.5.1.3

16 * Examples of homogeneity studies are given in ISO 33406, Annex D, LGC meat species RM



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Assessment of stability* (Section 5.6)

Mandatory for all assigned values (ISO 17034), where experimental stability study is required (ISO 33406, 5.6.1.3):

- determine the **change in proportion of units** responding correctly to qualitative test methods
- or, study **quantitative properties that can indicate degradation** of the material.

Contingency table analysis with numbers of unaffected and failed RM units at each time point in a stability design (5.6.2.3)

Regression of failure probability (5.6.2.4)

Survival analysis (reliability analysis) analysis of cumulative failures over time (5.6.2.5)

* *Examples of stability studies in ISO 33406*

17 *Annex D, LGC meat species RM*

*'... should be assessed in terms of stability of response to test methods **targeting the assigned qualitative property value(s)**.'*

ISO 33406, Section 5.6.1.2

*'It can be helpful ... to provide **information regarding the methods and properties used to investigate the stability of the material.**'*

ISO 33406, Note to Section 5.6.1.3

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Assessment of commutability ... (Section 5.7)

... is (also) important for qualitative property RMs that are **intended for use as positive or negative controls** in a qualitative testing procedure (5.7.2.2).

Note ... ISO 15194 requires evaluation of the commutability of certified RMs for in vitro diagnostics procedures.

ISO 33406, Section 5.7.1.2

*'The commutability of an RM relates to the **ability ... to act as a calibrator or quality control material for a second measurement or testing procedure applied to routine test materials.**'*

ISO 33406, Section 5.7.1.1

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Commutability assessment should include ...

'... one or more of the following:

- **consideration** of all ... properties of the material that could reasonably affect commutability, including the particular characteristics certified and any processing or other treatment of the RM ...;
- **review of published literature data** on commutability of closely related materials;
- **planned experiments** to test commutability.

ISO 33406, Section 5.7.2.2

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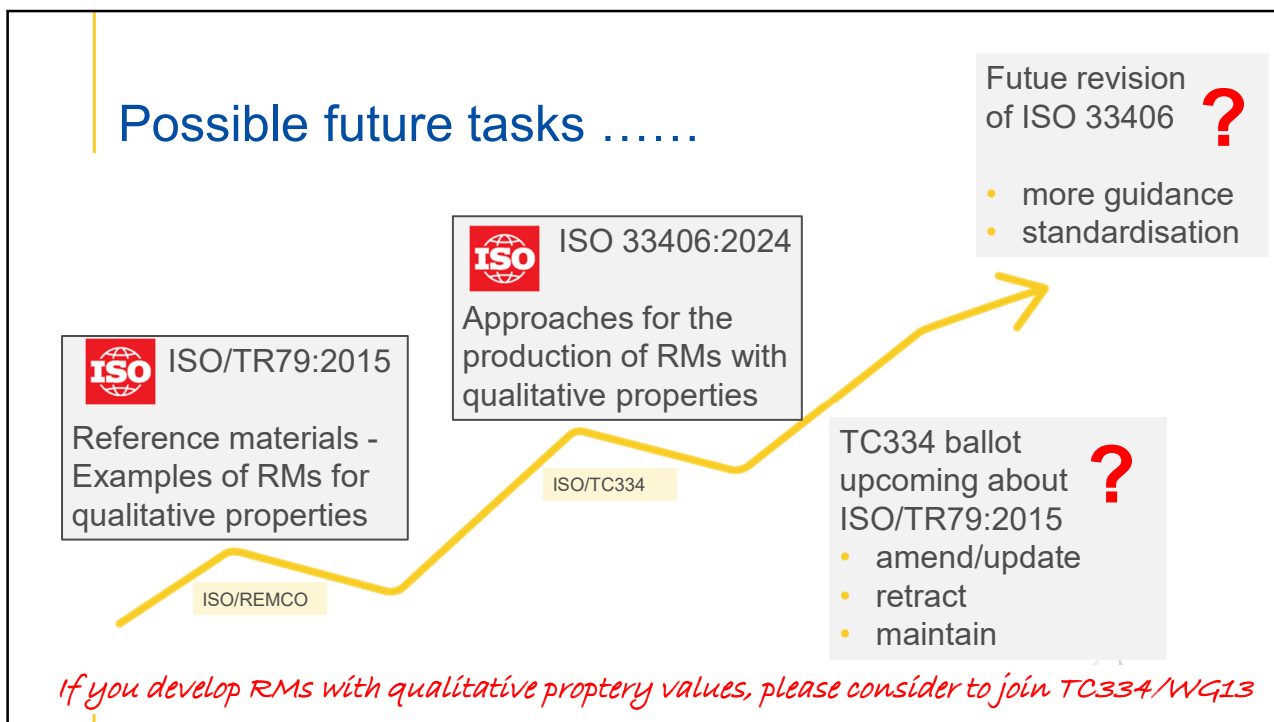
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Informative Annexes ISO 33406

- Annex A Guidance for DNA and protein RMs
- Annex B Expressing confidence in qualitative values
- Annex C Statistical procedures
- Annex D Examples of homogeneity and stability studies of RMs with qualitative properties



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Thank you for your attention!

THANK YOU

to the numerous WG13 members (and their patience), without them ISO 33406 would not exist!

ISO 33406:2024 Approaches for the production of reference materials with qualitative properties

S Trapmann, SLR Ellison, WC Davis, A Possolo, J Zheng, A Botha (2024) Approaches for the production of reference materials with qualitative properties – The new International Standard ISO 33406, Accred Qual Assur

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