Nederlandse Voedsel- en Warenautoriteit
Ministerie van Economische Zaken

Validation of alternative microbiological methods - the ISO 16140 series.

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ISO TC 34/SC 9/WG 3

- In 2003 the ‘old’ ISO 16140 (validation of alternative methods) was published.
- This standard was published after 10 years of development.
- Starting point was the EU (EURECA) project called Microval.
- In 2005 it became clear that there was a need to revise the 16140 and there was a need for more standards on validation.
- SC9 decided in 2006 to set up a working group for this.
- This working groups started in 2006 with the following mandate:

ISO TC 34/SC 9/WG 3

WG 3 (method validation) mandate:

- Revision ISO 16140 (2003)
- Development standard on verification
- Development standard on validation standardised reference methods
- Development standard on single lab validation
- Development standard on intermediate validation
- Development standard on validation confirmation methods

Standards (to be) published prepared by WG3

ISO 16140-1: **Vocabulary** (publised)

ISO 16140-2: **Protocol for the validation of alternative (proprietary) methods against a reference method** (publised)

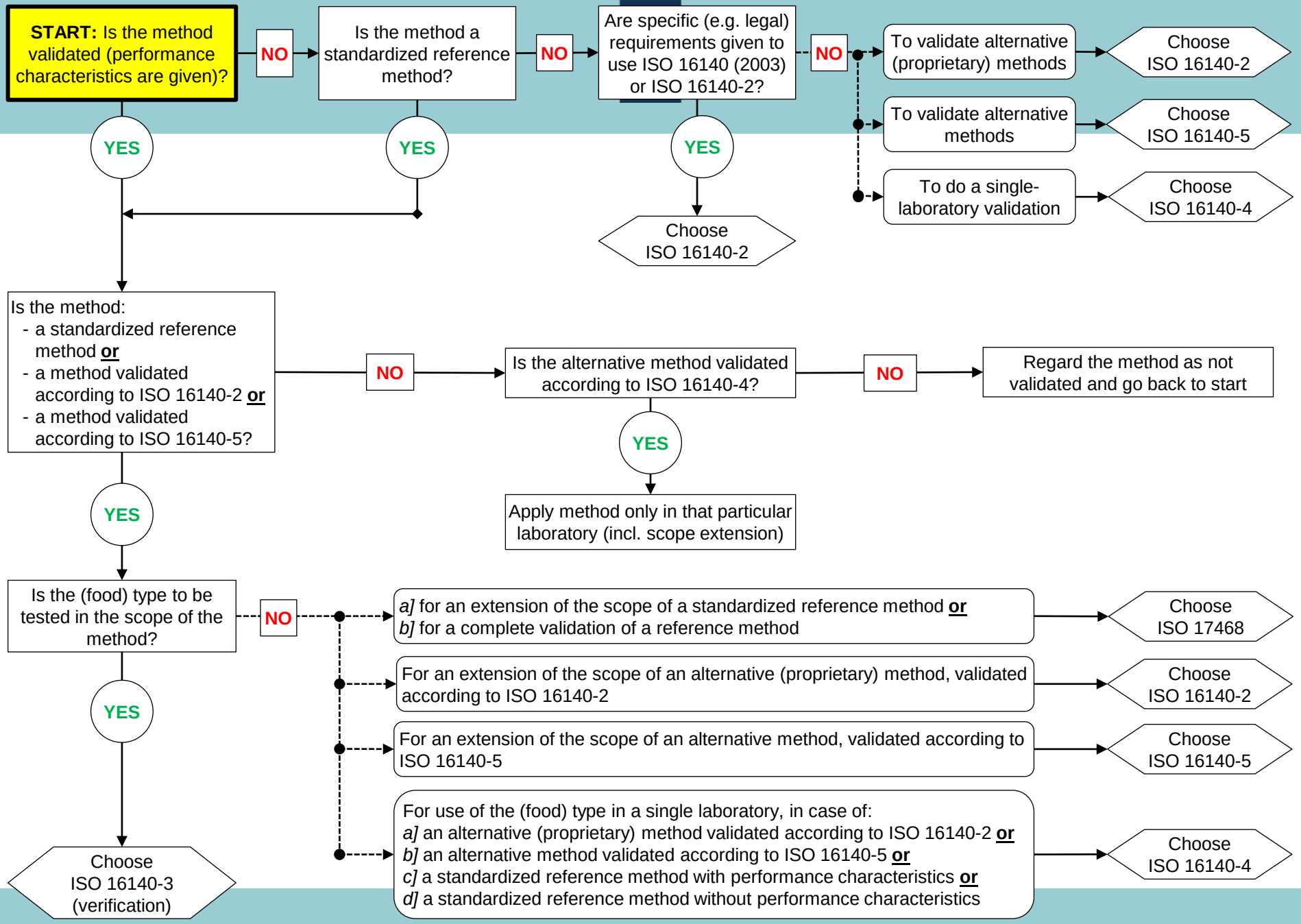
ISO 16140-3: **Protocol for the verification of reference and validated alternative methods implemented in a single laboratory** (in preparation for DIS vote)

ISO 16140-4: **Protocol for single-laboratory (in-house) method validation** (in preparation for DIS vote)

ISO 16140-5: **Protocol for factorial interlaboratory validation for nonproprietary methods** (in preparation for DIS vote)

ISO 16140-6: **Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures** (in preparation for DIS vote)

ISO 17468: **Technical requirements and guidance on establishment or revision of a standardized reference method** (published)


START: Is the method validated (performance characteristics are given)?
NO
Is the method a standardized reference method?
NO
Are specific (e.g. legal) requirements given to use ISO 16140 (2003) or ISO 16140-2?
NO
To validate alternative (proprietary) methods
Choose ISO 16140-2
To validate alternative methods
Choose ISO 16140-5
To do a single-laboratory validation
Choose ISO 16140-4
YES
YES
YES
Choose ISO 16140-2
Is the method:
- a standardized reference method or
- a method validated according to ISO 16140-2 or
- a method validated according to ISO 16140-5?
NO
Is the alternative method validated according to ISO 16140-4?
NO
Regard the method as not validated and go back to start
YES
Apply method only in that particular laboratory (incl. scope extension)
YES
Is the (food) type to be tested in the scope of the method?
NO
a] for an extension of the scope of a standardized reference method or
b] for a complete validation of a reference method
Choose ISO 17468
For an extension of the scope of an alternative (proprietary) method, validated according to ISO 16140-2
Choose ISO 16140-2
For an extension of the scope of an alternative method, validated according to ISO 16140-5
Choose ISO 16140-5
For use of the (food) type in a single laboratory, in case of:
a] an alternative (proprietary) method validated according to ISO 16140-2 or
b] an alternative method validated according to ISO 16140-5 or
c] a standardized reference method with performance characteristics or
d] a standardized reference method without performance characteristics
Choose ISO 16140-4
YES
Choose ISO 16140-3 (verification)

ISO 16140-2 (Validation of alternative (proprietary) methods)

- Successor of ISO 16140 (2003).
- Basis is the comparison between a reference method and an alternative method.
- Protocol for qualitative and quantitative methods
- Both protocols have 2 phases; a method comparison study and an interlaboratory study.
- Method comparison study focusses on testing a diversity of samples/matrices
- Interlaboratory study establishes the ‘reproducibility’ of the method using a single matrix.
- Evaluation of the data using preset criteria, alternative method can be better when proven.

ISO 16140-2 (Validation of alternative (proprietary) methods)

Qualitative study (MCS):

- Sensitivity study, comparison between (naturally) contaminated samples, minimum of 5 food categories each having a minimum of 60 samples. Separation in inter-pretation of data from a paired and unpaired study design.
- RLOD study, determination of the relative level of detection using artificially contaminated samples, 1 matrix per category, 30 samples per matrix.
- Inclusivity/exclusivity study using 50/30 strains.

ISO 16140-2 (Validation of alternative (proprietary) methods)

Quantitative study (MCS):

- Relative trueness study, comparison between (naturally) contaminated samples, minimum of 5 food categories each having 15 samples per category. Grafical interpretation of the data (Bland-Altman en scatter plots)
- Accuracy profile study, combination of evaluation of precision and trueness (= accuracy) of the method. Six samples each with 5 replicates for each category tested.
- Inclusivity/exclusivity study using 50/30 strains.

ISO 16140-3 (Verification of methods)

- Validation: establishment of the performance characteristics of a method and provision of objective evidence that the performance requirements for a specified intended use are fulfilled.

- Verification: demonstration that a validated method functions in the user's hands according to the method's specifications determined in the *validation* study and is fit for its purpose.

- Still heavily debated!

16140-3 (Verification of methods)

	Method with published validation data		Method without published validation data	
	Reference method	**Alternative method**	**Reference method**	**Alternative method**
Implementation verification	✓	✓		Not applicable [a]
(Food) type verification	✓	✓	✓	Not applicable [a]

[a] Not applicable: the method first needs to be validated (see Figure 1).

ISO 16140-3 (Verification of methods)

Current draft:

- Qualitative methods: estimated LOD_{50}
- Quantitative methods: estimated bias en reproducibility (S_{IR}) (link with measurement uncertainty ISO 19036)

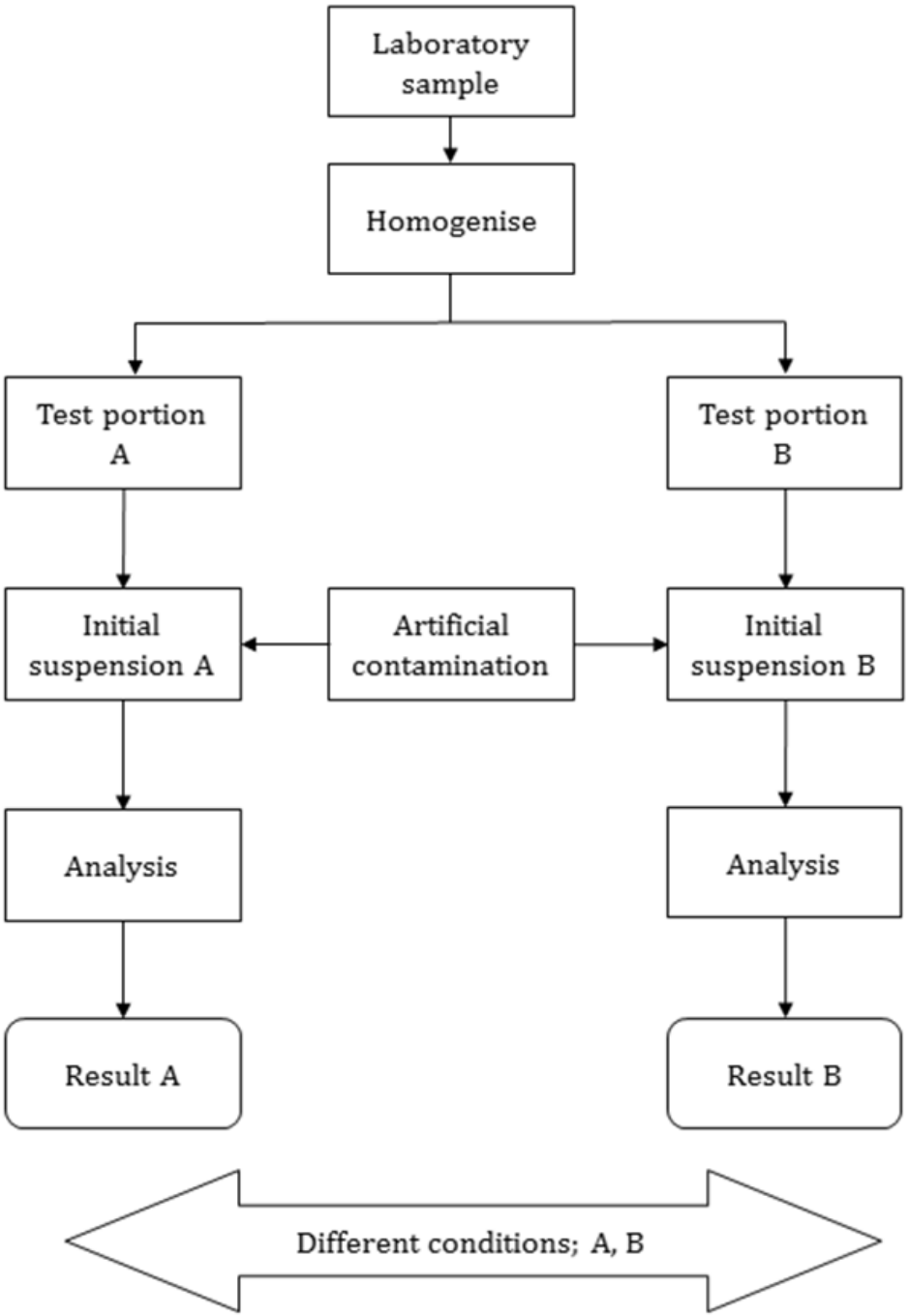
Table 3 — Estimation of LOD_{50} based on the number of positive results per level of contamination

High level of inoculation *(ca. 10 x LOD_{50} /test portion)*	**Intermediate level of inoculation** *(ca. 5 x LOD_{50} /test portion)*	**Low level of inoculation** *(ca. 1 x LOD_{50} /test portion)*	**Blank control**	**Estimated LOD_{50}** *(cfu/test portion)*
2/2	2/2	2/2	0/2	< 1×LOD_{50}
2/2	2/2	1/2	0/2	= 1×LOD_{50}
2/2	2/2	0/2	0/2	= 2×LOD_{50}
2/2	1/2	2/2	0/2	= 2×LOD_{50}
2/2	1/2	1/2	0/2	= 3×LOD_{50}
2/2	1/2	0/2	0/2	= 4×LOD_{50}
2/2	0/2	2/2	0/2	Unreliable result [a]
2/2	0/2	1/2	0/2	= 5×LOD_{50}
2/2	0/2	0/2	0/2	= 8×LOD_{50}

[a] This combination of results is very unlikely to occur with the levels of contamination tested and therefore leads to an unreliable result. The test shall therefore be repeated.

Table 5 — Test results

	Results *(log cfu/g)*		**Mean result per batch** *(log cfu/g)*		**Difference between alternative and reference method**
	Method to be verified	Reference method	Method to be verified	Reference method	
Batch 1, test portion 1	1,87	2,15	2,06	2,17	-0,11
Batch 1, test portion 2	2,25	2,20			
Batch 2, test portion 1	3,93	4,23	3,99	4,29	-0,30
Batch 2, test portion 2	4,04	4,35			
Batch 3, test portion 1	3,73	3,59	3,68	3,62	0,06
Batch 3, test portion 2	3,63	3,65			


Laboratory sample
Homogenise
Test portion A
Test portion B
Initial suspension A
Artificial contamination
Initial suspension B
Analysis
Analysis
Result A
Result B
Different conditions; A, B

ISO 16140-4 (Single-lab validation)

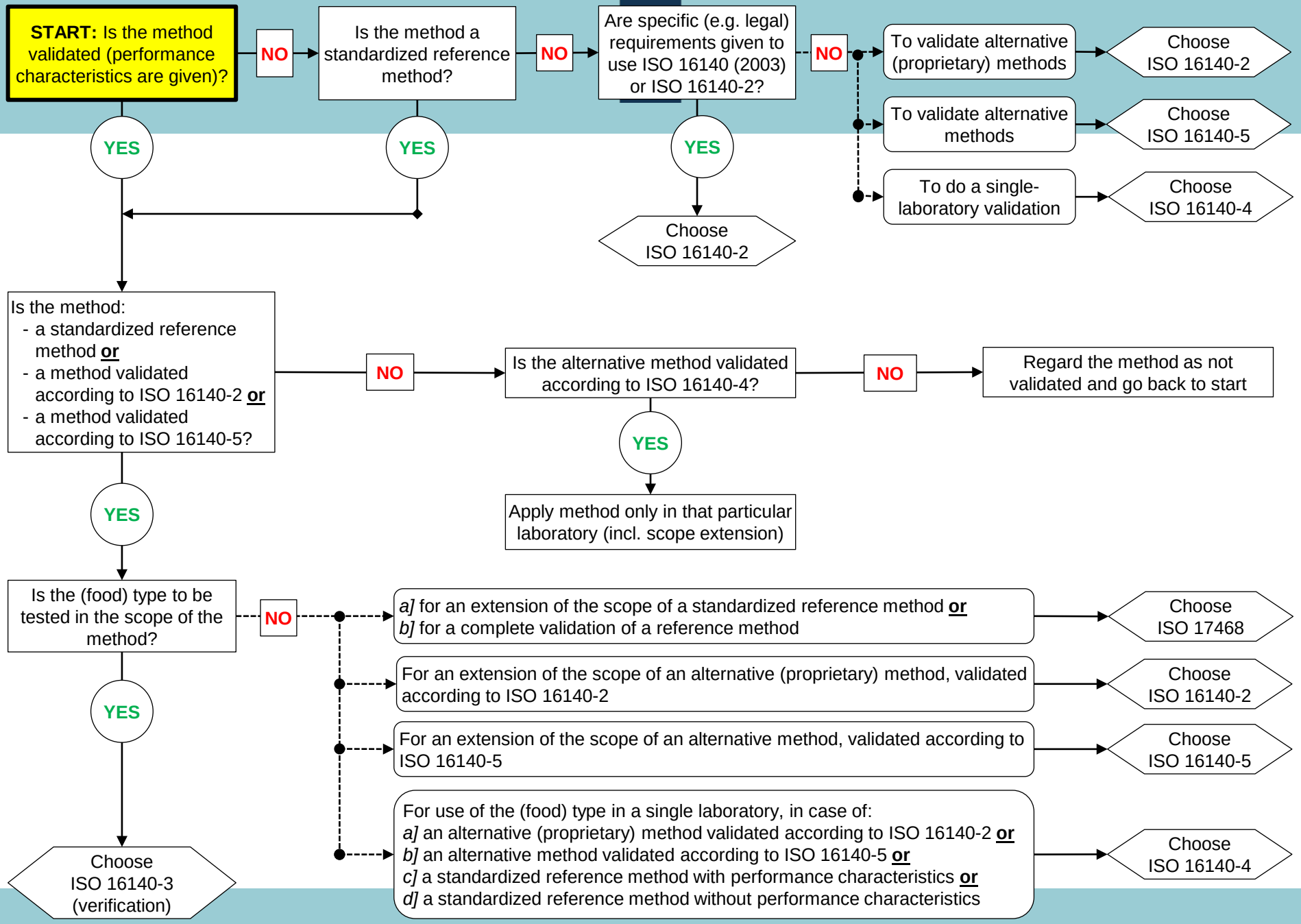
- Experimental design two-fold: classical approach and factorial design approach.
- For both experimental designs a protocol is described with and without the use of a reference method (using a reference value).
- Separate protocols for qualitative and quantitative methods
- Results of the validation study are only valid in the laboratory that conducted the study!

ISO 16140-5 (factorial interlab validation)

- Factorial interlaboratory study.
- By using the ‘factorial design’ less laboratories (≤ 4) are needed for the study (in comparison to 16140-2).
- Factorial approach cannot replace the interlaboratory study of an alternative (proprietary) method according to 16140-2.

ISO 16140-6 (validation confirmation methods)

- Validation of a part or complete confirmation procedure of a reference method against a (proprietary) alternative confirmation method (e.g. API 20 E or PCR test or Maldi-TOF).
- Validation starts with a suspected colony and not a (food) sample.
- Is based on the inclusivity/exclusivity study of 16140-2, using well characterised strains.
- Differentiation between validation at genus, species or subspecies level.
- Comparison between reference method and alternative method is basis. Comparison between identity of strain only done in cases where differences occur.
- Has both method comparison study and interlab study (use of reference method is optional for interlab study)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