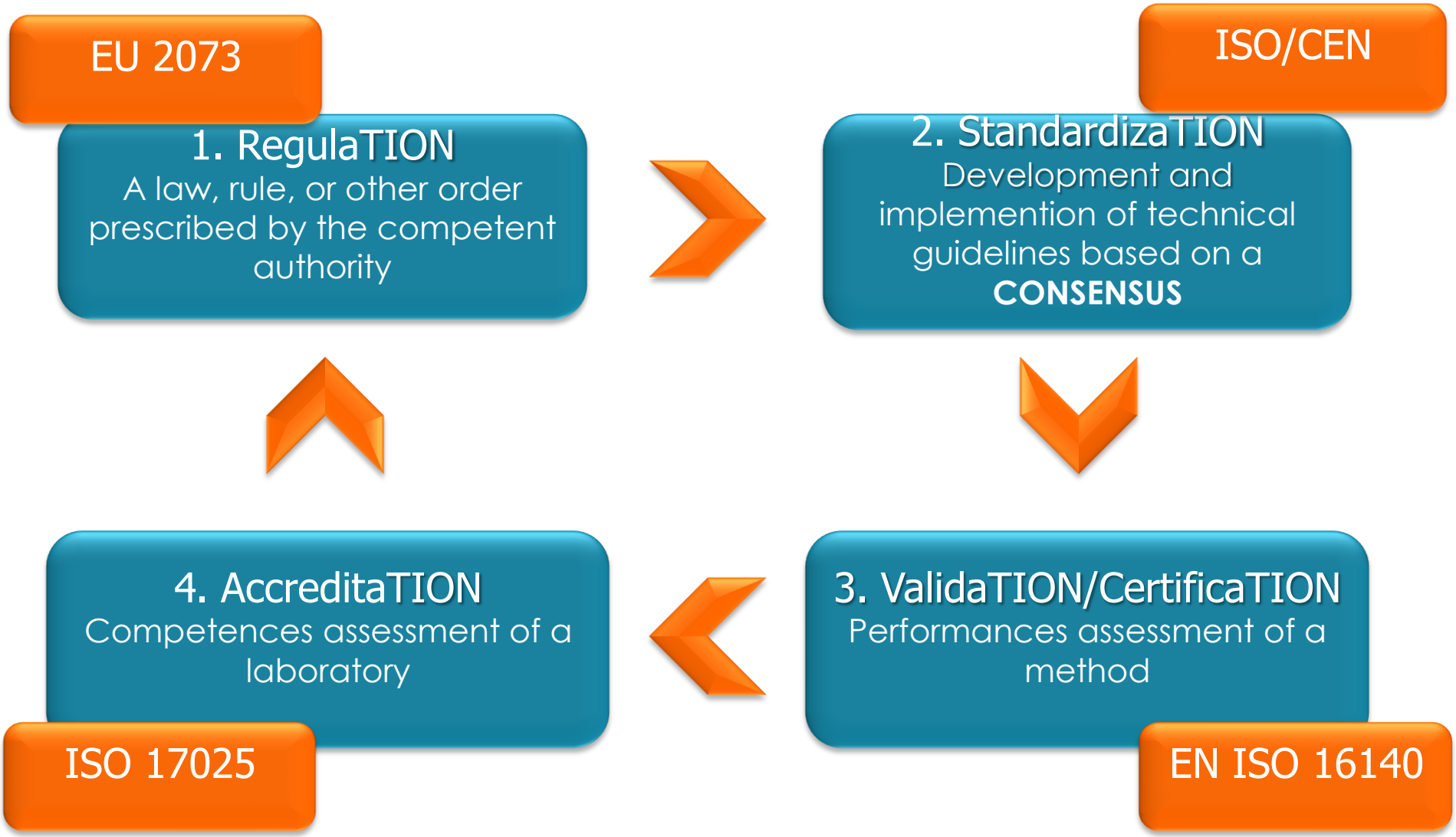
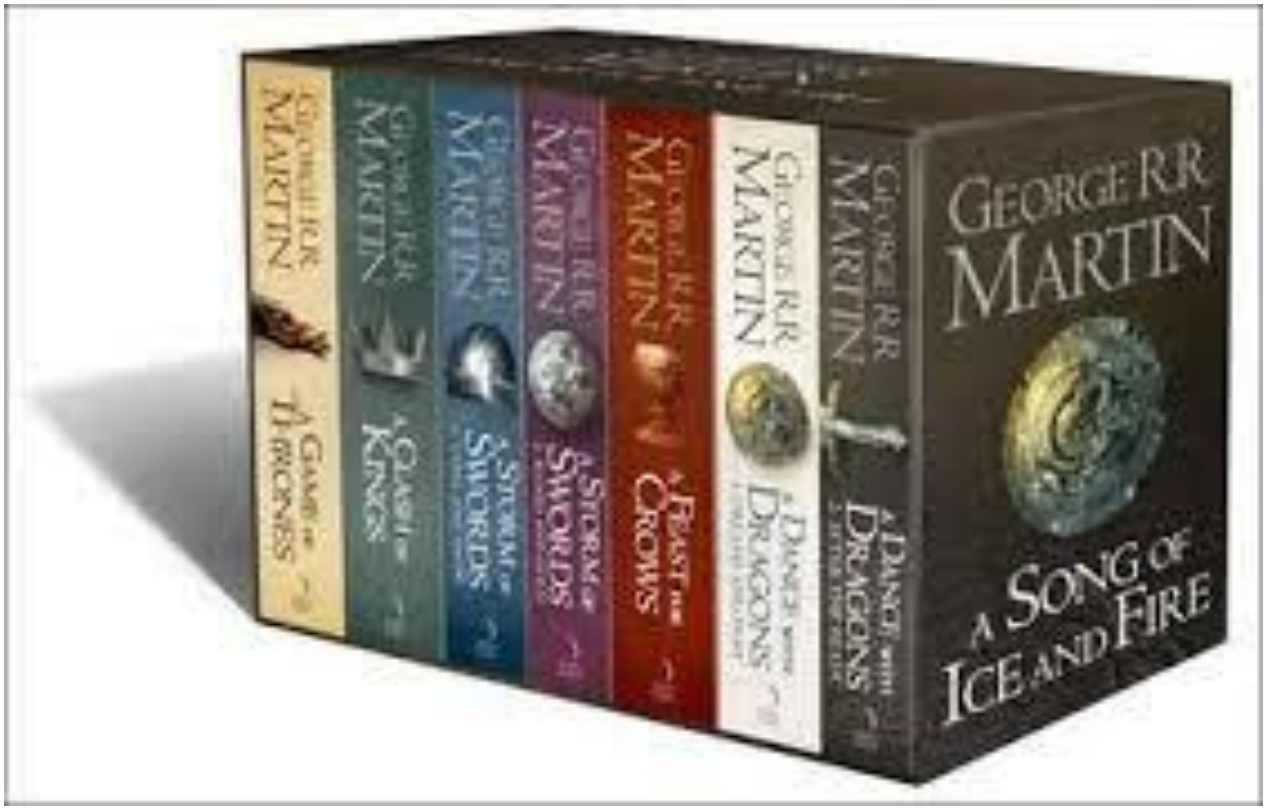
Methods Validation in Food Microbiology

The ISO 16140 series and their impact on routine laboratories

DEFINITIONS



THE SERIES OF THE ISO 16140 STANDARD



THE SERIES OF THE ISO 16140 STANDARD

Part 6 – Validation of confirmation methods

Part 5 - Factorial multi-laboratory method validation

Part4 – In house (single) method validation

Part 3 – Verification of reference and alternative methods

IMPACTS?

1 - Terminology	→	Parts 2 to 6
2 - Validation of alternative (proprietary) methods	→	Certification
3 - Method verification	→	Accreditation
4 - In house (single) method validation	→	Accreditation
5 - Factorial multi-laboratory method validation	→	Accreditation
6 – Validation for confirmation methods	→	Certification

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2 - Validation of alternative (proprietary) methods → Certification

Concerned methods	**Proprietary methods** ❶ Qualitative methods = detection method ❷ Quantitative methods = enumeration methods
Dedicated to	**Validation /Certification bodies** ➥ Qualified expert laboratories, which operate for the certification bodies
Added values **Assessment of performances by comparison to reference methods**	**Harmonization** with other Guidelines, particularly with AOAC / **Definition of the claim** ➥ Matrix study design, inclusivity/exclusivity New approaches, **user-friendly & clear rules** ➥ RLOD, Accuracy profile, Acceptability limits

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Process of the certification	❶**Technical study** based on the ISO 16140 – part 2 ➥Review and a vote on the technical study **(MCS & ILS)** ❷ **Audit** of the production plan ➥ Ruggedness & lot-to-lot studies, quality assurance
Recognition	**EU-Regulation 2073 (2005)** If comparison with the ISO/EN reference methods

Responsibility of the certification bodies to clearly define the scope of the validation (=categories and types of matrices), and ensure the link with the method verification

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3 - Method verification → Accreditation

Concerned methods	**All qualitative & quantitative methods** ❶ Reference methods ❷ Alternative methods
Dedicated to	**User Laboratories**
Added values	**Method implementation and verification** ❶ Reference methods ❷ Alternative methods
Process of implementation & verification	**Scope** of the reference method, **OR** the alternative method according to the ISO 16140-part 2 ❶ Matrices covered by the scope ❷ Matrices covered by the scope BUT NOT TESTED DURING THE VALIDATION STUDY

2 STUDIES

Dairy products ISO 16140 – part 2	❶ Raw milks and raw milks cheeses ❷ Heat treated milks and pasteurized cheeses ❸ Ice creams and dairy desserts

	Implementation Verification	**Sample type Verification**
Methods	1. Validated reference method 2. Validated alternative method	1. Reference method with no published validation data 2. Validated reference method 3. Validated alternative method
Items (Matrices)	**One representative item from a type tested in the validation and relevant to the user laboratory**	**"Challenging" items included in the scope of the method, but not tested in the validation and relevant for the user laboratory**

Ice creams **Caseinates**

❶ IMPLEMENTATION

→ **Qualitative methods**

Performance criteria: Range of detection

→ **Quantitative methods**

Performance criteria: Bias & Precision

❷ VERIFICATION

→ Qualitative methods

Performance criteria: Range of detection

→ Quantitative methods

Performance criteria: Bias

❷ VERIFICATION

- ✓ **Matrix Risk Analysis**
  - ❑ Microbial characteristics
    - Backgroud/technological microflora
  - ❑ Physical structure of the food
    - Viscosity, aw, pH…
  - ❑ Food Process characteristics
    - Inactivation processes

    ➥ **The laboratory is expected to perform a classification of the food matrices tested in routinely analyses based on their characteristics**

QUALITATIVE METHODS: RANGE OF DETECTION

✓ **Range**

Blank control	Low level of inoculation (LL) 1-3 cfu/test portion	Intermediate level of inoculation (IL) 5-15 cfu/test portion	High level of inoculation (HL) 10-30 cfu/test portion	Range of detection (cfu/test portion)
0	0	0	2	≥HL
0	0	1	2	IL-HL
0	0	2	2	IL-HL
0	1	0	2	Unreliable result
0	1	1	2	IL-HL
0	1	2	2	LL-IL
0	2	0	2	Unreliable result
0	2	1	2	Unreliable result
0	2	2	2	LL-IL

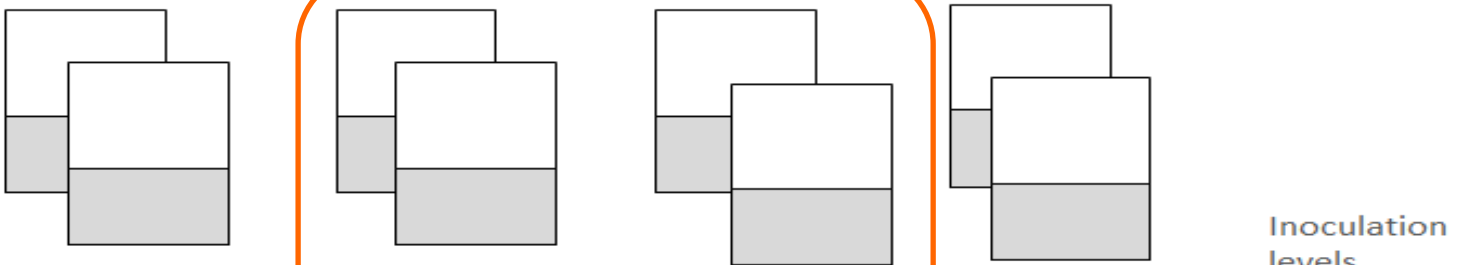
STEP 1

Dilute a pure cu

High level

STEP 2

Inoculate 6 stomacher bags containing the matrix to be tested diluted in the enrichment broth



STEP 3

Analyse each sample with the method to be verified

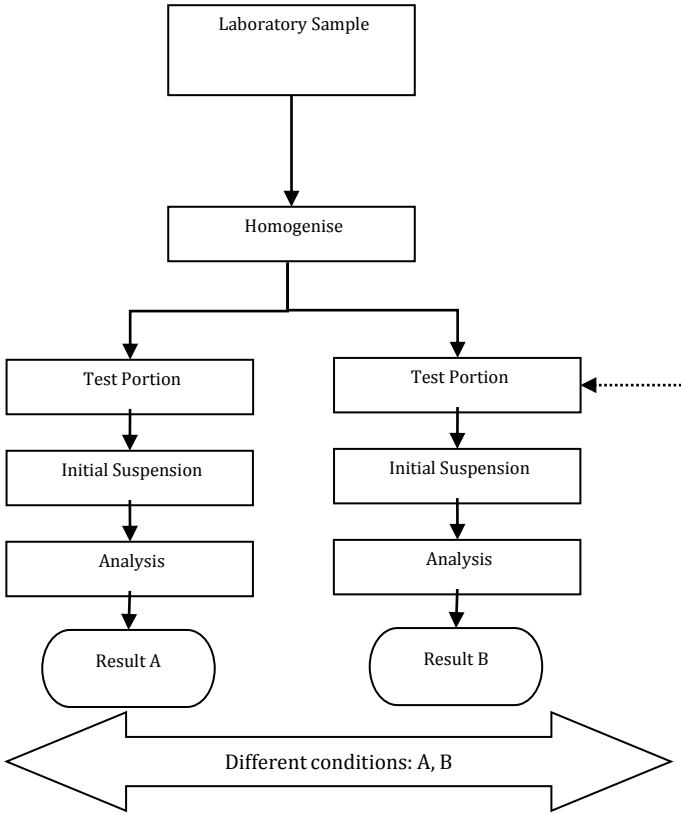
QUANTITATIVE METHODS: BIAS

- ✓ **Bias using duplicates of**
  - **LL** = A **low contamination level**, which corresponds to the lower limit of the expected specifications; this level could be a blank level.
  - **IL** = An **intermediate contamination level**;
  - **HL** = A **high contamination level**, which corresponds to the upper limit of the expected specifications.

Control enumeration data **Log CFU/g**	**Enumeration data of the method to be verified** **Log CFU/g**
HL	HL - 0,5 < x < HL + 0,5
IL	IL - 0,5 < y < IL + 0,5
LL	LL - 0,5 < z < LL + 0,5

QUANTITATIVE METHODS: PRECISION

✓ Precision (aligned with ISO19036)



- The standard deviation of the (intra-laboratory) reproducibility (SD_{IR}) of the verified method shall be smaller or equal to the standard deviation of the (inter-laboratory) reproducibility (SR) from the validation study.

Implementation ONLY!

3 - Method verification → Accreditation

Concerned methods	**All qualitative & quantitative methods** ❶ Reference methods ❷ Alternative methods
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Recognition	**Accreditation bodies**

Responsibility of the user laboratories to assess the performances of methods on matrices out of the initial scope

CONCLUSION

All the scenario?

- ☑ Proprietary and in-house methods
- ☑ Detection, enumeration & confirmation
- ☑ Validation, implementation, **verification**
- ☑ **Recognition** by
  - ➥ Certification
  - ➥ Accreditation
  - ➥ Regulation

Valuable standards that will facilitate the end-users' life

- ➥ **On the right way, as the part 1 & part 2 are already used!**
- ➥ **Hope the changes and opportunities will be welcomed…**

…Next scenario?

THE QUESTION OF TODAY

- ✓ **How the ISO 16140 series will impact on routine laboratories**
  - ❑ Part 2 : Method validation (certification)
  - ❑ Part 3 : Method verification
    - **Study design, training, budget….**

Thanks for your attention!

benjamin.diep@rdls.nestle.com
daniele.sohier@adria.tm.fr