



Validation of Methods in Accordance to ISO16140-2

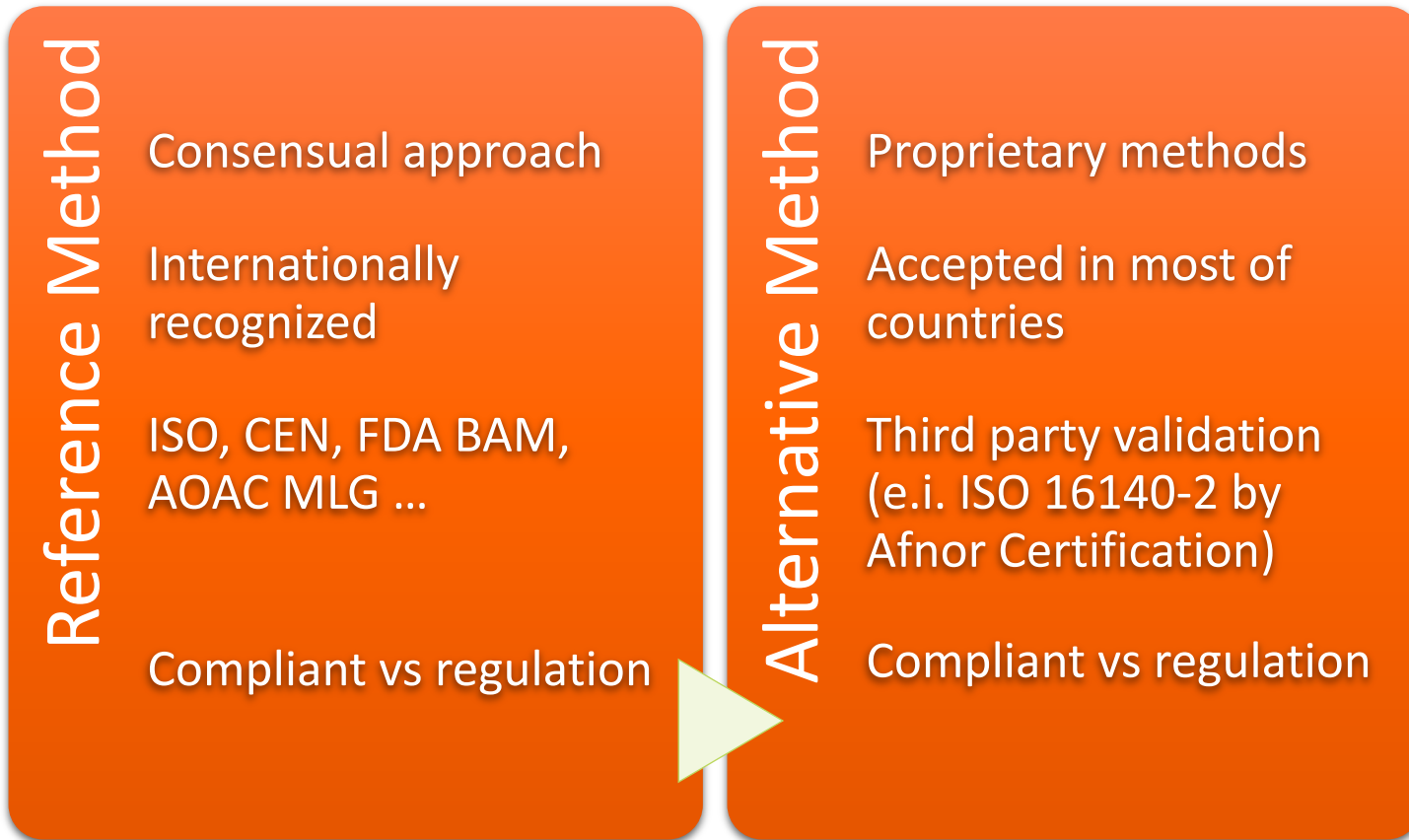
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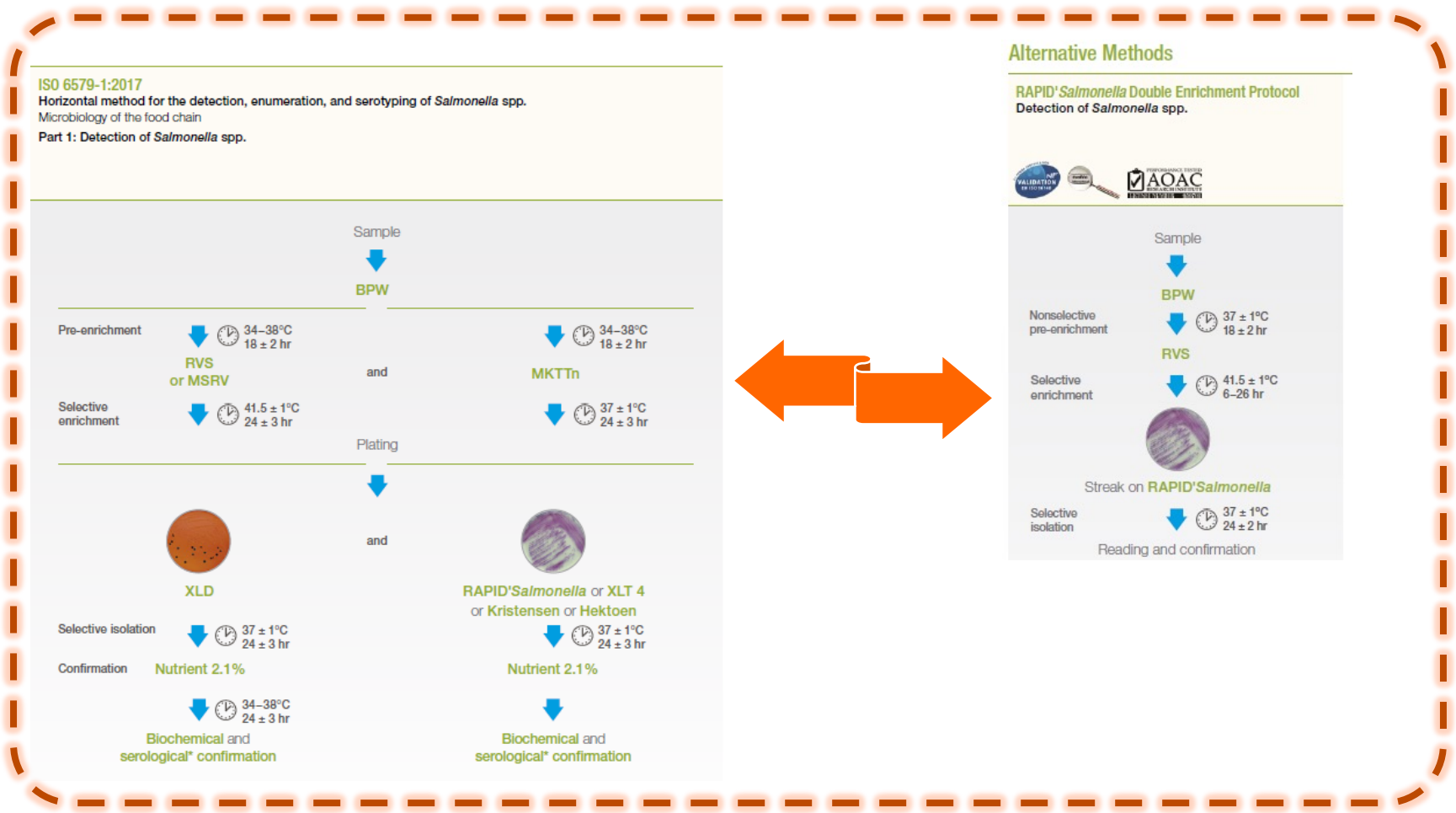
Validation of Alternative Methods

Objective:

Demonstrate the equivalence between an “Alternative method” and a “Reference Method”



Principle: validation in comparison with a reference method



ISO 16140-2:2016

Microbiology of the food chain — Method validation —

Protocol for the validation of alternative (proprietary) methods against a reference method

AFNOR Certification process

1

Candidate Method

Application for certification:
Validation project
submission
Audit of the production site

2

Validation Study

Performed by a qualified,
independent and expert lab
→ Study performed
according a standardized
protocol (ISO 16140-2 /
AOAC)

afnor
CERTIFICATION

4

Periodical monitoring

Certificate is granted for a 4
years period
New audit of the
production site (4 years)

3

Decision of certification

Technical Board review
→ Group of experts public /
private / research /
industrial / reference center
...

1st phase: characterization of the alternative method's performance

Method Comparison Study (MCS)

- Qualitative methods: Sensitivity study; RLOD; Inclusivity and exclusivity
- Quantitative methods: Relative Trueness; Accuracy Profil; Inclusivity and exclusivity

2nd phase: results variability of the alternative method

Inter-Laboratory Study (ILS)

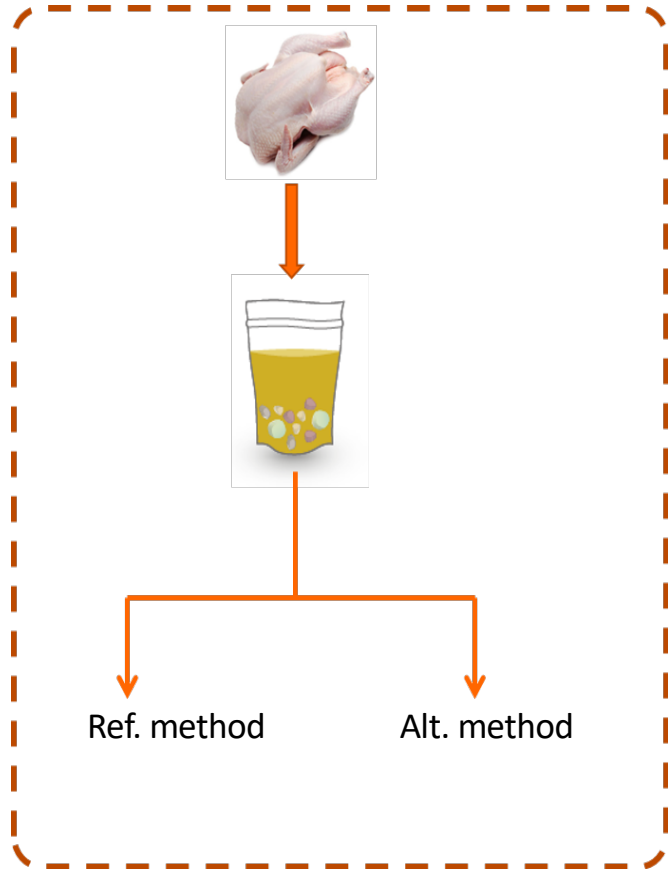
Determine the variability of the alternative method in several laboratories under defined conditions of reproducibility

Food categories and validation claim

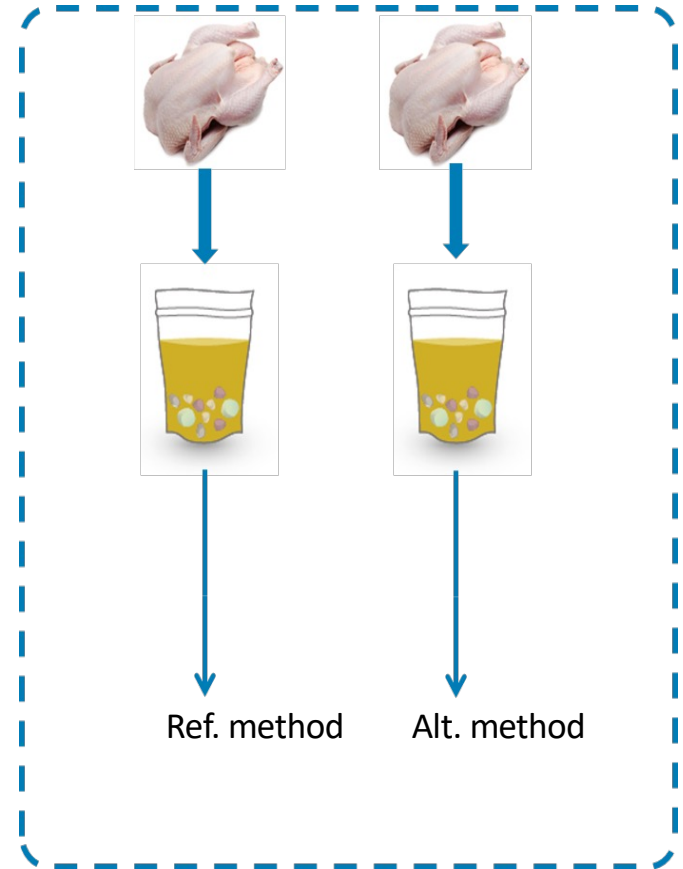
| | | | | | |
|-----------------------------------|--|--|--|--|---|
| Raw milk and raw milk product | Heat processed milk and dairy product | Raw meat and ready to cook meat products | Ready to eat, ready to reheat product (except poultry) | Raw poultry and ready to cook poultry products | Ready to eat, ready to reheat meat poultry products |
| Eggs and eggs products | Raw and ready to cook fish and seafoods products | Ready to eat, ready to reheat fishery products | Fresh produces and fruits | Processed fruits and vegetables | Dried cereals, fruits, nuts, seeds and vegetables |
| Infant formula and infant cereals | Chocolate, bakery products and confectionary | Multicomponent foods and meal components | Primary production samples | Pet food and animal feed | Environmental samples |

18 categories: 15 "Human food" and 3 additional
 "Broad range of food" = minimum of 5 "human food" categories
 Categories selected must be linked with the target selected

Detection methods: MCS study design



One enrichment bag: Paired



Two enrichment bags: Unpaired

1/ Sensitivity Study:

- 60 samples per category (30 pos. & 30 neg.)
 - Artificial contamination with stressed bacteria or natural contamination
 - 3 types per category
- Sensitivity, relative trueness and false positive rate calculation
- Acceptability Limits per category and for the whole method

| | Reference-method positive (R+) | Reference-method negative (R-) |
|-------------------------------------|-----------------------------------|-----------------------------------|
| Alternative-method positive (A+) | +/+ Positive Agreement (PA) | -/+ Positive Deviation (PD) |
| Alternative-method negative (A-) | +/- Negative Deviation (ND) | -/- Negative Agreement (NA) |

Acceptability Limits of ISO 16140-2

Acceptability limit parameters and values for a paired and unpaired study design in relation to the number of categories used

| Number of categories | Paired study | | Unpaired study |
|----------------------|--------------------------------------|-----------|----------------|
| | (ND ^a - PD ^b) | (ND + PD) | (ND - PD) |
| 1 | 3 | 6 | 3 |
| 2 | 4 | 8 | 4 |
| 3 | 5 | 10 | 5 |
| 4 | 5 | 12 | 5 |
| 5 | 5 | 14 | 5 |
| 6 | 6 | 16 | 6 |
| 7 | 6 | 18 | 7 |
| 8 | 6 | 20 | 7 |

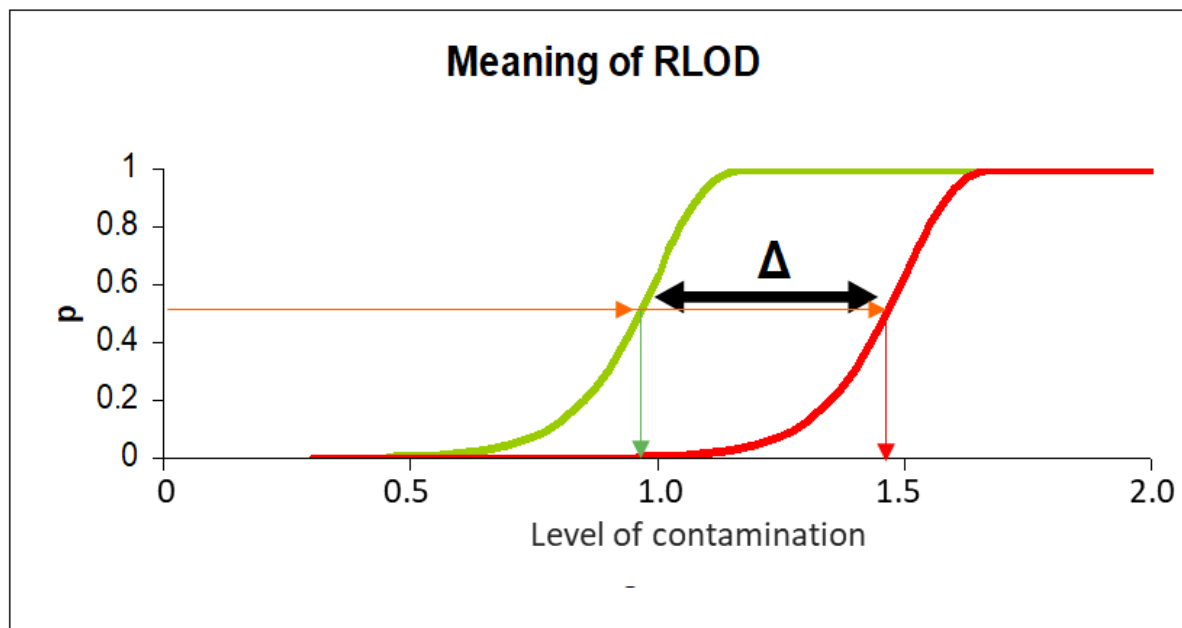
a ND = number of samples with Negative Deviation results.

b PD = number of samples with Positive Deviation results.

2/ Relative Limit Of Detection:

- Evaluation of the level of detection of 2 methods
- 1 for each category
- Artificial contamination at 3 different levels:
 - L0: Non contamination – 5 samples
 - L1: Low level of contamination – 20 samples
 - L2: high level of contamination – 5 samples
- LOD₅₀ calculation of each method
- $$RLOD = \frac{LOD_{Alt}}{LOD_{Ref}}$$

Acceptability Limits of ISO 16140-2



- $$RLOD = \frac{LOD_{Alt}}{LOD_{Ref}}$$

| | Paired | Unpaired |
|----|--------|----------|
| AL | 1.5 | 2.5 |

3/ Inclusivity and exclusivity study:

Inclusivity:

50* targeted strains diluted at a low level

Exclusivity:

30* non-targeted strains cultivated in non-selective broth

Results analyzed by the expert lab. and technical board

*for Salmonella studies, 100 and 50 respectively

Inter-Laboratory Study

- Determine the variability of the alternative method in several laboratories under defined conditions of reproducibility

Minimum of 10 sets of data (10 collaborative labs)

1 matrix artificially contaminated at 3 levels

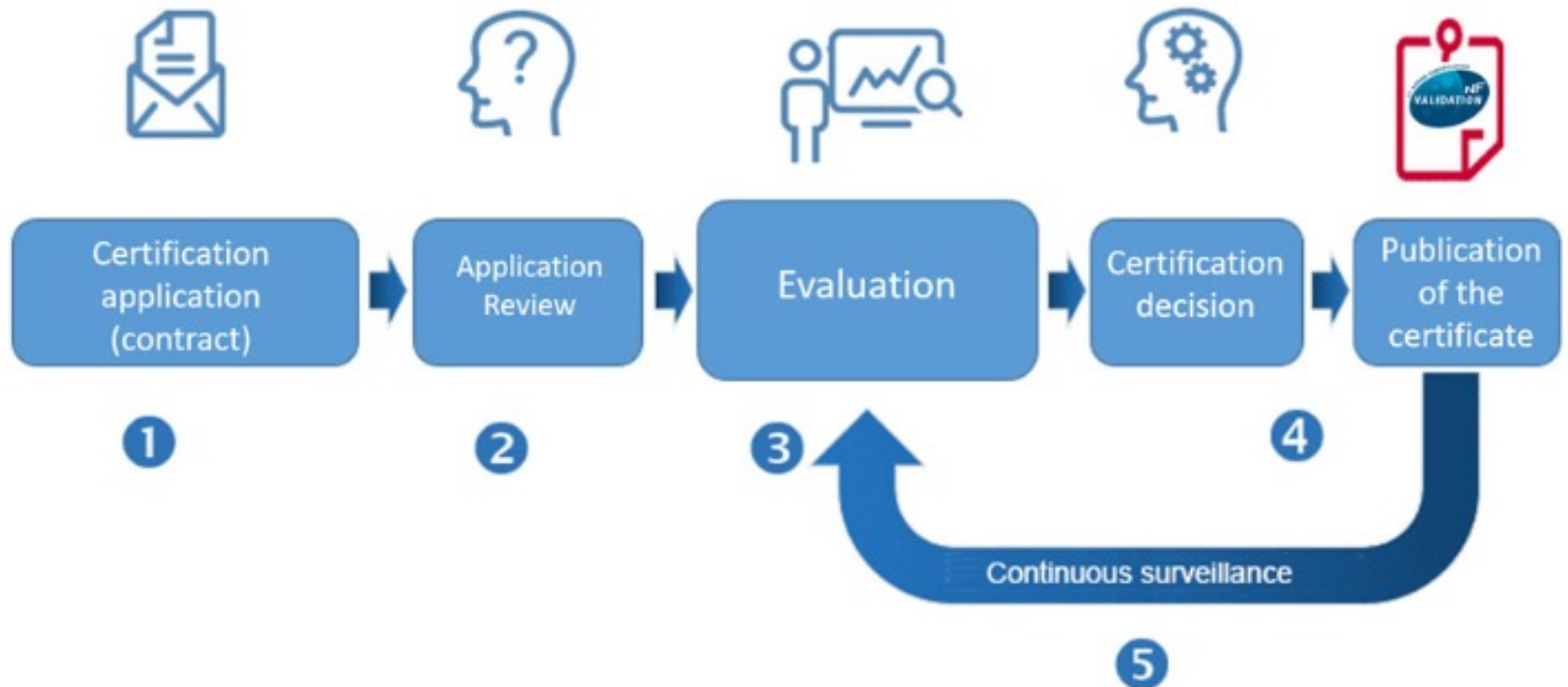
- Negative sample (L0) - 8 samples
- Low level (L1) - 8 samples
- High level (L2) - 8 samples

Analyzed by the ref. and alt. Methods in blind

48 results generated for each lab



Surveillance



Other validation systems

| | AOAC PTM | AOAC OMA | ISO 16140-2 |
|---------------------------|--|--|---|
| Type of methods | Proprietary Methods | | |
| Reference methods | AOAC, FDA, USDA, ISO, Health Canada | AOAC, FDA, USDA, ISO, Health Canada | ISO, CEN, other reference methods |
| Claim | Variety-10 matrices/5 groups Selected-5 matrices/2 groups Group-5 matrices/1 group | Per matrix basis- all matrices in Method Developer Claim | Broad Range of Foods- (5 cat.) Restricted categories (< 5 cat.) Additional cat. (Feed, env. and PP) |
| Time to validation | ~ 6 months | ➤ 12 months | ~12 months |
| Statistical calculations | POD | POD | AL and RLOD |
| Expert lab. accreditation | N/A | N/A | ISO 17025 |
| Validated method review | Yearly | First action for 2 years Final action vote by OMB | Every 4 years + manufacturer audits |
| Method published | ILM, Journal of AOAC | Journal of AOAC, Official Methods of Analysis | AFNOR / Microval / Nordval website |

User laboratory adoption: verification process

STAY TUNED

Validation

- *Performance characteristics*
- *Versus a reference methods*
- *Validation protocol*
- *Third party recognition*

Method provider

ISO 16140-2

Verification

- *Implementation*
- *Comparison & demonstration*
- *According to the method's specifications*

Verification protocol

User laboratory

ISO 16140-3





Thank
you

BIO-RAD