Enhancing trueness and accuracy of procedures for food, feed and environmental samples

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Norwegian Veterinary Institute

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- Rational for certification of alternative (proprietary) microbiological methods
- Method performance characteristics & acceptance criteria
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Microbiological methods
(foods, feeds, environment)

- **Reference methods**
  elaborated by standardisation org.: ISO, NMKL, IDF
  ISO methods given in EU regulation 2073/2005

- **Alternative methods (proprietary methods)**
  certified by AFNOR NF Validation, MicroVal, NordVal International
  validated according to ISO 16140-2:2016

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NordVal International

– an independent third party

Certifying alternative methods (proprietary methods, test-kits)

- To ensure reliable analytical results
- To ensure a high level of food safety
- Demonstrate fit for purpose
- Demonstrate equivalence with ref. method (in accordance with EU regulations)
- To be in compliance with the manufacturer's claims

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NordVal International

- Independent: Steering group (5 Nordic Countries) + Technical Committees
- Secretariat: NMKL, Nordic Committee on Food (Denmark) www.nmkl.org
  - Linked to the Nordic Council of Ministers
- Reviewing results from independent expert laboratories

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Validation of qualitative and quantitative methods for food, feed and environmental samples

1. Comparison study performed at an expert lab alternative method vs reference method

2. Interlaboratory study performed by 10 collaborators (5 different organisations/locations)

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Qualitative & Quantitative Selectivity study

1. Inclusivity: detection of the target microorganism from a wide range of strains, (50 (100) pure cultures of target org.)
   10-100 x LOD

2. Exclusivity: the lack of interference from a relevant range of non-target microorganisms (30 pure cultures non-target microorganisms known to cause interferences)

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Qualitative: Method Comparison Study

Sensitivity: positives obtained/ positives expected

Specificity: negatives obtained/ negatives expected

Agreement between the methods.

Level of Detection (LOD) – the concentration at a certain rate of sensitivity (LOD_{50} – the concentration where the sensitivity is 50%)

Relative LOD – LOD for alternative / LOD for reference method

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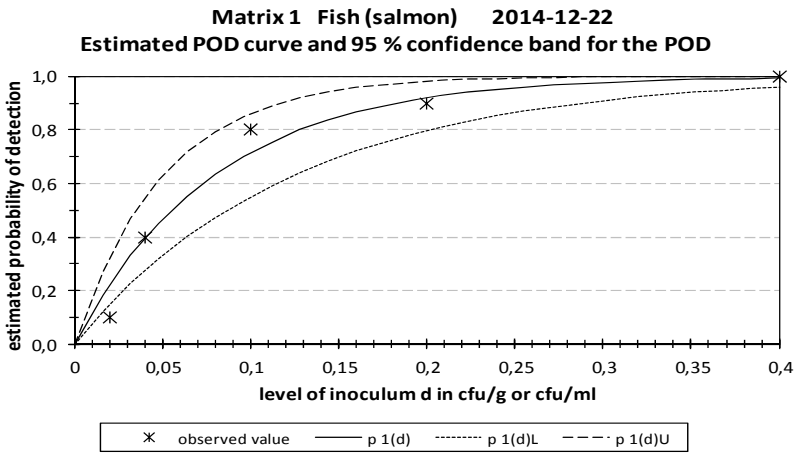
Probability of detection (POD)



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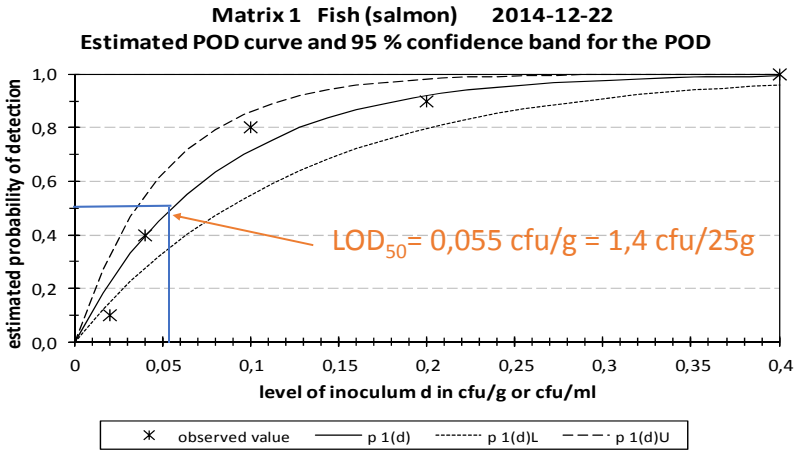
Level of Detection (LOD_{50})



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Sensitivity study - samples

- Naturally and/or artificially contaminated samples
- Broad range of foods, at least 5 matrix group
  - 3 types x 20 samples = 60 samples each group -> 300 samples

Example: *Salmonella* spp. in foods

1. Milk & dairy (raw, pasteurized, dry)
2. Meat (fresh (unprocessed, ready-to-cook (processed), ready-to-eat)
3. Eggs (unprocessed, processed, dry)
4. Fish & Seafood (raw, cooked, smoked)
5. Vegetables (fresh, processed, dry)

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Sensitivity study - samples

2 strains relevant for the matrix should be used

50% of the samples positives + 50 % negative

For each group:

Responses	Reference method Positive (+/)	Reference method Negative (-/)
Alternative method Positive (/+)	+/+ positive agreement (PA)	-/+ positive deviation (PD)
Alternative method Negative (/-)	+/- negative deviation (ND)	-/- negative agreement (NA)

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Sensitivity study – acceptability limit

- How is the agreement between the methods?
- False positives?
- False negatives?

Statistical: Cohen's kappa ≥ 0.80 & sensitivity ≥ 95%

Empirical: Tabled values (ISO 16140-2) for max permitted deviations depending on the number of matrices and study design.

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Relative Level of Detection (RLOD) Study

1 food type each category, 3 levels:

- negative [5 samples],
- low (0.7 cfu), fractional recovery 25-75% [20 samples],
- higher (1-1.5 cfu/test portion)[5 samples]

RLOD = LODalt /LODref

Excel Spreadsheet
Prof. Dr. Wilrich, Freie Universität Berlin

Acceptance Criteria
Paired study:
RLOD ≤ 1.5
Unpaired study:
RLOD ≤ 2.5

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Inter Laboratory Study (ILS)

- 1 food matrix, 3 levels, 8 replicates – 10 collab.
- Example

  L_0: negative control,
  L_1: 1-5 cells per 25 g and
  L_2: 5-50 cells per 25 g.

Both methods: 48 results per collab -> 480 results

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ILS

Responses	REF (+/)	REF (- /)
ALT (/+)	(+/+) PA	(-/+) PD
ALT (/-)	(+/-) ND	(-/-) NA

- Agreement between the methods?

  Statistical: Cohen's kappa ≥ 0.80

  Empirical: table 10 collab: ND-PD ≤ 3 & ND+PD ≤ 4

Acceptance criteria + any additional information

- > fit for purpose or not

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Quantitative methods

Comparison Study

- Relative Trueness Study
- Accuracy Profile Study
- Selectivity Study (inclusivity and exclusivity)

At independent expert lab

ILS

- Accuracy Profile

Org. by expert lab

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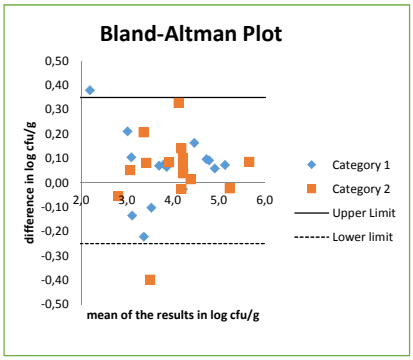
Quantitative methods – comparison study

- Relative trueness study
  - 5 food categories, 3 different types, 5 samples = 75 samples
  - Bland-Altman Plot; diff = alternative – reference
  - ≤ 1 in 20 should be outside the 95% confidence levels*

  *D ± 2SD, D= average of the differences, SD = standard deviation of the differences for all categories



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Quantitative methods– comparison study

Accuracy Profile study

- 5 food categories, 1 type each category
  - 6 samples (2 low, 2 intermediate, 2 high)
  - 5 replicates

-> 5 x 6 x 5 each method

Calculations: median, mean, SD, combined SD, confidence levels, bias

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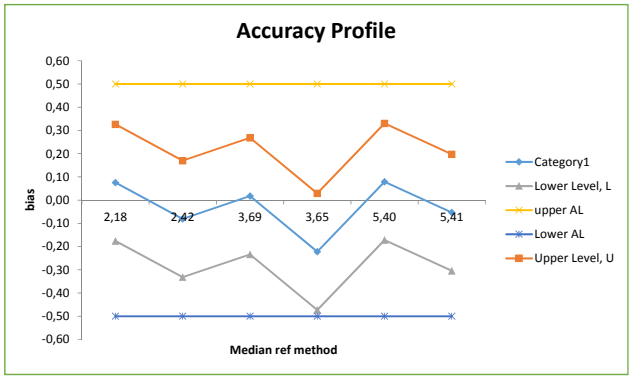
Quantitative methods– comparison study



Acceptance criteria, AL, ± 0.5 log cfu/g

The alternative method is accepted as being equivalent to the reference method if the results falls within the ALs.

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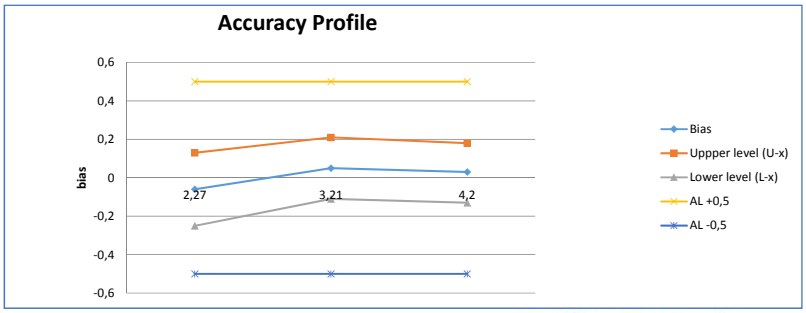
ILS – quantitative methods

- 8 collaborators (min. 4 organizations)
- 1 matrix, 3 levels, 2 replicates



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NMKL

NordVal International / NMKL
c/o Danish Technical University
Mørkhøj Bygade 19, DK-2060 Søborg, DK
www.nmkl.org

NordVal International Certificate

Issued for:	Salmonella Velox
NordVal No:	046
First approval date:	7 June 2016
Renewal date:	25 May 2017
Valid until:	25 May 2019

Salmonella Velox

Manufactured and supplied by:
DNA Diagnostic A/S
Voldbjergvej 14
8240 Risskov
Denmark

fulfils the requirements for NordVal Certification. The reference method was EN ISO 6579:2002: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

NordVal International has studied the enclosures to the application and evaluated the results obtained in the validations conducted by the expert laboratory AnalyTech Miljølaboratorium A/S, Denmark. The comparison study is conducted according to ISO/DIS 16140-2. The interlaboratory study is carried out according to NordVal Protocol of 2009. Salmonella Velox

Validation - certification

- Expensive for the manufactures - quality sells
- Cheaper for the lab - they do not need to validate (only verify)
- Required in food legislation - EC 2073/2005 Microbiological criteria

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Verification

- What to examine when taking a validated method into use?
- Qualitative: LOD
- Quantitative: Precision
- Proficiency testing scheme (z-score / zeta score)

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References

- NordVal Validation Protocol Microbiology 2016 www.nmkl.org Nordval
- ISO 16140-2: 2016: Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method
- NMKL Procedure No. 32 (2017): Verification of microbiological methods

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