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NordVal Validation Protocols

NordVal's 10 Anniversary Symposium

Rapid Methods

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The NordVal validation protocols

- Protocol for the validation of alternative microbiological methods, (March 2009)
- Guide in validation of alternative proprietary chemical methods (NordVal Protocol 2, May 2010)



Objective:

- to guide applicants, laboratories, NordVal technical committees and the NordVal steering group in the validation, evaluation and certification of test kits



- **Qualitative** methods (+ / -)
- **Quantitative** methods (levels / amount)
- **Proprietary methods** - methods which someone has ownership too
- **Alternative methods** - proprietary and non proprietary rapid methods



Microbiology:

- method comparison study of the alternative method against a reference method (performed by an expert laboratory)

- collaborative study of the alternative method. Valid results from at least 8 laboratories (include 10-12 labs). In some case 5 laboratories are considered acceptable.
(organised by an expert laboratory)



Chemistry:

- method comparison study of the alternative method against a reference method.
(performed by an expert laboratory)
When no reference method: use CRM, control materials and spiked samples at different levels in various matrixes.
- intermediate study confirm the obtained results on at least one additional laboratory.



Method Comparison Study

- **ruggedness testing** – how sensitive a method is to minor deviations in the experimental conditions of the method.

A method which is not influenced by such minor deviations, is said to be rugged when it comes to these experimental conditions.

Parameters to be tested depends on method in question

- the composition of the samples
 - pH
 - timing of individual (assay) steps
 - temperature
-
- Blank samples can be used.
 - Information from the ruggedness test can be used to specify the conditions under which a method should be used.



Method Comparison Study

- Batch-to-batch variation

The expert laboratory review the manufacturer's descriptions and documentation on how they have ensured that there is no significant batch-to-batch variation of their products.



Method Comparison Study

Performance characteristics:

Qualitative methods

- field of application
- selectivity (inclusivity/exclusivity)
- limit of detection
- relative accuracy
- relative sensitivity
- relative specificity
- false positives
- false negatives
- the agreement between the methods, κ

Quantitative methods

- field of application, concentration range
- limit of detection / quantification
- selectivity (inclusivity/exclusivity)
- repeatability
- trueness
- recovery



Method Comparison Study - Qualitative methods

Sensitivity, accuracy, specificity, false positives, false negatives, agreement between methods:

- Artificially contaminated samples (when real incurred samples are not available).
- For food: at least 5 relevant food matrices
- For other categories: a relevant number of matrices.

Microbiological:

3 inoculation levels:

- 0 = negative control,
- 1-10 cells/ 25 g
- 10-100 cells/ 25 g

Usually 2 strains are selected.

60 samples for each matrix ~30 positive + 30 negative.

No replicates

Chemical:

- Levels: Low, medium, high + blind
- Replicates: ~10

Each sample is analysed by the reference method and the alternative method



Method Comparison Study - Qualitative methods

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)

n+

n-

N+

N-

N



Estimation of accuracy, sensitivity, specificity and agreement

Matrix	PA	NA	ND	PD	Sum	Relative Accuracy AC (%)	Relative Sensitivity SE (%)	Relative Specificity SP (%)	Agreement kappa
					N	$\frac{(PA+NA) \times 100}{N}$	$\frac{PA \times 100}{PA+ND}$	$\frac{NA \times 100}{PD+NA}$	$\kappa = \frac{AC - p_e}{1 - p_e}$
									$p_e = \frac{(N+ \cdot N-) + (n+ \cdot n-)}{N^2}$
Food cat 1									
Food cat 2									
Food cat 3									
Food cat 4									
Food cat 5									
Other									

Example

Responses	Reference method positive (+/)	Reference method Negative (-/)	
Alternative method positive (/+)	23	13	36
Alternative method negative (/-)	4	30	34
	27	43	70 13



Example cont.



Results after screening

Matrices	PA	NA	ND	PD	Sum	AC (%)	SE (%)	SP (%)	Kappa
Meat	23	30	4	13	70	76	85	70	0.52

PA = + / +, NA = - / -

ND = - / +; PD = + / -



Acceptance criteria - qualitative methods

- Fit for its purpose
- The sensitivity should $\geq 95\%$
- The agreement between method, kappa should indicate very good agreement, i.e. $\kappa > 0.80$.

Example cont.

Results after screening

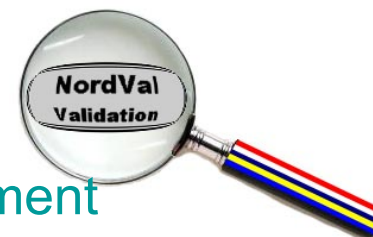
Matrices	PA	NA	ND	PD	Sum	AC (%)	SE (%)	SP (%)	Kappa
Meat	23	30	4	13	70	76	85	70	0.52

PA = + / + , NA = - / -
 ND = - / + ; PD = + / -

Results after confirmation

Matrices	PA	NA	FN	TP	FP	Sum
Meat	23	30	4	11	2	70

TP = True positive, FP = False positive
 FN = False negative



Estimation of accuracy, sensitivity, specificity and agreement

Matrix					Sum	Relative Accuracy AC (%)	Relative Sensitivity SE (%)	Relative Specificity SP (%)	Agreement kappa
					N	$\frac{(PA+NA+FP) \times 100}{N}$	$\frac{(PA + TP) \times 100}{PA+FN}$	$\frac{NA \times 100}{FP+NA}$	$\kappa = \frac{AC - p_e}{1 - p_e}$
									$p_e = \frac{(N+ \cdot N-) + (n+ \cdot n-)}{N^2}$
Food cat 1									
Food cat 2									
Food cat 3									
Food cat 4									
Food cat 5									
Other									



Example cont.

Results after screening

Matrices	PA	NA	ND	PD	Sum	AC (%)	SE (%)	SP (%)	Kappa
Meat	23	30	4	13	70	76	85	70	0.52

PA = + / +, NA = - / -
 ND = - / +, PD = + / -

Results after confirmation

Matrices	PA	NA	FN	TP	FP	Sum	AC (%)	SE (%)	SP (%)	Kappa
Meat	23	30	4	11	2	70	79	126	94	0.59

TP = True positive, FP = False positive
 FN = False negative

Alternative 1: LOD – limit of detection

Results at different levels for the determination of LOD

Concentration (µg/g)	n	Positive	Negative	Positive (%)
25	10	0	10	0
50	10	1	9	11
75	10	5	5	50
100	10	10	0	100
200	10	10	0	100

The reliability of the detection method becomes less than 100% at concentrations below 100 µg/g.

Method Comparison Study - Qualitative methods

Alternative 2: LOD – limit of detection

based on the trimmed Spearman-Kärber method.

$$\text{LOD}_{50} = e^m \text{ where } m = \sum_{i=1}^{k-1} (p(i) + (p(i+1) - p(i)) \frac{(x(i) + x(i+1))}{2})$$

k = number of levels

p(i) = the proportion of positives for the level i (i = 1, 2, 3) – the sensitivity for each level

x(i) = the log concentration i

	Level 1	Level 2	Level 3
Concentration	50	75	100
No of analyses	10	10	10
No of positives	1	5	10
In concentration, x(i)	3,91	4,32	4,61
Sensitivity p(i)	0,10	0,50	1,00
$(x(i) + x(i+1))/2$	4,11	4,46	
m_i	1,75	2,73	
LOD₅₀	88		



Method Comparison Study - Qualitative methods

Alternative 3: **Detection capability (CC β)**

- tested against the method's claimed screening/threshold limit.

Analyse at least 20 blank materials and 20 blanks per matrix fortified with the analyte(s) at the screening limit. Analyse the samples and identify the analyte(s). No more than one sample obtained can be false negative ($\beta = 5\%$, 95% confidence).

Commission decision 2002, implementing Council Directive 96/23/EC the performance of analytical methods and the interpretation of results



Qualitative methods

Collaborative study

-to determine the variability of the results obtained by the alternative method in different laboratories using identical samples

No. of labs: ≥ 8

No. of matrix: 1

Levels: Negative,
1-10 cells/25 g and
10-100 cells/25 g

No. of replicates: 0

Intermediate study

- to confirm the obtained results on at least one additional laboratory.

No. of labs: ≥ 1

No. of matrix: 3

Levels: 4 (neg, low, medium, high)

No. of replicates: 5

Compare AC, SE and SP with the results obtained in the comparison study.



Method Comparison Study - Quantitative methods

Precision:

- Artificially contaminated samples (when real incurred samples are not available).
- For food: at least 5 relevant food matrices
- For other categories: a relevant number of matrices.

Microbiological:

- Levels: 5 covering low, medium and high
- Replicates: 5

Chemical:

- Levels: Low, medium, high + blind
- Replicates: 5
- Repeated at another day

Each sample is analysed by the reference method and the alternative method



Method Comparison Study - Quantitative methods

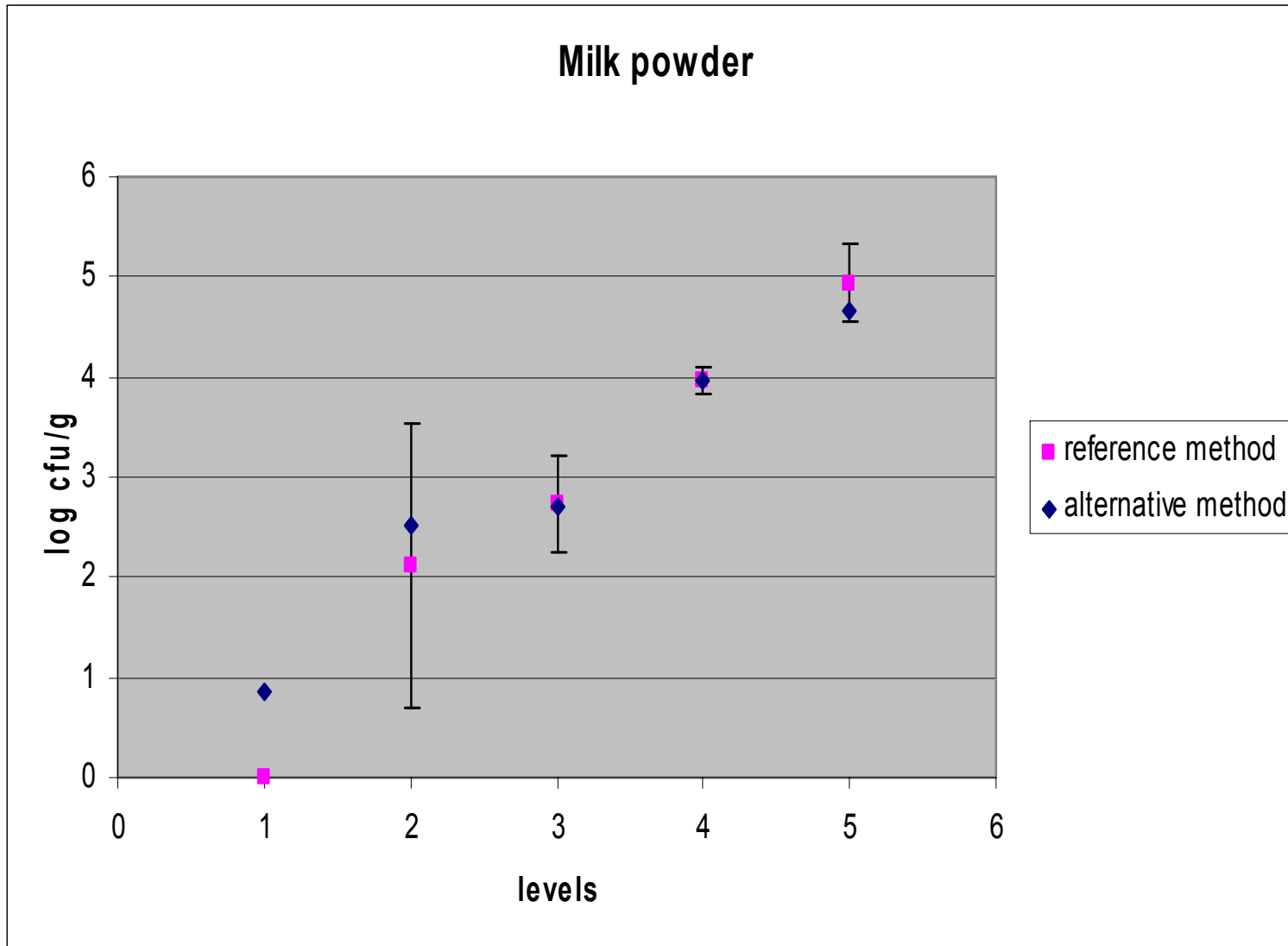
Precision:

For each matrix, calculate the mean and the SD of the results obtained by the reference method and its confidence level $2 \cdot SD$

Calculate the mean and the SD of the results obtained by the alternative method.

Plot the results

Quantitative Methods





Acceptance Criteria – Quantitative Methods

- Fit for purpose
- If the mean of the alternative method is included in the confidence interval of the reference method (i.e. $\text{ref}_{\text{mean}} \pm 2 \text{ SD}$), there is no significant deviation between the methods.
- Micro: Repeatability of less than 0.4 log cfu/g illustrates satisfactory precision.
- Chem: $\text{HorRat} \leq 2$ (internal reproducibility)



Quantitative methods

Collaborative study

-to determine the variability of the results obtained by the alternative method in different laboratories using identical samples

No. of labs: ≥ 8

No. of matrix: 1

Levels: 4 (from 0 to 10^4 per 1 g)

No. of replicates: 2

Intermediate study

- to verify the obtained results on at least one additional laboratory.

No. of labs: ≥ 1

No. of matrix: 3

Levels: 4 (neg, low, medium, high)

No. of replicates: 5



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How to get method criteria based on ML

Procedure no. 4:

Validation of chemical analytical methods

Annex 1

Bilag 1

Bilag 2

Bilag 3

Bilag 4

Bilag 5

Bilag 6

Procedure no. 5:

Estimation and expression of measurement uncertainty in chemical analysis

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