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JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

Working document

EURL-FA Guide: Protocol for verification studies of single-laboratory/in-house validated methods

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Document History

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1.00	24/05/2007	Document created by Giuseppe Simone	
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1. INTRODUCTION

The purpose of this document is to provide guidance on how to implement the requirements of *verification* for analytical methods introduced by the Commission Regulation (EC) No 429/2008[1].

> *"Performance characteristics of in-house validated methods shall be **verified** by testing the method in a second, accredited, and independent laboratory […]".*

According to the paragraph 2.6.1.1 and 2.6.2.1 of Annex II of the Commission Regulation (EC) No 429/2008[1] the analytical methods shall meet the same requirements as those used for official control purpose as laid down in Article 11 of Regulation (EC) No 882/2004[2].

When only in-house[3] method validation is provided the verification of the performance characteristics by an independent expert laboratory is required (*cf.* paragraph 2.6.1.3 of Regulation (EC) No 429/2008[1]).

The verification study aims to demonstrate that the method can be transferred reliably to another laboratory.

With this guidance document the European Union Reference Laboratory for Feed Additives recommends a harmonised reporting format to enable an effective evaluation of dossier submitted for authorisation.

Although this is not a legal text, applicants are recommended to take into account this document when preparing an application for authorisation of feed additives, in compliance with Article 7 of Regulation (EC) No 1831/2003[4] of the European Parliament and of the Council.

For the purpose of this guidance document the definitions laid down in the relevant Community legislation and ISO standards shall apply.

[1] OJ L133, 22.05.2008, p.1-65

[2] OJ L165, 30.04.2004, p.1

[3] The term of "*in-house*" validation – meaning "*single-laboratory*" validation - is used throughout this document for consistency with the Regulation.

[4] OJ L268, 18.10.2003, p.29-43

2. PROCESS OVERVIEW

- ☐ The **Applicant** shall select and define the intended scope of use of the quantitative analytical method(s) for determining compliance with maximum or minimum proposed levels of the active substance(s)/agent(s) (denoted hereafter as analytes) in the additive, premixtures, feedingstuffs[5], water, target tissues and animal products (denoted hereafter as matrix(ces)).
- ☐ The Applicant shall perform the *in-house* validation, applying the relevant recommendation of international standards or guidance documents, such as the IUPAC *harmonised protocol*[6], the ISO 16140 standard of relevance for microbiology[7]. The performance characteristics to be investigated - according to Regulation 882/2004 - are listed in Annex I.
- ☐ The applicant shall provide the validation report including performance characteristics and experimental data/evidence.
- ☐ The Applicant shall draft a detailed description of the analytical method – defined hereafter as Operating Procedure (OP) using the format recommended in the ISO 78-2[8] standard. Annex II lists the topics/*"clauses"* in the preferred order of presentation.
- ☐ The Applicant shall select and entrust an **independent expert laboratory**, denoted hereafter as Laboratory 2 (Lab.2), with well established experience and demonstrated competence in the field related to the relevant analyte/matrix/method combination.
- ☐ The Applicant shall provide Lab.2 with the relevant information and sufficient amount of material to properly perform the analyses. This includes:
  - Clear statement of the scope and the objective of the study;
  - The draft of the operating procedure mentioned above;
  - Standards for calibration and blank samples (when relevant);
  - Samples with declared analyte content allowing in the first phase Lab.2 to get familiar with the method;
  - Samples with undisclosed content (blind samples) to assess the quality of the work performed by Lab.2;
  - The draft of the experimental protocol to be followed by Lab.2;
  - The form to be used by Lab.2 to report about the *verification* study (Verification Study Report, Annex III).

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[5] For the analyte/feedingstuff combination a representative feed should be investigated according to the authorisation sought.

[6] M. Thompson et al.: Harmonised Guidelines For Single Laboratory Validation Of Methods Of Analysis (IUPAC Technical Report) Pure Appl. Chem., Vol. 74, No. 5, pp. 835-855, 2002.

[7] EN ISO 16140: Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods (2003)

[8] EN ISO 78-2: 1999: Chemistry – Layout standards. Part 2: methods of chemical analysis

- Lab.2 shall contact the Applicant whenever clarification about the OP is needed. The Applicant shall keep track of these interactions and implement the relevant modifications (e.g. rephrase the text where appropriate) to ensure the unambiguous interpretation of the OP. These clarifications may require a revision of the OP.
- For each analyte, Lab.2 shall analyse the blank-, known- and unknown-samples and determines the relevant performance characteristics defined in the above mentioned experimental protocol. This includes - at least - repeatability, intermediate precision, recovery rate, limit of detection (LOD) and limit of quantification (LOQ), when applicable, specifying the concentration/activity range investigated.
- For each analyte, Lab.2 shall report to the Applicant the results of the sample analysis and the performance characteristics, using the *Verification Study Report* Template (Annex III). Additional information may be included if deemed necessary (i.e. chromatograms, photographs, etc.).
- The Applicant shall evaluate for each analyte the Verification Study Report prepared by Lab.2 and finalises the technical dossier in accordance with Regulation (EC) No 429/2008[1], including the Verification Report (Annex IV), containing for each analyte the following four sections:
  - The list of modifications implemented in the OP and the corresponding impact on the original in-house validation;
  - The evaluation of the results obtained for the blind sample;
  - The comparison of performance characteristics obtained by the Applicant and Lab.2;
  - Conclusion about the method *verification*.

3. REQUIREMENTS

The mandate of the independent expert laboratory (Lab.2) is to verify the analytical method selected by the Applicant. Lab.2 will analyse all the samples provided and will report the experimental results. In particular, Lab.2 shall investigate - under appropriate quality standards, such as GLP in accordance with Directive 2004/10/EC or ISO standards (cf. Commission Regulation (EC) 429/2008[1]) - the relevant performance characteristics of the method(s) in the same concentration range and matrix(ces) used during the in-house validation study.

Lab.2 can be a public or private organisation, preferably independent from the applicant's company. Lab.2 could belong to the same company of the Applicant, provided that it did not contribute/participate to the in-house validation study. If a NRL from the EURL-FA network is selected by the Applicant to be Lab.2, this NRL shall inform the EURL-FA.

Lab.2 should be familiar with the type of method (technique, matrices, etc…) under investigation and should be a laboratory where similar method(s) is(are) applied on a routine basis.

A preparatory meeting and/or a training session should be organised by the Applicant in order to introduce, explain and demonstrate the method to Lab.2. This would allow clarifying practical details of the protocol, when appropriate.

The Applicant shall adapt and revise the document describing the operating procedure, based on the comments raised by Lab.2.

The Applicant shall provide all the required samples with appropriate homogeneity and stability, including standards for calibration, blank(s), known sample(s) with declared concentration of the target analyte, and blind sample(s) with undisclosed concentration of the target analyte. The Applicant shall provide the list of samples provided.

Lab.2 shall declare deviations from the operating procedure and describe problems encountered in the conduct of the study. Lab.2 may provide additional information (e.g. chromatograms, photographs, etc…), if deemed relevant.

Lab.2 shall prepare a report according to the template provided (see Annex III) addressing in particular the performance of the method, ease of use of the method, and general comments.

4. EXPERIMENTAL PROTOCOL

It is the responsibility of the Applicant to perform proper in-house validation and to provide the adequate documentation. The scope of the Verification Study is to confirm the performance characteristics of the submitted analytical method, not to reproduce a new complete validation study.

In the frame of such a *verification* study, the following issues shall be ensured:

- Analyte/matrix combinations to be investigated are fixed. Extrapolations to other combinations have to be justified.
- The concentration ranges for the verification study shall be similar to those used in the in-house validation.
- No deviations from the analytical protocol are to be implemented by Lab.2 without prior information to the Applicant and formal agreement.
- All samples provided to Lab.2 for analysis are to be characterised by the Applicants. Concentration values are disclosed by the Applicant only for standards for calibration and "known" samples, while they remain undisclosed for the "blind" samples.

The experimental design included in Annex III is presented hereafter. If necessary this design can be modified, provided that it is fully described and justified.

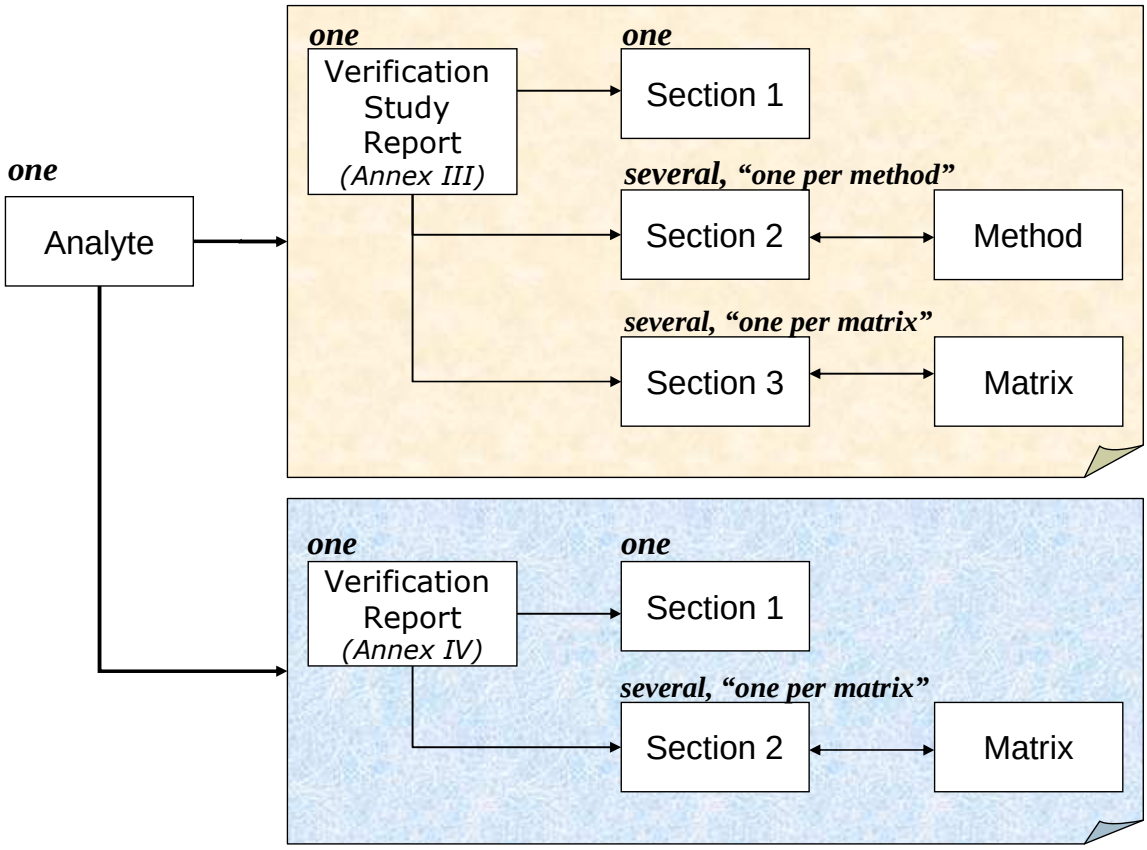
One Verification Study Report is expected for each analyte in relation with the scope of the method, including:

- Information about the five points calibration;
- Estimation of Limit of Detection (LOD) and Limit of Quantification (LOQ) by analyzing three sub-samples of the blank on two different days (when relevant);

Note: For all analyte/matrix combinations falling under Council Directive 96/23/EC[9] validation should be performed according to Commission Decision 2002/657/EC[10].

- Estimation of relative standard deviations for repeatability (RSDr), intermediate precision (RSD_R), and recovery rate by analyzing six sub-samples of known samples on two different days;
- Results of analysis of three sub-samples of the blind samples, if possible in one day.

Overview of the Sections to be compiled



"The same analytical method may have different performance characteristics for different matrices"

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[9] OJ L 125, 23.5.1996, p. 10–32

[10] OJ L 221, 17.8.2002, p. 8-36

ANNEX I:
PERFORMANCE CHARACTERISTICS TO BE INVESTIGATED IN THE FRAME OF A METHOD VALIDATION

According to Regulation (EC) No 882/2004[2], methods of analysis should be characterised by the following characteristics –to be considered during *in-house* validation, if applicable:

- ☐ Accuracy;
- ☐ Applicability (matrix and concentration range);
- ☐ Limit of detection;
- ☐ Limit of determination/quantification;
- ☐ Precision;
- ☐ Repeatability;
- ☐ Reproducibility (intermediate precision);
- ☐ Recovery;
- ☐ Selectivity;
- ☐ Sensitivity (and interferences);
- ☐ Linearity;
- ☐ Measurement uncertainty;
- ☐ Other characteristics that may be selected as required.

ANNEX II:
RECOMMENDED STRUCTURE FOR A DESCRIPTION OF A METHOD OF ANALYSIS

Preferred titles of the clauses in the methods of chemical analysis, and preferred order of the clauses, according to ISO 78-2:1999 (EN)

- ☐ Foreword
- ☐ Introduction
- ☐ Title
- ☐ Warnings
- ☐ Scope
- ☐ Normative references
- ☐ Definitions
- ☐ Principle
- ☐ Reactions
- ☐ Reagents and materials
- ☐ Sampling
- ☐ Procedure
- ☐ Calculation
- ☐ Precision
- ☐ Quality assurance and control
- ☐ Special cases
- ☐ Test report
- ☐ Annexes
- ☐ Bibliography

ANNEX III: VERIFICATION STUDY REPORT

⚐ One report for each analyte, to be compiled by the Independent Expert Laboratory

Section 1

1.1. Laboratory Identification

Company / Institute	
Department	
Laboratory / Group	

1.2. Experience in the field, *related to the method(s) under investigation*

- Your laboratory carries this type of analyses
  ☐ Often ☐ Seldom ☐ Never
- Accreditation: ☐ Yes ☐ No ☐ Pending
  - according to/compliant with: *(specify standard)*
  - specify scope of accreditation :

1.3. List of samples provided by the Applicant

	Description, *specify analyte, matrix (specify major constituents)*	**Amount delivered & units**
Standard(s) for Calibration	▪ . ▪ .	▪ . ▪ .
Blank(s), if applicable	▪ . ▪ .	▪ . ▪ .
Known Samples	▪ . ▪ .	▪ . ▪ .
Blind Samples	▪ . ▪ .	▪ . ▪ .

Sample Delivery Date	
Storage conditions used (*short description)*[11]	
Date (s) of Measurement campaign	

[11] *Specify relevant information, such as temperature, humidity, darkness/light, etc.*

Verification Study Report approved by:

Name	
Function	
Date	
Signature	

Send the completed Report to the Applicant. Thank you

Section 2 (⚐ One for each **method**)

2.1. Scope: Verification of

Title of the Method

for the determination of (specify analyte)

in the following matrices

- ☐ **Feed Additive**
- ☐ **Premix** for *(specify species)*.
- ☐ **Feedingstuffs** for *(specify species)*
- ☐ **Water**
- ☐ **Target tissues/animal products** *(specify tissues/product)*

2.2. Review of the Operating Procedure (OP)

(list of comments discussed with the Applicant)

Num	Describe problem	Modification suggested
1		
2		
3		
…		

2.3. Overall evaluation of each method

Is the Operating Procedure clear & understandable?	☐ Yes ☐ No ☐ Could improve (cf. Section 2.2.)
Is the Operating Procedure easy /practical?	☐ Yes ☐ No
Do your results confirm the in-house validate characteristics?	☐ Yes ☐ No ☐ Not quite
Would you implement this method in your laboratory?	☐ Yes ☐ No Explain why?
Do you have knowledge of similar methods fit for the purpose?	☐ Yes ☐ No If Yes, please provide reference:

Section 3 (⚐ One for each **matrix**)

3.1. Calibration *(when applicable)*

⚐ Provide one set of calibration **for each** matrix.

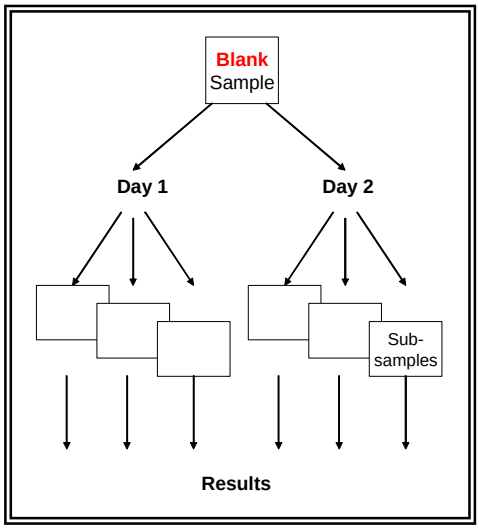
Method	
Analyte	
Matrix	

Calibration date (Day 1)	
Standard for calibration	
Calibration Equation & correlation coeff.	
Calibration Graph (*insert Graph*➔)	

Calibration date (Day 2)	
Calibration Equation & correlation coeff.	
Calibration Graph (*insert Graph*➔)	

Comments - describe experimental problems encountered (if any)

3.2. Blank *(when applicable)*



Method	
Analyte	
Matrix	

	Date	Sample ID	Sample intake[12]	Result (*) or less than value
Day 1				
Day 2				
		Units:		

(*) Provide (when possible) 2 significant digits *(i.e. 0,12 or 1,2 or 12 or 120)*

Estimates of Limit of Detection (LOD) and Limit of Quantification (LOQ)

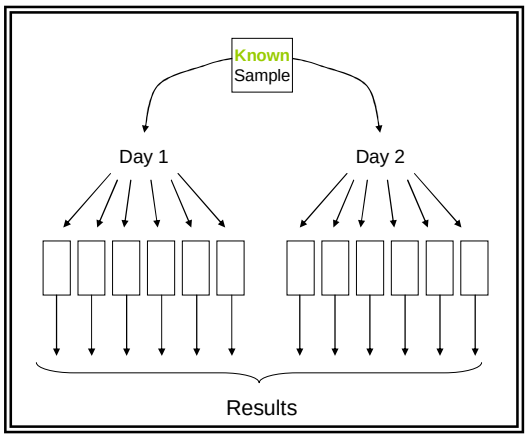
LOD	
LOQ	
Units:	

Comments

- Explain / specify how LOD and LOQ were calculated
- describe experimental problems encountered (if any)

[12] Amount of sample used for the analysis

3.3. "Known" samples



Method	
Analyte	
Matrix	
Expected content, unit	

	Date	Sample ID	Sample intake[13]	Results (a)
Day 1				
Day 2				
		Units:		

(a) Provide (when possible) 3 significant digits *(i.e. 0,123 or 1,23 or 12,3 or 123)*

[13] Amount of sample used for the analysis

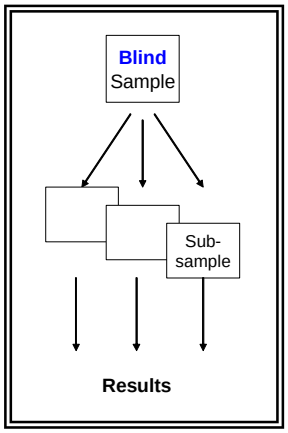
Estimates of relative standard deviations for repeatability (RSD_r) and intermediate precision (RSD_R); and Recovery

RSD_r (%)	
RSD_R (%)	
Recovery	

Comments

- Specify calculation of RSD_r, RSD_R and Recovery rate
- describe experimental problems encountered (if any)

3.4. "Blind"/*unknown* samples



Method	
Analyte	
Matrix	

Measurement Date	Sample ID	Sample intake[14]	Results (*)
	Units:		

(*) Provide (when possible) 3 significant digits *(i.e. 0,123 or 1,23 or 12,3 or 123)*

Computed mean, standard deviation and RSD%

Mean (#)	
Repeatability Standard Deviation (#)	
RSD%	
(#) Units:	

Comments

- describe experimental problems encountered (if any)

[14] Amount of sample used for the analysis

ANNEX IV: VERIFICATION REPORT

⚐ One report for each analyte, to be compiled by the Applicant

Section 1

1.1. Introduction:

One/Several method(s) has/have been in-house validated:

- ☐ Method 1 (short descriptor) for the determination of (specify active substance) in the **Feed Additive**.
- ☐ Method 2 *(short descriptor)* for the determination of *(specify active substance)* in **Premix**.
- ☐ Method 3 *(short descriptor)* for the determination of *(specify active substance)* in **Feedingstuffs**
- ☐ Method 4 (short descriptor) for the determination of (specify active substance) in the **Water**.
- ☐ Method 5 (short descriptor) for the determination of (specify active substance) in the **target tissues/animal products** (specify tissue/product).

(adapt accordingly - add or remove)

The following independent expert laboratory (denoted here after as Lab.2) was selected to confirm the outcome of the validation study(ies):

Company / Institute	
Department	
Laboratory / Group	

This report:
a) presents the comments made by Lab.2 concerning the Operating Procedure document and the consequent corrections implemented;
b) (if required) provides additional experimental evidence resulting from a major modification in the experimental protocol (see previous point);
c) compares the performance characteristics submitted by Lab.2 to those obtained during the in-house validation study;
d) draws conclusions about the successful verification study.

1.2. Review of suggested modifications for the Operating Procedure(s):

OP Method #	Comment #	Modification Suggested *by Lab.2*	Reply/Justification *by Applicant*	Category (*)
1	1			
1	2			
1	3			
…				

(*) E: editorial; m: minor; M: major/critical

☐ All modifications are implemented accordingly. The final operating procedure is included in Enclosure .

☐ At least one "Major/critical" modification was implemented; the following additional experimental data are submitted to complement the in-house validation study. See Enclosure .

Section 2 (⚐ One for each **matrix**)

2.1. Comparison of performance characteristics

Method	
Analyte	
Matrix	

	Applicant	Lab.2	Significance test used	Acceptable? Yes or No
LOD			---	
LOQ			---	
RSD_r %			---	
RSD_R %(*) intermediate precision			Compare with Target (*)	
Concentration **Known** Sample		---	---	
Recovery (%)			t-test	
Concentration **Blind** sample (X)			z-score[15]	

(*) *Target derived from - Legislation or - the Horvitz equation or - an expert opinion; Target to be specified in the comments.*

Comments

Conclusion

Successful Verification Study: ☐ Yes ☐ No

[15] z-score defined as: $(X_{App.} - X_{Lab.2})/(RSD_R * X_{App.})$. The result is considered *satisfactory* when $|z| \leq 2$.

Verification Report Approved by:

Name	
Function	
Date	
Signature	

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This document is the result of the activities of an ad-hoc working group established by the EURL (former CRL) for Feed Additives and composed by experts from the EURL and from the consortium of National Reference Laboratories assisting the EURL for the tasks related to Regulation (EC) No 1831/2003 listed in Annex II of Commission Regulation (EC) No 378/2005, as amended by Commission Regulation (EC) No 850/2007. The members of the working group were: Maria Cesarina Abete, Jacob de Jong, Jozsef Dömsödi, Krzysztof Kwiatek, Annette Plöger, Piotr Robouch and Giuseppe Simone.

FEFANA (EU Association of feed additives and premixtures operators) has been consulted during the preparation of this document.

This document has been endorsed by the above mentioned consortium of National Reference Laboratories.