EURL GUIDANCE DOCUMENT ON SCREENING METHOD VALIDATION

(FOCUS ON MICROBIOLOGICAL, IMMUNOLOGICAL AND PHYSICO-CHEMICAL SCREENINGS)

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Anses VMPR website : http://eurl-veterinaryresidues.anses.fr/

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a short story …

for the validation of VDR screening methods

from Decision (EC) 657/2002 to Regulation (EU) 2021/808

2002-2004

. Entry into application of the **Decision (EC) 657/2002**

. Expression of need by NRLs of a Technical Guidance for Screening issues

2005-2010

. Rounds of tech discussions with EU-MS NRLs during EURLs Workshops <=> Questionnaires

Jan 2010

. Launch of the 1st Tech **Guidance for Validation of Screening**

March 2021

. Entry into application of Regulation 2021/808

. and Repeal of CD 657/2002

2021 > 2022

. Revision of the Jan2010 Technical Guidance for Screening

From previous steps

1. Decision EC/657/2002 from 12 Aug 2002

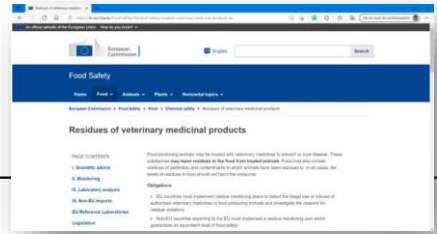
=> “screening methods” criteria of performance **not fully described**

1.35 – Definition : Screening method means methods that are used to detect the presence of a substance or class of substances at the level of interest. These methods have the capability for a high sample throughput and are used to sift large numbers of samples for potential non-compliant results. They are specifically designed to avoid false compliant results.

2.2 – SCREENING METHODS : Only those analytical techniques, for which it can be demonstrated in a documented traceable manner that they are validated and have a false compliant rate of < 5 % (β-error) at the level of interest shall be used for screening purposes in conformity with Directive 96/23/EC. In the case of a suspected non-compliant result, this result shall be confirmed by a confirmatory method.

2. Guideline for the validation of screening methods (launch Jan 2010) available from: https://**ec.europa.eu/food**/system/files/2016-10/cs_vet-med-residues_guideline_validation_screening_en.pdf

III. Laboratory analysis :



Starting questions discussed with the EU-MS NRLs network:

- Looking back at the content of the Guidance from Jan 2010:

  - How to technically validate a screening method ?

  - News related to Reg 2021-808 ?

  - What will not have to change when bridging from Guidance 2010 to the new Guide « 2022 » ?

  - To which extent the Regulation 2021/808 brings changes ?

Revision of the Guideline from Jan 2010 according to Reg 2021/808

Part 1 What does not change with the new regulation?

Part 2 What changes will impact

the future screening guidance 2022?

What does not change with the new regulation?

What does not change ?

- Fundamental principles of the validation of screening methods (guideline 2010)

Jan 2010 – Chap 2.2 - Screening methods:

Only those analytical methods, for which it can be demonstrated in a documented traceable manner that they are validated and have a **false compliant rate lower than or equal to 5 % (β error)**, shall be used for screening purposes. In the case of a suspected non-compliant result, that result shall be confirmed by a confirmatory method.

1. Detection capability for screening CCβ

   with Beta error β 5% (probability to give false negatives ≤ 5%)

2. Screening Target Concentration (STC)

3. Specificity/selectivity

1. Detection capability for screening (CCβ)

Decision EC/2002/657 (Screening guidance (2010)) vs Regulation 2021/808/EC (Future Screening guidance (to be acted 2022))

Definition

The smallest content of the analyte that may be detected, ~~identified and/or~~ **or** quantified in a sample with an **error probability of β**.

(a) **Prohibited or unauthorised substances: detect or quantify samples containing residues**

(b) **Authorised substances**: detect concentrations **below the permitted limit**

CHAPTER 1 PERFORMANCE CRITERIA AND OTHER REQUIREMENTS FOR ANALYTICAL METHODS

Prohibited or unauthorised substances: CCβ as low as reasonably achievable and ≤ RPA (Regulation (EU) 2019/1871)

Authorised substances: CCβ ≤ MRL or ML

2. Screening Target Concentration (STC)

Decision EC/2002/657 (Screening guidance (2010)) vs Regulation 2021/808/EC (Future Screening guidance (to be acted 2022))

Not defined in the decision EC/2002/657

Introduced in the **Screening guidance (2010)**

Regulation 2021/808/EC

STC : **the concentration at which a screening test categorises the sample as "Screen Positive" (potentially non-compliant) and triggers a confirmatory test**

(39) the concentration **lower than or equal to the CCβ** at which a screening measurement categorises the sample as potentially non-compliant 'Screen Positive' and triggers a confirmatory testing

1- Authorised analytes: STC ≤ MRL (preferably ½ MRL wherever possible)

2- Prohibited & unauthorised analytes: STC ≤ RPA

3- Analytes for which no MRL: STC ≤ RC* (CRL Guidance Paper 2007)

=> Will be kept in the future Screening guidance (2022)

** RC recommended concentration (MRPL => MMPR)*
MRPL : minimum required performance limit (<2019)
MMPR : minimum method performance requirement (> 2019)

3. Specificity / Selectivity and Detection capability CCβ

Specificity / selectivity:

- Simultaneously determined with CCβ
- Investigation of *blank samples*: at least 20 samples => Calculation of false positive rate => No regulatory maximum value for this rate

Detection capability CCβ:

- Investigation of *fortified blank samples* at the STC (< regulatory limit). At least 20 fortified blanks shall be analysed in order to ensure a reliable basis for this determination.

	STC ≤ ½ MRL or ½RPA or ½MMPR	≤ ½ MRL or ½RPA or ½MMPR < STC ≤ 90% MRL, RPA or MMPR	STC > 90% MRL, RPA or MMPR
Number of samples	20	40	60
5% of false negative	1	2	3

CCβ = The concentration level (STC), where only ≤ 5 % false compliant results remain.

Method 2 (Regulation EC/2021/808)

Specific case of microbiological methods

- Screening guidance (2010):
- Choice of analytes used for validation and selectivity of the method : **representative analytes**

- Preparation of **"simulated tissue"** for validation of microbial growth inhibition tests : Tissue is minced, weighed, spiked and frozen. Pieces of frozen spiked tissue are placed directly on the plates.

(NB This procedure may not be applicable to kidney samples - due to false positive results

Cut-off values (T and Fm)

Not defined in decision 2002/657/EC and in Regulation 2021/808/EC.

Defined in **screening guidance (2010) (Annex II) (kept in future screening guidance 2022)**

Applicable to immunological methods (ELISA, RIA, biosensors) (signal inversely proportional to the concentrations), physico-chemical methods

Cut-off values (T and Fm)

Calculation of two cut-off values: **Threshold value T and cut-off Level Fm**

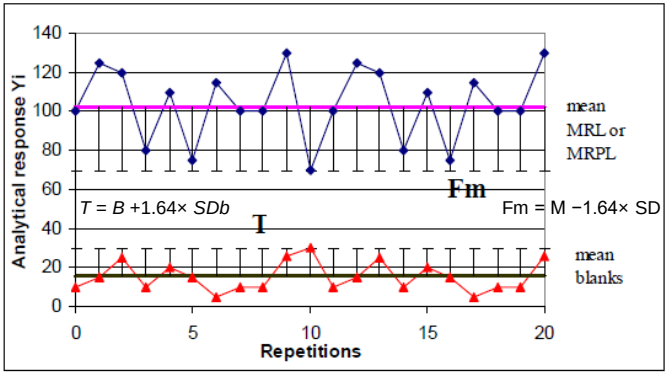
Initial validation: Matrix blank samples (T) + replicates of same samples spiked at the STC (Fm).

Cut-Off Level Fm = the response or signal from a screening test which indicates that a sample contains an analyte at or above the STC => test results are considered "positive".

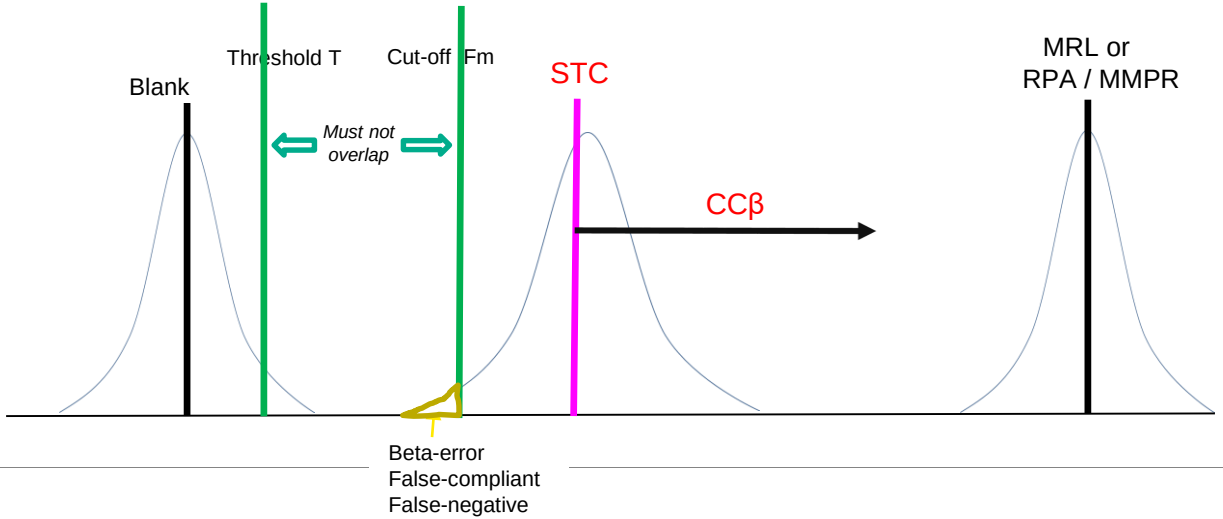
Routine (QC spiked at the STC): If the Cut-Off Level Fm is exceeded a subsequent confirmatory test is carried out.

CCβ = STC when at least 95 % of spiked samples at STC gave positive results (response/signal ≥ Fm) (Method 2 regulation EC/2021/808).

Cut-off values (T and Fm)



Case 1: The signal is proportional to the concentrations (eg. HPLC, LC-MS/MS).



What changes with the new regulation that will impact the future screening guidance 2022?

Categories of methods and their Screening Performance

Decision EC/2002/657 vs Regulation 2021/808/EC

- **Introduction of semi-quantitative methods:**

CHAPTER 1 PERFORMANCE CRITERIA AND OTHER REQUIREMENTS FOR ANALYTICAL METHODS

1.1. Requirements of screening methods

1.1.1. **Categories of suitable screening methods**

Qualitative, semi-quantitative or quantitative methods shall be used as suitable screening methods.

- **Categories based on the detection principle:**

1.1.2. Requirements for **biological, biochemical or physico-chemical** screening methods

Only those analytical methods, for which it can be demonstrated in a documented traceable manner that they are validated and have a **false compliant rate lower than or equal to 5 % (β error)**, shall be used for screening purposes. In the case of a suspected non-compliant result, that result shall be confirmed by a confirmatory method.

Classification of analytical methods

Guideline (Jan 2010)

3. Screening Method classification

3.1. Classification by detection principle

- Biological methods
- Biochemical methods
- Physicochemical methods

3.2. Classification by their degree of quantification

- Qualitative
- Semi-quantitative
- Quantitative

Revised guideline (2022)

Screening Method classification

. Classification by detection principle

- Biological methods
- Biochemical methods
- Physicochemical methods

. Classification by their degree of quantification

- Qualitative
- **Semi-quantitative**
- **Quantitative**

Classification of analytical methods : performance characteristics

From Decision EC/2002/657

2 types of methods: qualitative and quantitative methods

Table 9

Classification of analytical methods by the performance characteristics that have to be determined

		Detection limit CCß	Decision limit CCα	Trueness/recovery	Precision	Selectivity/ specificity	Applicability/ ruggedness/ stability
Qualitative methods	S	+	–	–	–	+	+
	C	+	+	–	–	+	+
Quantitative methods	S	+	–	–	+	+	+
	C	+	+	+	+	+	+

S = screening methods; C = confirmatory methods; + = determination is mandatory.

(Semi-)Quantitative methods

Regulation (EU) 2021/808

CHAPTER 2 VALIDATION

2.1. Performance characteristics to be determined for analytical methods

Which performance characteristic shall be verified for which type of method?

Table 5

Classification of analytical methods by the performance characteristics that have to be determined

Method	Confirmation		Screening		
	Qualitative	Quantitative	Qualitative	Semi-quantitative	Quantitative
Substances	A	A, B	A, B	A, B	A, B
Identification in accordance with 1.2	x	x			
CCα	x	x			
CCβ	-		x	x	x
Trueness		x			x
Precision		x		(x)	x
Relative matrix effect/absolute recovery *		x			x
Selectivity/Specificity		x	x	x	x
Stability *		x	x	x	x
Ruggedness		x	x	x	x

x: It is required to prove by means of the validation that the requirements for the performance characteristic are met.

(x) The precision requirements of Chapter 1.2.2.2 do not need to be met for **semi-quantitative screening methods**. However, **the precision shall be determined** to prove the suitability of the method for avoiding false compliant analytical results.

Quantitative screening methods, used for **both screening and confirmation** shall meet the same requirements for **accuracy, range, and precision** as described in 1.2.2.1 and 1.2.2.2. for confirmatory methods.

A: Prohibited subst.
B: Authorised subst.

Methods for calculation of CCβ (2.7.)

2.7.1 - prohibited substances (a) (b) (c) **2.7.2 - authorised substances (a) (b) (c)**

(a) **Method 1**: **The calibration curve procedure according to ISO 11843-1-2:1997**

In this case, representative blank material shall be used, which is fortified at and below the permitted limit (authorized), at and below the RPA, or if no RPA has been established, around the STC in equidistant steps (unauthorised or prohibited).

CCβ = The corresponding concentration at the STC plus 1.64 times the standard deviation of the within-laboratory reproducibility of the mean measured content at the STC **(CCβ > STC)**.

(b) **Method 2**: **Twenty batch-independent fortified blanks**

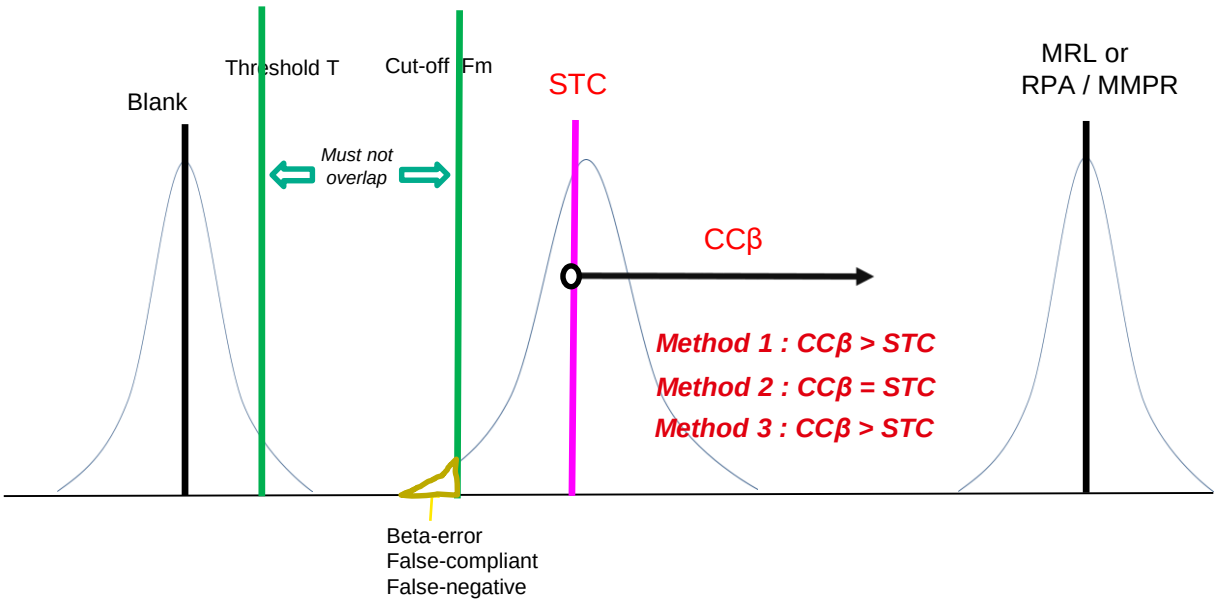
Investigation of fortified blank material at concentration levels at and above the STC (below the permitted limit (authorized)). For each concentration level 20 fortified blanks shall be analysed in order to ensure a reliable basis for this determination.

CCβ = The concentration level, where only ≤ 5 % false compliant results remain **(CCβ = STC)**.

(c) **Method 3**: **Based on combined standard measurement uncertainty**

CCβ = STC + k(one-sided, 95 %) × (combined) standard measurement uncertainty at or above the STC **(CCβ > STC)**.

The within-laboratory reproducibility and the trueness are suitable to define the (combined) standard measurement uncertainty, if determined by taking into account all relevant influencing factors.


Blank
Threshold T
Cut-off Fm
Must not overlap
STC
MRL or
RPA / MMPR
CCβ
Method 1 : CCβ > STC
Method 2 : CCβ = STC
Method 3 : CCβ > STC
Beta-error
False-compliant
False-negative

Conclusions: What will change in the revised guidance?

- Biological methods: no major changes

  but validation procedures will be detailed

- Physico-chemical methods used as screening methods (qualitative or semi-quantitative):

  validation procedures will be detailed

Take home message

Classification of analytical methods by the performance characteristics that have to be determined

Method	Confirmation		Screening		
	Qualitative	Quantitative	Qualitative	Semi-quantitative	Quantitative
Substances	A	A, B	A, B	A, B	A, B
Identification in accordance with 1.2	x	x			
CCα	x	x			
CCβ	-		x	x	x
Trueness		x			x
Precision		x		(x)	x
Relative matrix effect/absolute recovery *		x			x
Selectivity/Specificity		x	x	x	x
Stability *		x	x	x	x
Ruggedness		x	x	x	x

VALIDATING SCREENING METHODS :

- **Qualitative screening methods**

  Specificity/selectivity, detection capability CCβ, stability, ruggedness

- **Semi-Quantitative screening methods**

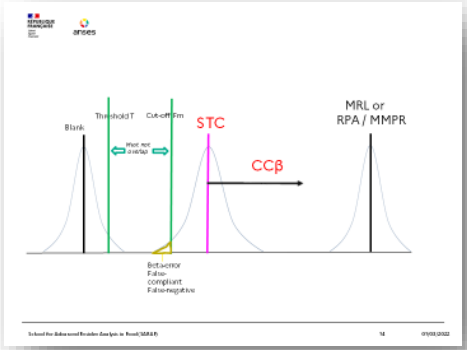
  same + precision (determined, but criteria not required)

- **Quantitative screening methods**

  same + precision, trueness, relative matrix effect/absolute recovery:

  « all criteria required » ⇔ Confirmatory method

Take home message



VALIDATING SCREENING METHODS :

At the detection capability CCβ (β: 5%) ≥ STC Screening Target Concentration

1 – Screening for MRL-authorised substances :

Detection capability CCβ ≤ MRL

2 – Screening for prohibited / non-authorised substances :

Detection capability CCβ ≤ RPA* or MMPR**

** RPA : Reference Point for Action (from Reg (EU) 2019/1871)*

*** MMPR : Minimum Method Performance Required (VMPR EURLs Guidance Paper – v2.0 March 2021)*

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Thank you for your attention

QUESTION?

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