REVISION OF REGULATION 2021/808
A laboratory's (not so quiet) journey through the process of revalidating its methods

TRACES Symposium 2025
Ghent, Belgium

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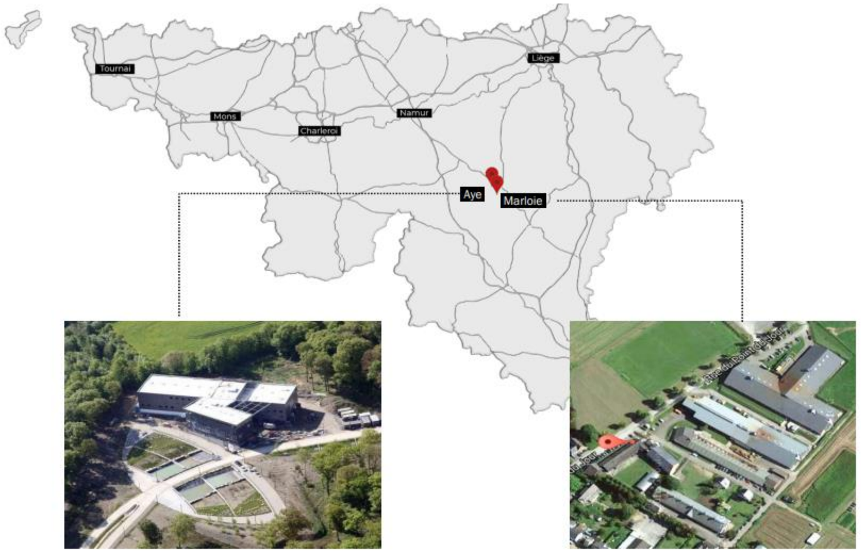
Wednesday, June 04th, 2025.

CER Groupe & Food Safety



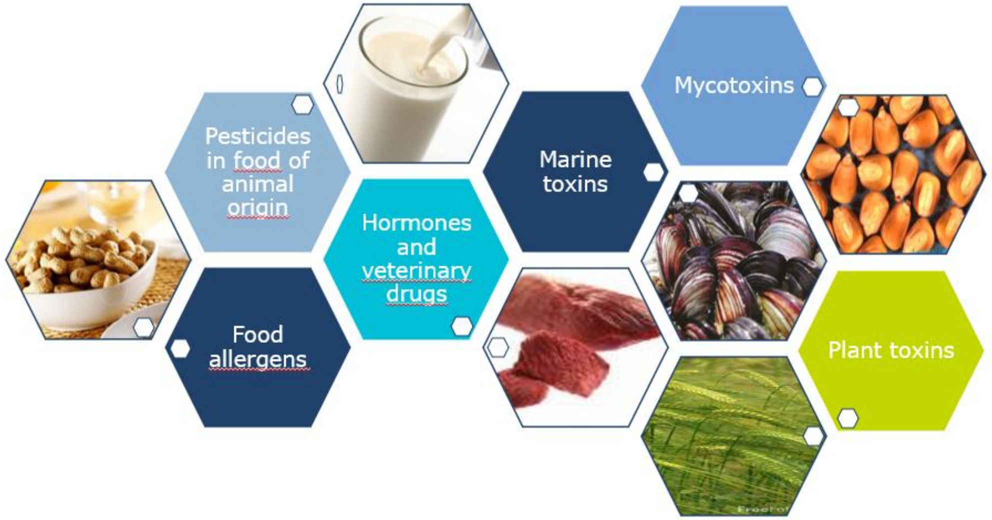
Created 1977

> 200 p. incl. 60 DVM/PhD/MSc

2 sites / 15000 sqm

Activities in Food Safety

- Official Control Laboratory, ISO17025 flexible scope
- National Reference Laboratory
- R&D



ILVO

fasfc

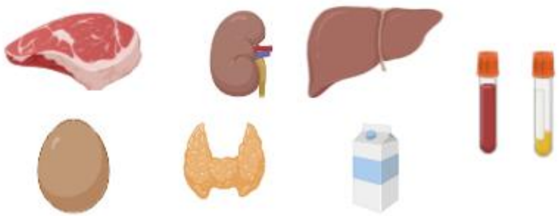
Revision of VMPR regulation and its impact on CER's scope

<u>Global scope</u> (May 2025)
13930 combinations*

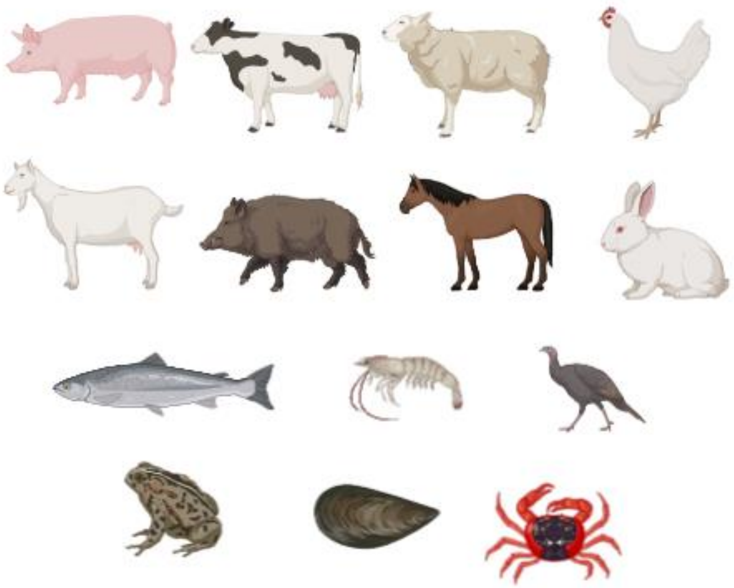
<u>VMPR scope</u>
9790 combinations*
5377 group A
4413 group B
= 367 validations to be done

* method/matrix/species/compounds

tissues from food-producing animal



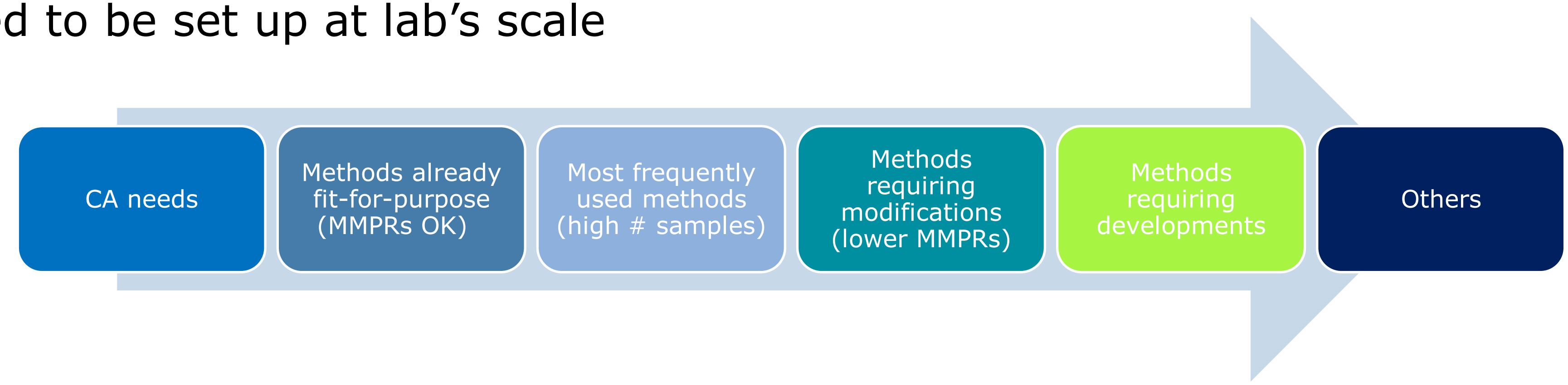
for various species



but also fluids, excrements, animal by-products, feed, water, material

Revision of VMPR regulation - CER's observations

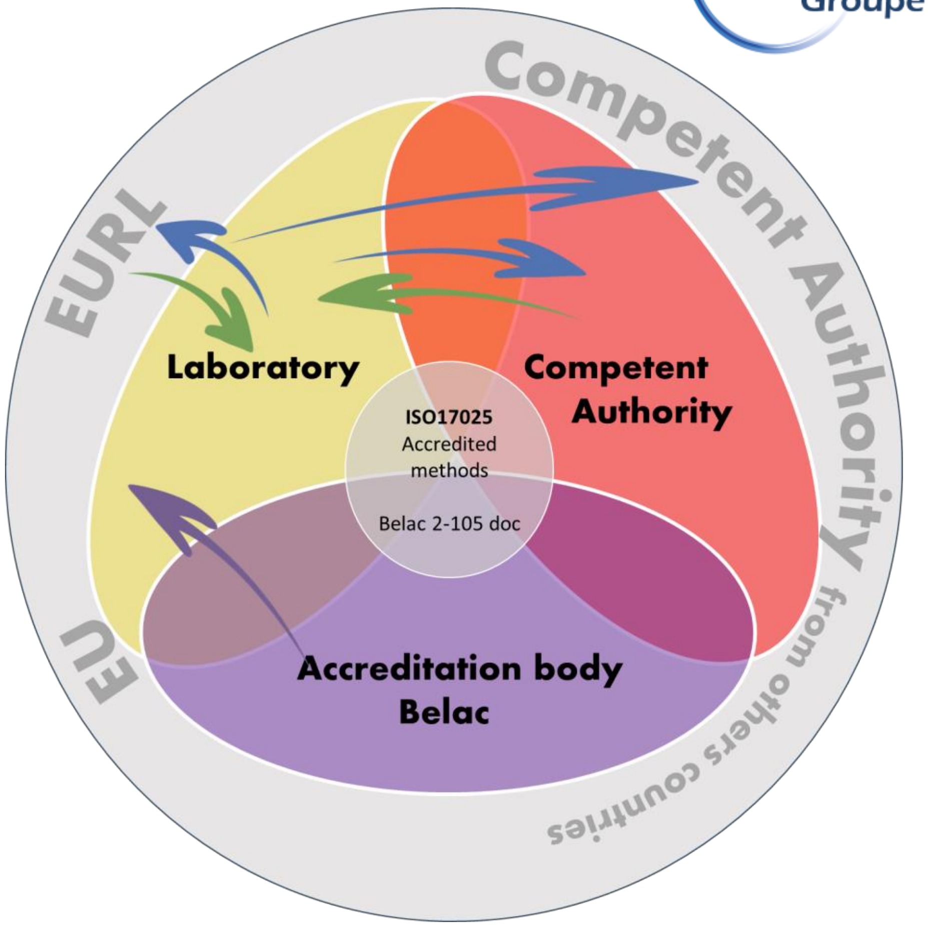
- ✓ Additional workload and costs
  - For lab: resources to be allocated for the validation work (HR, equipments, reagents, consumables) without additional incomes
  - For Competent Authority : increased costs
- ✓ A full validation (2021/808) cannot be applied for all combinations (not achievable)
- ✓ Alternative strategies need to be set up
- ✓ Alignment with CA & accreditation bodies are crucial
- ✓ Priorities need to be set up at lab's scale



- ✓ Alignment of screening/confirmatory methods are crucial
- ✓ Having a flexible scope is a major advantage to reduce validation workload

How do we start this validation journey ?

- ✓ CER = active player in the european network
  - ✓ Participation to working groups on standard addition and stability
  - ✓ Sharing of experience during workshops, webinars, congres
- ✓ Strong partnership with the belgian Competent Authority and the belgian accreditation body (BELAC)
  - ✓ Revision of BELAC 2-105 document
  - ✓ Building of a stability database



P83 A competent authority is nothing without a competent laboratory: mutual benefit of a close collaboration [C. Robert and N.Gillard]

New EU regulation for VMPR : reference documents

- Regulation 2021/808 regarding method validation
- Regulation 2022/1644 regarding new classification of substances
- Technical EURL Guidance Documents were planned:
    1. *on the quality control during routine analysis (ongoing method performance verification) (finalized, version 1.1)*
    2. *on confirmation method validation (finalized, version 1.1)*
    3. *on the extension of methods (finalized, version 1.0)*
    4. *on validation of screening methods (not yet finalized)*
    5. *EURL Guidance Document on the validation of HRMS methods*
    6. *Standard addition*
    7. *Stability studies (upcoming)*

https://eurl-residues.eu/eurl-portal/portal-guidance-documents/

In Belgium, additional guideline for flexible scope management of VMPRs

BELAC 2-105 R3-2015 F (fgov.be)

Belgian guideline for flexible scope management of VMPR (BELAC 2-105)

BELAC 2-105 Rev 4-2023

BELAC

CRITÈRES AUXQUELS DOIVENT RÉPONDRE LES LABORATOIRES ACCRÉDITÉS DEMANDEURS D'UN DOMAINE D'APPLICATION FLEXIBLE POUR LES ANALYSES CONCERNANT LES RÉSIDUS DE MÉDICAMENTS VÉTÉRINAIRES, SUBSTANCES PHARMACOLOGIQUEMENT ACTIVES AUTORISÉES COMME ADDITIFS ALIMENTAIRES ET SUBSTANCES PHARMACOLOGIQUEMENT ACTIVES INTERDITES OU NON AUTORISÉES VALIDES D'APRES LE RÈGLEMENT D'EXÉCUTION 2021/808/UE

Publication planned for 01/2024
(french & dutch)

✓ Historical content :

- how to obtain a flexible scope
- validation concepts

✓ Modifications linked to :

1. Update to fit with new Regulations/guidance
   - new classification of substances
   - new classification of matrices
   - modification of validation requirements
2. News solutions to be implemented
   - On-going validation
   - Standard addition
   - Target concentration based on ALARA principle for not authorized compounds
   - Analysis of dual use substances

Belgian guideline for flexible scope management of VMPR (BELAC 2-105)

- ✓ Classification of matrices/parameters allows:
  - ✓ Flexible scope attribution/request by labs
  - ✓ Extension of methods with lighter validation scheme, based on lab experience

- ✓ <u>Classification of matrices</u>
  Flexibility possible at different levels (if ≥ 50% already validated/accredited)
  - sub-matrices
  - principal matrices
  - groups of matrices

- ✓ <u>Classification of parameters</u>
  Flexibility possible at different levels (if ≥ 50% already validated/accredited)
  - principal parameters
  - sub-groups

BELAC 2-105 guideline : groups of matrices

I. Biological matrices including food and water

1. Matrices of animal origin

2. Milk and dairy products

3. Animal excretion products and water

4. Hairs, fleece, eyes, products containing keratin

5. Animal feed, foodstuffs of plant origin and raw materials

6. High-carbohydrate animal and plant matrices

7. Singular matrices

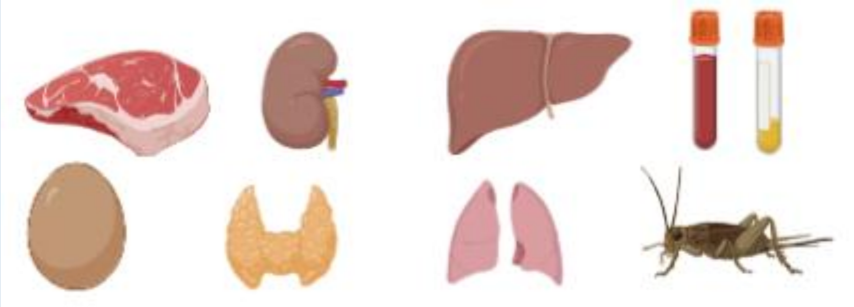
II. Material samples

High concentration samples

BELAC 2-105 guideline : groups of matrices

I. Biological matrixes including food and water

1. Matrices of animal origin



If a matrix is fully validated for bovine muscle, the method could be extended with a secondary validation for

- Other muscle species (porcine, poultry,…)
- Other matrices from the same group (liver, kidney, plasma, egg, thyroid,…)

BELAC 2-105 guideline : groups of parameters

Parameter group	Subgroup	Main parameters
Group A : Prohibited or unauthorised substances	1. Substances having a hormonal or thyrostatic action and beta-agonists whose use is prohibited by Directive 96/22/EC	a. Stilbenes, c. Steroids, d. Resorcylic acid lactones, including zeranol b. Antithyroid agents e. Beta-agonists
	2. Prohibited substances listed in table 2 of the annex to regulation (EU) 37/2010	a. Chloramphenicol b. Nitrofurans c. Dimetridazole, metronidazole, ronidazole and other nitroimidazoles d. Other substances
	3. Pharmacologically active substances not listed in Table 1 of the Annex to Regulation (EU) 37/2010 or substances not authorised for use in food-producing animals in accordance with Regulation (EU) 1831/2003	a. Dyes b. Plant protection products within the meaning of Regulation (EU) 1107/2009 (Pesticides) and biocides within the meaning of Regulation (EU) 528/2012 which may be used in the rearing of food-producing animals; d. Coccidiostats, histomonostats and other antiparasitic agents. (dual-use substance) c. Antimicrobial substances e. Protein and peptide hormones f. Anti-inflammatory substances, tranquillisers and any other pharmacologically active substance g. ***Antiviral*** substances
Group B Authorised substances *if there are restrictions such as "do not use in aquaculture" or "only for poultry", the classification as substance B does not change.	1. Pharmacologically active substances listed in table 1 of the annex to regulation (EU) 37/2010.	a. Antimicrobial substances b. Insecticides, fungicides, anthelmintics and other pest control agents. c. Tranquilizers d. Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and glucocorticoids. e. Other pharmacologically active substances
	2. Coccidiostats and histomonostats authorised under Regulation (EU) 1831/2003, for which MRLs are set under EU legislation and for which maximum levels are set under Regulation (EC) 124/2009.	

BELAC 2-105 : validation concepts

Full validation

L 180/84 EN Official Journal of the European Union 21.5.2021

COMMISSION IMPLEMENTING REGULATION (EU) 2021/808

of 22 March 2021

on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC

Alternative validations :

- Secondary validation
  - New parameters
  - New matrix (! Same group)
  - Extension of the calibration range
- On-going validation
- Standard addition (verification)

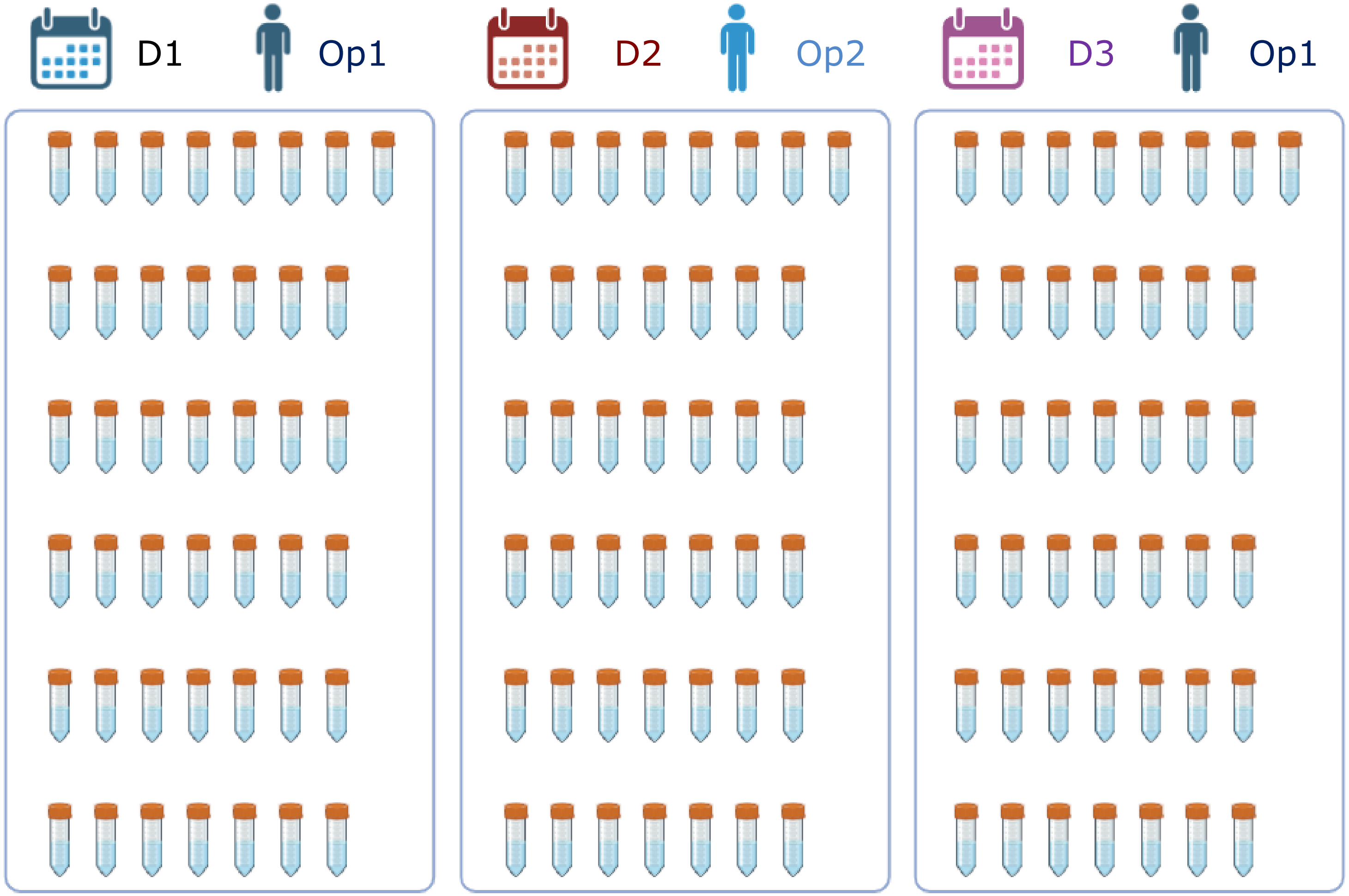
Dual-use substances

No discussion with ISO17025 auditor for the approval of alternative validation schemes

Validation of quantitative confirmatory methods – full validation

?

- New method or new group of matrices
- Revalidation after modification of the sample prep



Calibration curve (n=8)
0.05 -2 MRL

Blank (n=7)

QC level 1 (n=7)
0.1 MRL

QC level 2 (n=7)
0.5 MRL or LCL

QC level 3 (n=7)
MRL

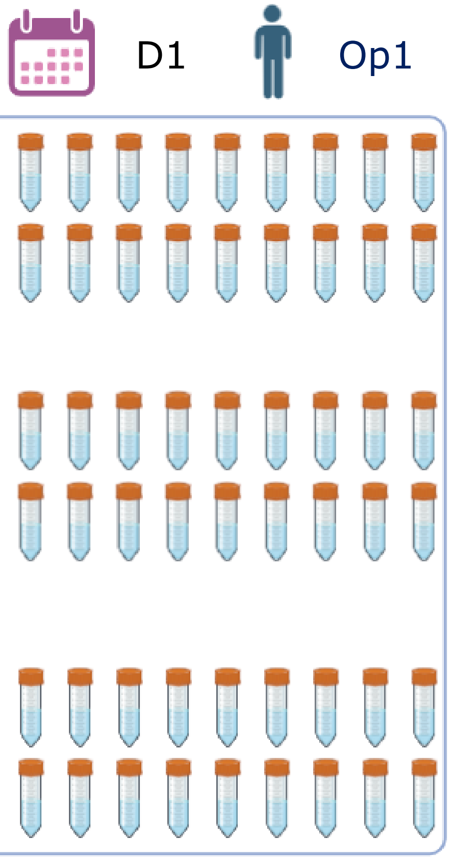
QC level 4 (n=7)
1.5 MRL

Performance criteria
CCα
Identification (IR/RT)
Trueness
Precision
Selectivity/ specificity
Ruggedness
Stability (matrix)
Relative matrix effect

Validation of qualitative confirmatory methods – full validation

?

- Applied for group A/non authorized parameters
- New method or new group of matrices
- Revalidation after modification of the sample prep



Blank (n=20)

QC level 1 (n=20)
0.1 MRL/STC

QC level 2 (n=20)
0.5 MRL

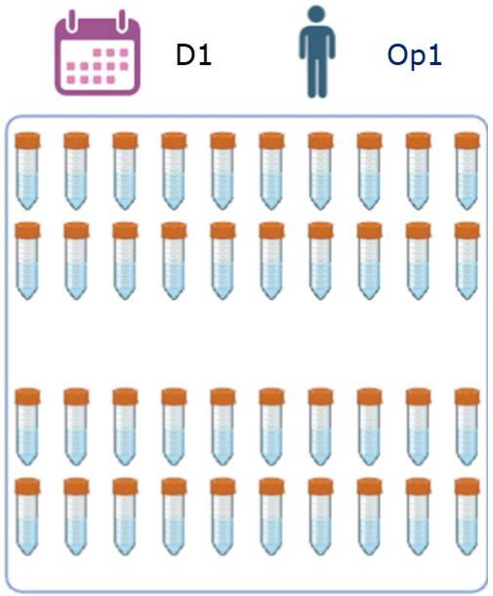
Performance criteria
CCβ
Identification (IR/RT)
Selectivity/ specificity
Ruggedness
Stability

Alternative validation scheme : mix of species/matrix

ANA28 – antiectoparasitics compounds (A3f) in food commodities by UPLC-MS/MS

Strategy :

- Method with a unique sample prep protocol for several matrices/species
- Non-authorized parameters, no MRL, no MMPR
- Qualitative screening method
- Full validation tested for several matrices/species with the same STC



20 blank/ 20 QC at STC:
-4 liver
-5 egg
-5 poultry muscle
-3 fatty fish muscle
-3 lean fish muscle

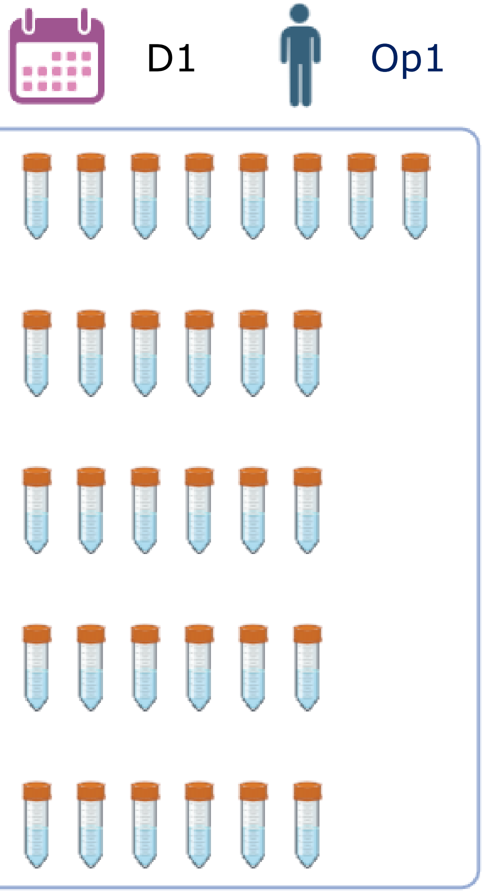
Advantages :

- Reduced validation workload
- common CCβ
- single mixture of standards

Validation of quantitative confirmatory methods – secondary validation

?

- Extension to new parameters
- Extension to new matrices (within same group) or species
- Revalidation at lower concentration (0.1 MRL or lower MMPRs)



Calibration curve (n=8)
0.05 -2 MRL

Blank (n=6)

QC level 1 (n=6)
0.1 MRL

QC level 2 (n=6)
0.5 MRL or LCL

QC level 3 (n=6)
MRL

Performance criteria
CCα
Identification (IR/RT)
Trueness
Precision
Selectivity/ specificity

Validation of quantitative confirmatory methods – secondary validation

ANA63-Aminoglycosides in honey

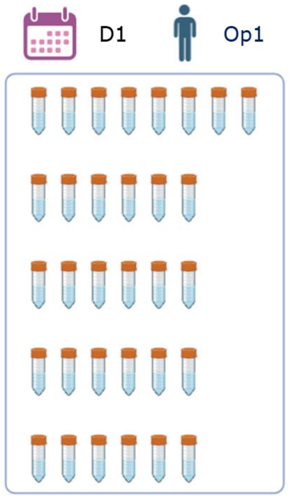
I. Biological matrixes including food and water

1. Matrices of animal origin
2. Milk and dairy products
3. Animal excretion products and water
4. Hairs, fleece, eyes, products containing keratin
5. Animal feed, foodstuffs of plant origin and raw materials
6. High-carbohydrate animal and plant matrices
7. Singular matrices

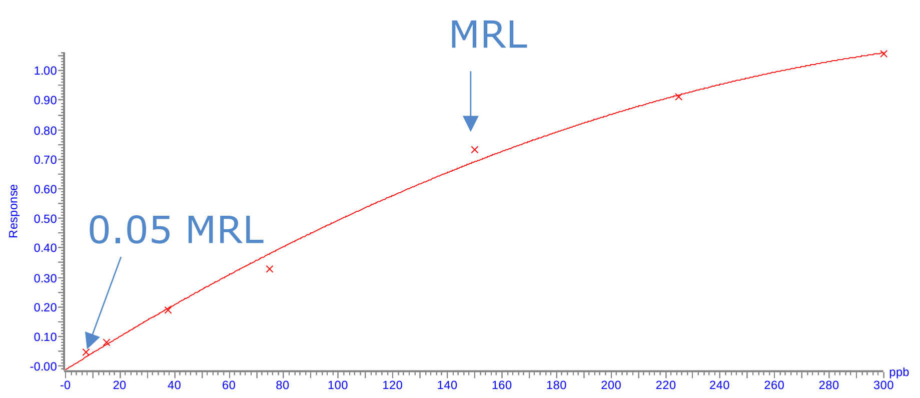
II. Material samples

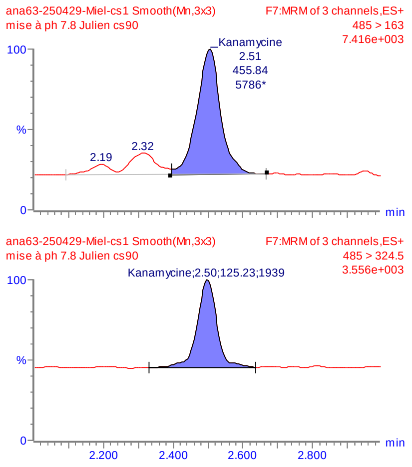
High concentration samples

- Method already validated for honey (quantitative)
- Extension of the calibration curve to 0.05 MRL
- Validation tested at 0.1 MRL and 0.5 MRL



Kanamycine



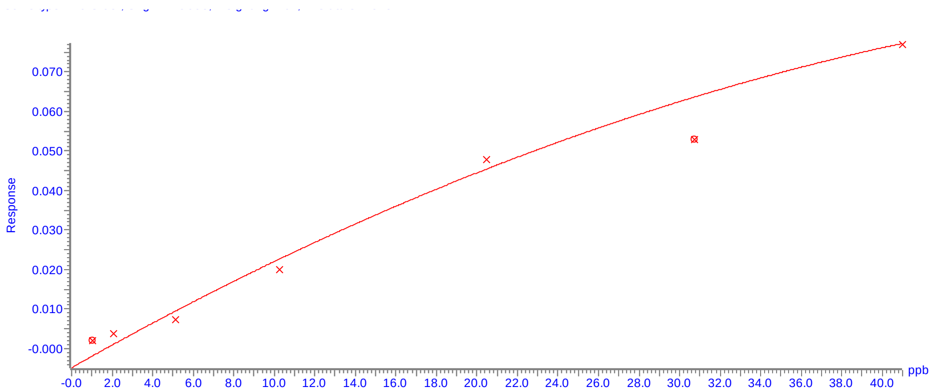


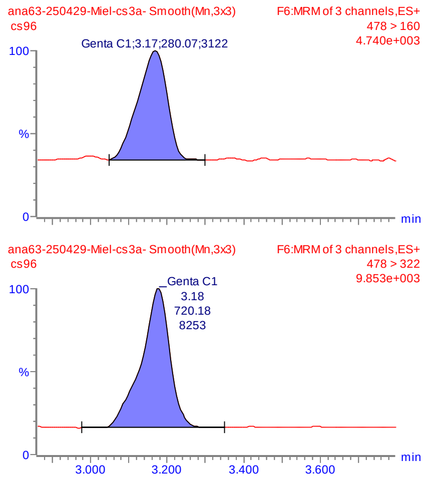
Performance criteria	0.1 MRL	0.5 MRL	MRL
Ion ratio	✓	✓	✓
RRT	✓	✓	✓
Trueness	✓	✓	✓
Precision	✓	✓	✓
Calibration range	0.05 -2 MRL		

Validation of quantitative confirmatory methods – secondary validation

ANA63-Aminoglycosides in honey

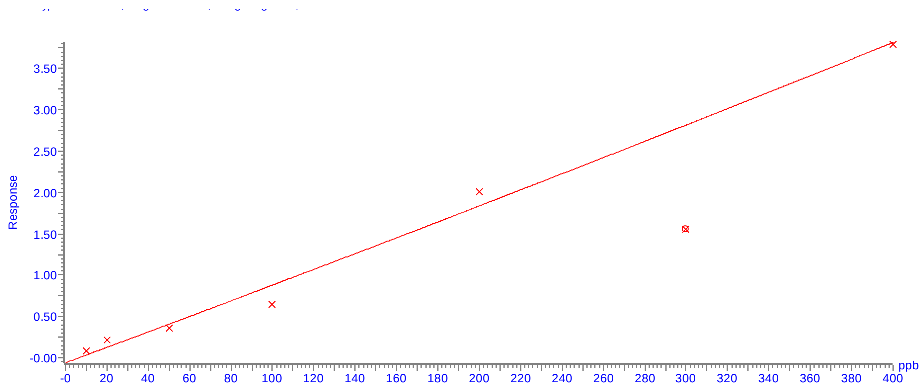
Gentamycine C1a

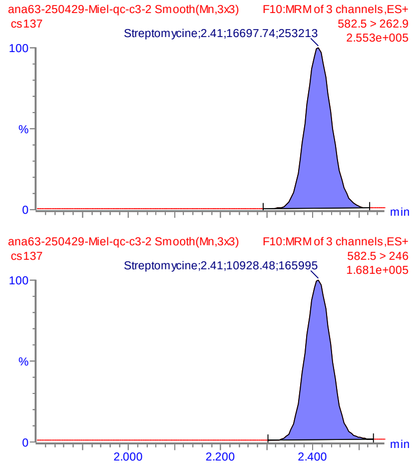




Performance criteria	0.1 MRL	0.5 MRL	MRL
Ion ratio	✗	✓	✓
RRT	✓	✓	✓
Trueness	✓	✓	✓
Precision	✓	✓	✓
Calibration range	0.1 -2 MRL		

Streptomycine





Performance criteria	0.1 MRL	0.5 MRL	MRL
Ion ratio	✓	✓	✓
RRT	✓	✓	✓
Trueness	✗	✓	✓
Precision	✓	✓	✓
Calibration range	0.1 -2 MRL		

Alternative validation scheme : mix of species/matrix

D1 Op1

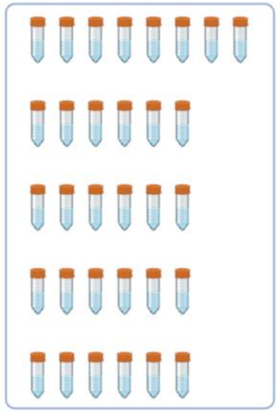
Calibration curve (n=8)
0.05 -2 MRL

Blank (n=6)

QC level 1 (n=6)
0.1 MRL

QC level 2 (n=6)
0.5 MRL or LCL

QC level 3 (n=6)
MRL

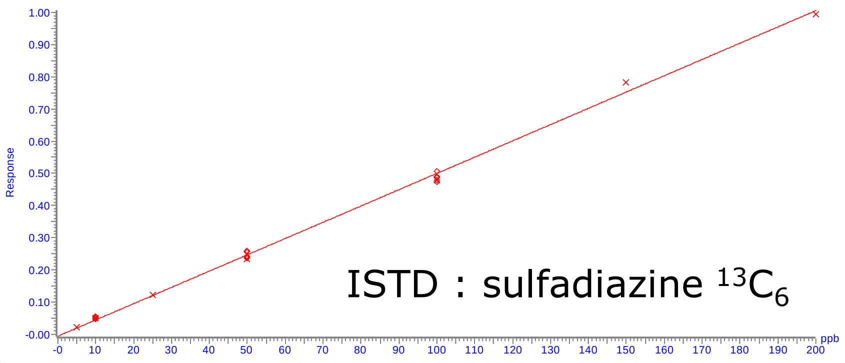


ANA65 – Tetracyclines/sulfonamides in muscle by UHPLC-MS/MS

Strategy :

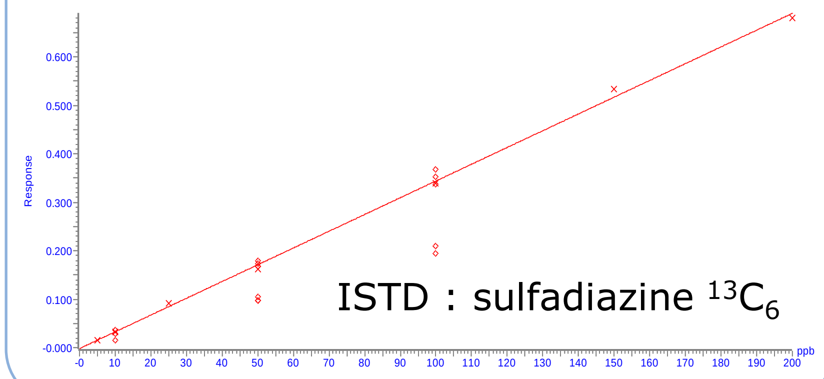
- Quantitative method, already fully validated for fish muscle
- Same MRL for all species for tetracyclines and sulfonamides
- Secondary validation tested for bovine and shrimp together
    - Calibration cuve in shrimp muscle
    - Blank and QC samples in bovine (n=3) or shrimp (n=3) muscle

Sulfadiazine



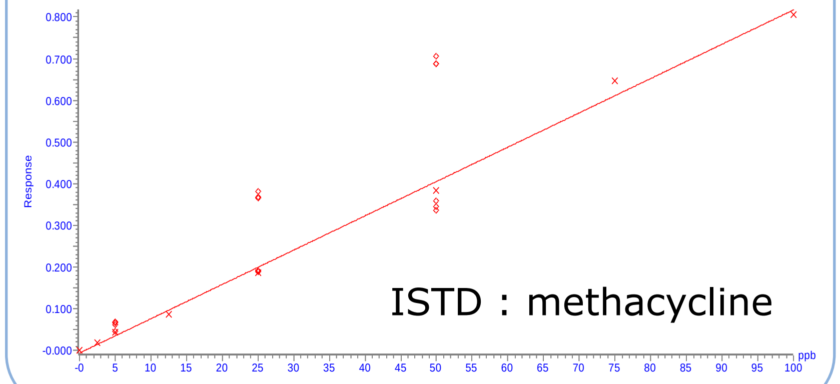
Sulfadoxine

Trueness
Precision



Tetracycline

Trueness
Precision



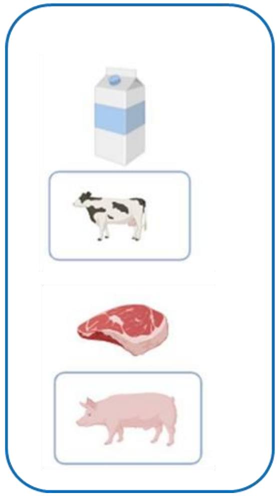
→ 2 separated validation needed for bovine and shrimp muscle

Alternative strategy: extension by on-going validation

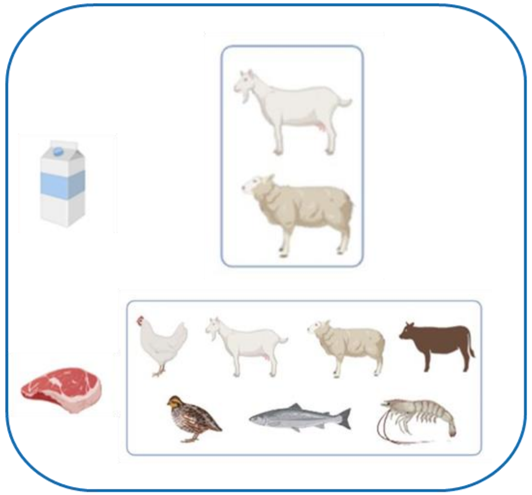
- Blank and QC samples collected from several analytical run
- Same requirement regarding QC number as for a secondary validation

Example : method ANA53 – screening of antibiotics by FCIA

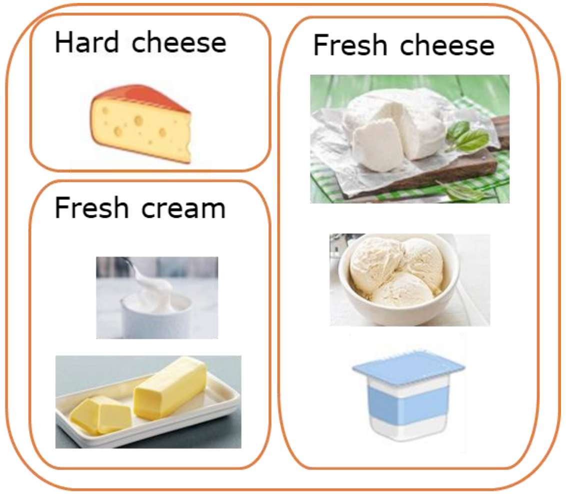
Full validation



Secondary validation



On going validation



Category	# QC (1 year)
Fresh cheese	29
Hard cheese	19
Fresh cream	4

Too low #

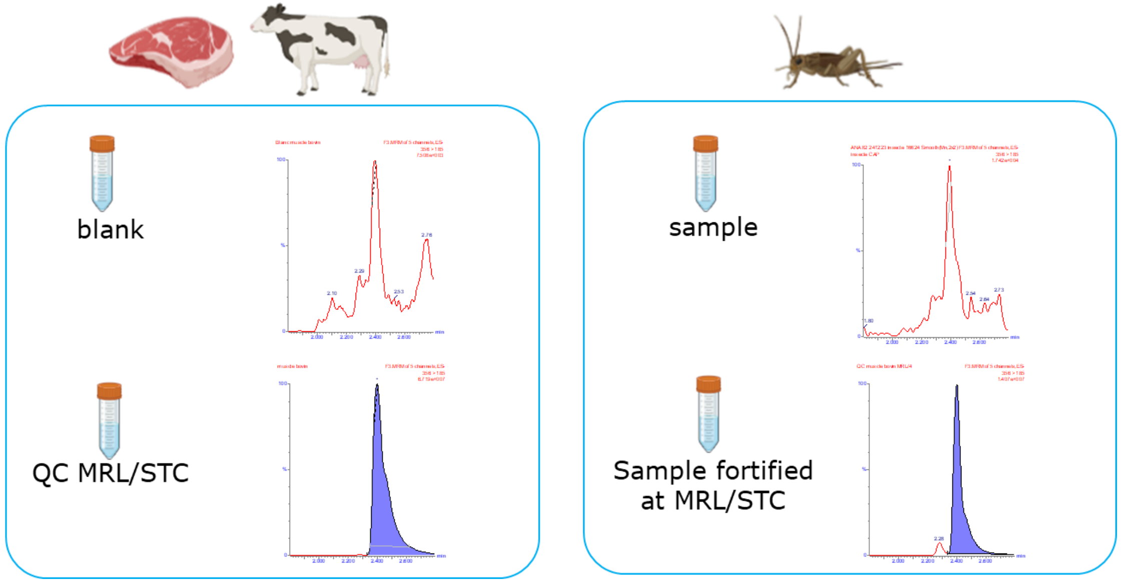
P74 Innovative ongoing validation of 10 antibiotic families in processed foods by flow cytometry immunoassay: the example of dairy products [F. Pineux, A-C Huet, A. Lamote and N.Gillard]

Alternative strategy : standard addition (BELAC 2-105)

- Strategy applied to avoid multiple validation for scarce matrices
- method validated for at least one main matrix
- ‘non regular matrix » (exotic, unidentified, scarce or blank not easliy available)
- Maximum 5 samples/year

Case 1

- Insect sample received for the analysis of phenicolated antibiotics
- Method ANA82 validated for « Matrices of animal origin »
- Analysis realized with standard addition, together with suitable controls



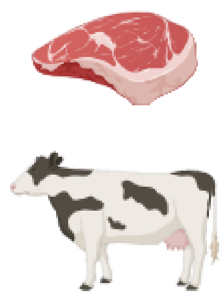
Chromatograms of florfenicol

Alternative strategy : standard addition (BELAC 2-105)

Case 2

- Muscle samples received for the analysis of coccidiostats
- Targeted compounds of method ANA86 validated for bovine muscle
- Standard addition performed for other species

Validated matrix/species



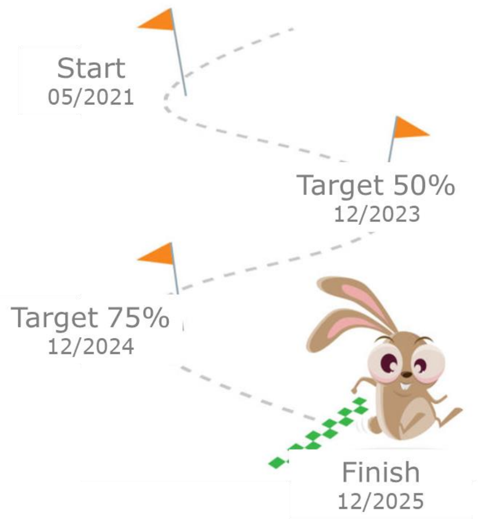
Standard addition

Méthode-ana	Resp Méthode	Description	Date demande analyse	Equivalence tissus accrédité (max 5 par an/sur 3 run par méthode/matrice/espéce)	ID CER
86a	CR	Poisson	10-11-23	Muscle-bovin	23/1xxx
86a	CR	Poisson	18-12-23	Muscle-bovin	23/14621
86a	CR	Poisson	18-12-23	Muscle-bovin	23/14622
86a	CR	muscle caprin	20-09-24	muscle bovin	24/12xxx
86a	CR	muscle caprin	20-09-24	muscle bovin	24/13117
86a	CR	muscle caprin	29-10-24	muscle bovin	24/14017
86a	CR	muscle cerf	26-11-24	muscle bovin	24/15520
86a	CR	muscle caprin	31-12-24	muscle bovin	24a/17003
86a	CR	muscle caprin	07-02-25	muscle bovin	25/0707

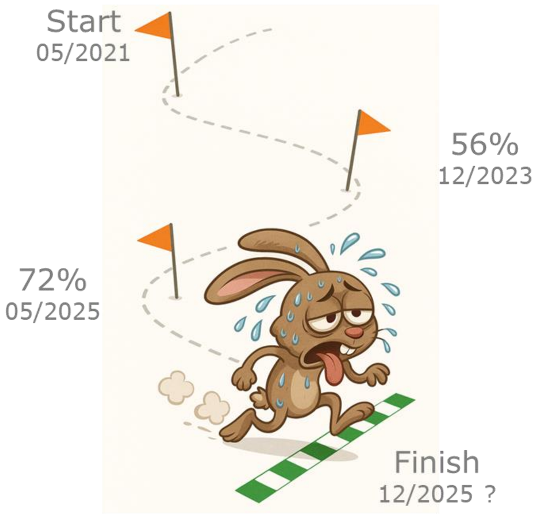
Validation for next goat sample (< 20/09/2025)

In conclusion….

- ✓ Revalidation of methods represent a high workload for laboratories
- ✓ Alternative strategies are helpful to reduce this workload
- ✓ Collaboration at EU and national levels is critical
  - ✓ Exchanges of experience
  - ✓ Stability data
  - ✓ Exchange of samples when no PT available…
- ✓ Still a lot to do for CER….



From target…



….to achievements

Thank you for you attention!