

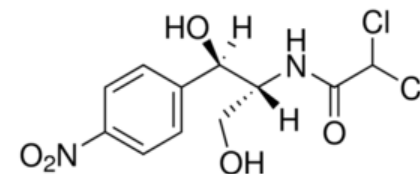
EVALUATION OF 5 ELISA KITS FROM DIFFERENT SUPPLIERS FOR THE DETECTION OF CHLORAMPHENICOL (CAP) RESIDUES IN MEAT AND AQUACULTURE PRODUCTS

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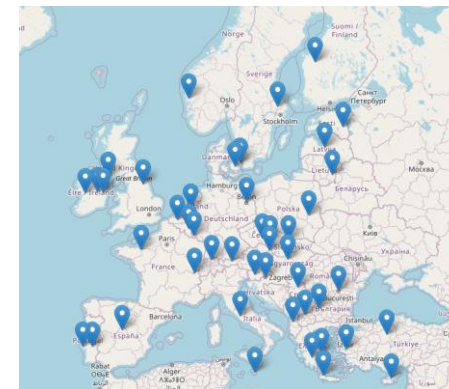
1. Context and objectives

Chloramphenicol (CAP)



- Efficient, broad spectrum against Gram – and Gram + bacterias
- Cheap
- Widely used
- Banned since 1994 in Europe due to its toxicity (eg. bone marrow suppression and aplastic anemia)
- Subject to illegal use : need to control

Member States need available detection methods for the control of this molecule



EU Regulation

EC 1430/94: CAP becomes an unauthorised substance

EC 2005/34:
Minimum Required Performance Limit (MRPL) (CAP) = 0.3 µg/kg

CR (EU) 2021/808: Fixes Performance Criteria for analytical methods for pharmacological active substances

EC 2002/657:
Performances of analytical methods and interpretation of results



CR (EU) 2019/1871:
Reference Point for Action (RPA) = 0.15 µg/kg

2023: EURL Guidance Document on Screening Method Validation

Screening methods: ELISA

Many methods are used for the screening of CAP

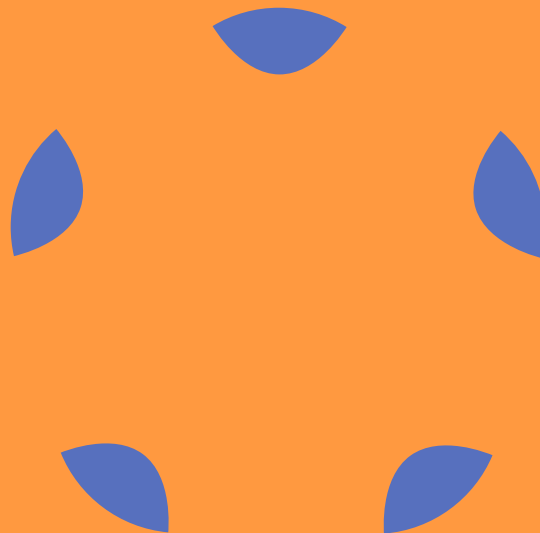
- Selection of the most relevant ELISA kits: best performances, reputation of the supplier (= spread of use)
- Evaluation and validation of the performances



- Cheap
- Fast
- Sensitive
- Highly specific

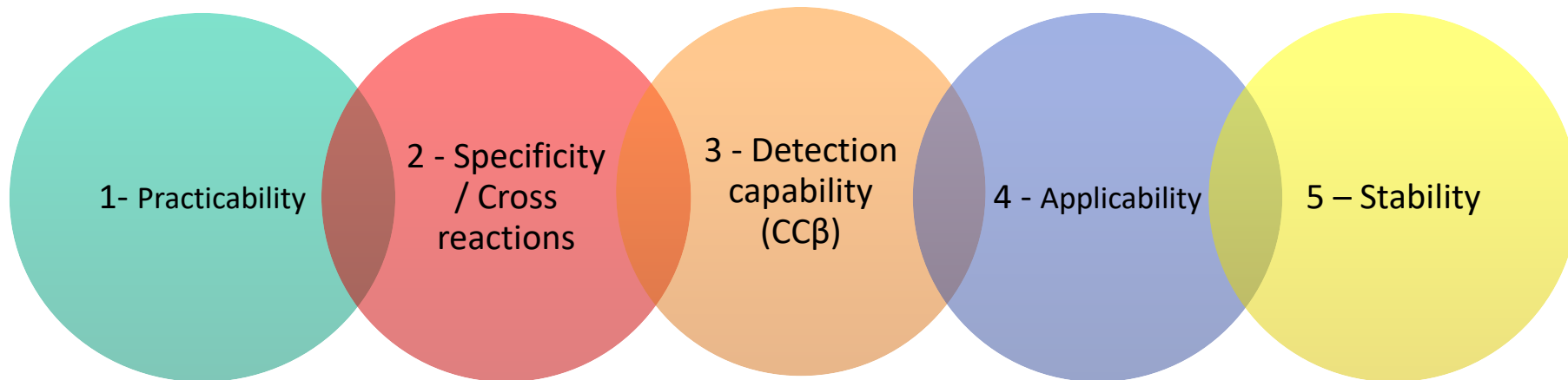
- Can detect only one molecule at a time
- Molecule can't be identified
- Less sensitive than instrumental methods

2. Materials and methods



Performance parameters

5 parameters to evaluate in line with EU/2021/808 and EURL Guidance Document (2023)



Determination of the Screening Target Concentration (STC) for the CC β

1. Evaluation step: STC determines the spiking concentration for the validation

$$STC \leq RPA$$

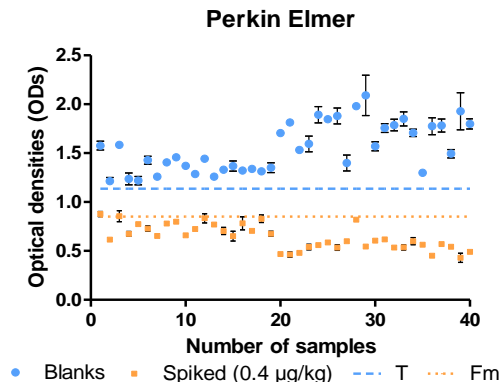
2. Determination of CC β : EURL Guidance document on screening methods (2023) – Method 2

Optical densities (OD) inversely proportional to the concentration

$$T = B - 1.64 * SD_B$$

$$Fm = M + 1.64 * SD$$

If $T > Fm$; STC valid
If $T < Fm$; increase STC



STC \leq ½ RPA	½ RPA < STC \leq 90% RPA	90% RPA < STC \leq RPA	STC > RPA
20 blanks + 20 spiked (2 days)	40 blanks + 40 spiked (4 days)	60 blanks + 60 spiked (6 days)	20 blanks + 20 spiked (2 days)

Matrices : aquaculture products (fish, shrimps), muscles (ovine, bovine, poultry, pork)

Selection of the kits

Supplier	Kit reference	LOD ($\mu\text{g}/\text{kg}$)
R-Biopharm	Ridascreen [®] CAP (R1511)	0.005-0.008
Randox	CAP Fast ELISA (CN10171)	0.02
Perkin Elmer	MaxSignal [®] CAP ELISA Kit (FOOD-1013-02F)	0.025
Biorex Food Diagnostics	CAP Fast ELISA (BXEFB03A)	0.05 - 0.1
EuroProxima	CAP Fast ELISA (5091CAPF)	0.02

3. Results and discussion



Practicability

- Similar procedures
- Assay procedure 45-50 min
- More reagent preparations for Europroxima and Radox : dilution, reconstitution → source of error
- Mix isooctane-chloroform (2:3) vs *n*-hexane

Specificity / Cross reactions

- All kits: 0% false positive rate
- Cross reactions not investigated; negligible
- 4 stereoisomers of CAP
- RR-CAP bioactive, antimicrobial activity, detected by kits
- SS-CAP and RR-CAP found in food matrices
- Only **R-Biopharm (kit Ridascreen®)** stated the cross reactivity with SS-*p*-CAP

Stability

- Bibliographical study

Detection capability and applicability

Perkin Elmer and Europroxima

Perkin Elmer: $CC\beta = 0.075 \mu\text{g}/\text{kg}$

20 samples (20 blanks, 20 spiked)

0 false negative ; 0 false positive

inter-matrices ; inter-batches

$T > F_m \rightarrow$ **Valid**

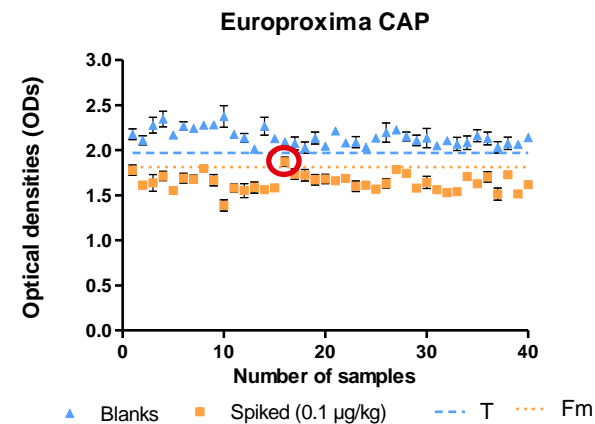
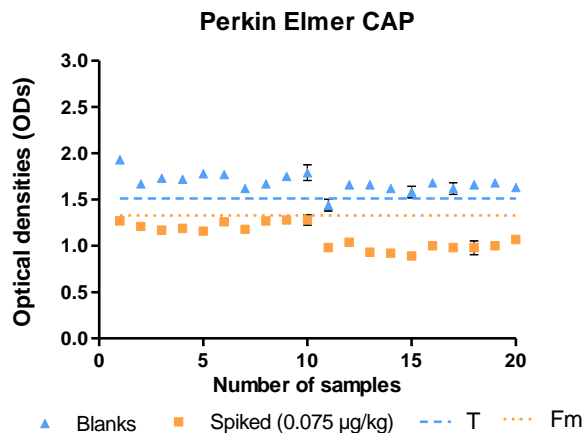
Europroxima: $CC\beta = 0.1 \mu\text{g}/\text{kg}$

40 samples (40 blanks, 40 spiked)

one false negative = **5%** ; 0 false positive

inter-matrices ; inter-batches

$T > F_m \rightarrow$ **Valid**



Detection capability and applicability

R-Biopharm

Global validation : $CC\beta = 0.075$

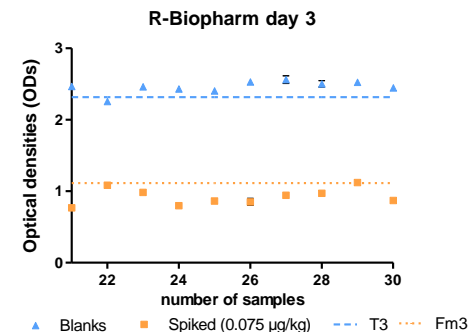
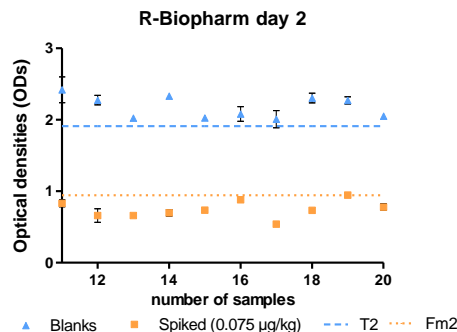
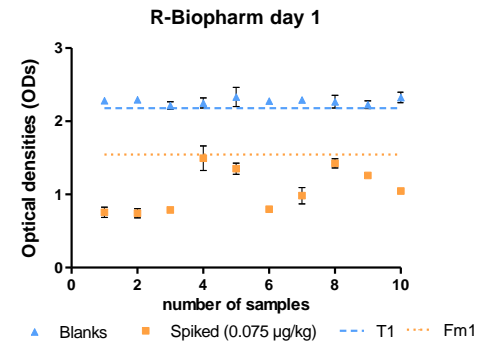
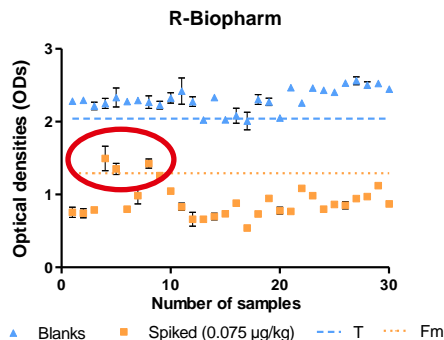
$\mu\text{g}/\text{kg}$

$T > Fm$

3 false negative (10%) \rightarrow **Non-compliant**

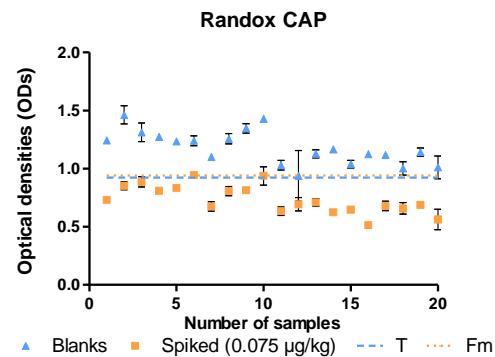
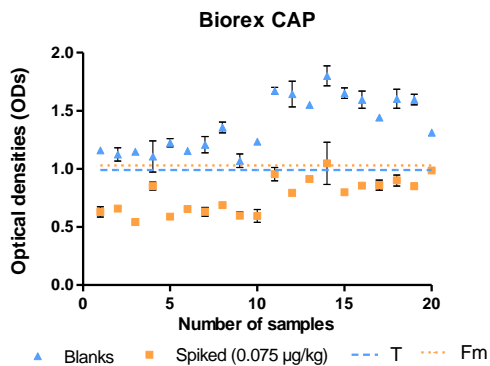
Intra-day: $T > Fm$

QC required for each day of analysis ; kits **valid**



Detection capability and applicability

Biorex and Radox: general validation



CC β = 0.075 µg/kg ; Both T < Fm → increase STC?

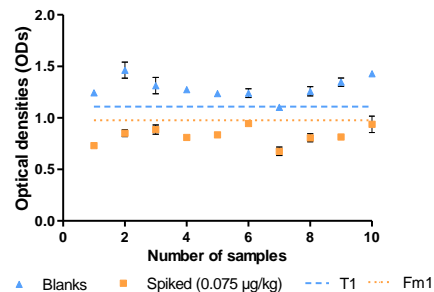
Detection capability and applicability

Biorex and Radox: Intra-day validation

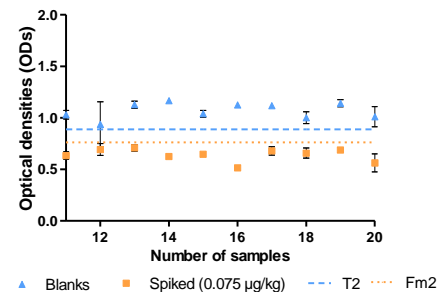
Intra-day: $CC\beta = 0.075 \mu\text{g}/\text{kg}$; T>Fm each day

QC required for each day of analysis ; kits **valid**

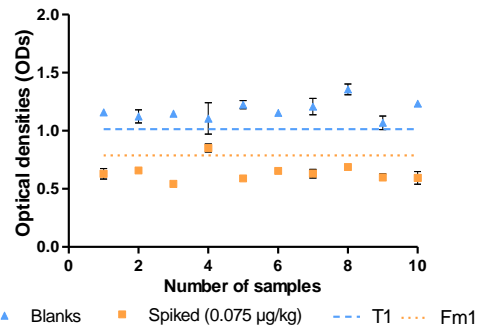
Radox day 1



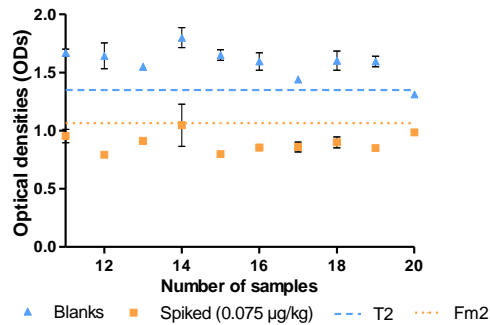
Radox day 2



Biorex day 1



Biorex day 2



Conclusion



Conclusion

Differences LOD/CC β

$$LOD = \bar{x}_{blanks} + 3 * SD_{blanks}$$

- Kits applicable to the screening of CAP
- All CC β < RPA
- Satisfactory specificity

5 ELISA kits are **valid**

Manufacturer	LOD ($\mu\text{g}/\text{kg}$) (CAP)	Measured CC β ($\mu\text{g}/\text{kg}$) (CAP)
Biorex	0.05-0.1	0.075
Europroxima	0.02	0.1
Perkin Elmer	0.025	0.075
R-Biopharm	0.005-0.008	0.075
Randox	0.02	0.075

First independent study for the validation of 5 ELISA kits for the detection of CAP in line with EU Reg 2021/808

Official and autocontrol laboratories: help in the selection of the kits and initial validation




Thank you for your attention

FOOD ADDITIVES & CONTAMINANTS: PART A
<https://doi.org/10.1002/9781118469932.ch143226>



Check for updates

Comparative assessment of commercial ELISA kits for the screening of chloramphenicol residues in meat and aquaculture products according to European Regulation (EU) 2021/808 and to the new Reference Point for Action (Commission Regulation (EU) 2019/1871)

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ABSTRACT

This study covered the evaluation of performance characteristics and validation of five commercial ELISA kits for the detection of a banned antimicrobial, chloramphenicol (CAP), in muscle and aquaculture products. CAP has been banned in the European Union since 1994, but is still authorized in some countries in the world. In 2019, the European Union set a new Reference Point for Action (RPA), decreasing the acceptable limit of CAP in animal tissues for human consumption from 0.20 µg/kg to 0.15 µg/kg. Validations were performed according to the European Regulation EC/2021/808 and to the European Guideline on Screening Method Validation (2023). The detection capabilities (CCP) were all determined under the RPA, but were 3 to 15 times higher than the announced limit of detection (LOD). False negative rates were satisfactory for all the kits (< 5%) and false positive rates were acceptable. All of them were found out to be applicable to aquaculture products and meat at a common CCP.

ARTICLE HISTORY

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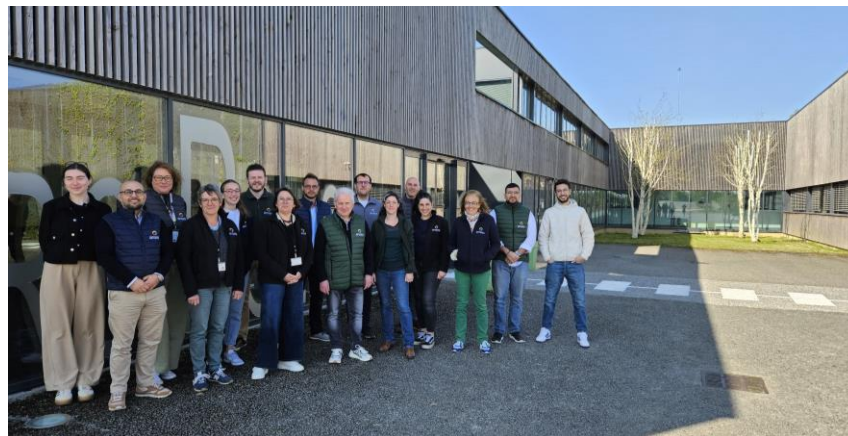
CAP; screening; ELISA; meat; aquaculture

Introduction



Chloramphenicol is a broad-spectrum antibiotic with bacteriostatic action originally used in human and veterinary medicine (EFSA Journal 2014). Many reports indicate the toxicological effects of CAP for humans such as aplastic anaemia and bone marrow suppression (EFSA Journal 2014) resulting in the ban of this substance in European Union from 1994 (CR 1438/94, Commission Regulation (EC) 1994), followed by other countries. However, CAP is subject to illegal use in aquaculture products (Oliveri Conti et al. 2015; Dogan et al. 2020; Rimkus et al. 2020), inducing a need to control this drug. After animal treatment, CAP residues could persist in foodstuffs of animal origin.

In 2002, a European Commission Decision stated the use of minimum required performance limits (MRPLs) for unauthorized substances, including

CAP as the 'minimum content of an analyte in a sample which at least has to be detected and confirmed. It is intended to harmonise the analytical performance of methods for substances for which no permitted limit has been established' (2002/657/EC, Commission Decision 2002). Later, the Commission Regulation (EU) 2019/1871 set a lower Reference Point for Action (RPA) for CAP residues at 0.15 µg/kg instead of 0.2 µg/kg, as defined before as MRPL. The RPA takes into account both the analytical considerations and the toxic potential of banned substances. Analytical methods should be able to detect the lowest possible concentrations of banned substances (CR 2019/1871, Commission Regulation (EU) 2019). According to the evaluation of 2020's national control plans (NRMIP), 21 out of 30 members of the European Economic Area use ELISA kits for the screening of CAP. Analyzed matrices were mainly muscles, milk, eggs and



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