Introduction

Chloramphenicol is a broad spectrum antibiotic used in cattle farming to treat multiple infections, being effective against many bacteria, gram negative and positive as well as large number of anaerobic organisms. However, the abuse of this antibiotic can cause many problems for human health, remain banned according to Council Directive 96/22/EC and Council Regulation (EEC) No 2377/90 establishing a minimum required performance limit of 0.3 ppb. In this work, an indirect competitive ELISA for the detection of Chloramphenicol (CAP) in milk samples has been described, reaching a detection limit in milk of 0.009 ± 0.004 µg·Kg⁻¹ (90% response of the zero dose). The ELISA was validated according to the Commission Decision 2002/657/EC criteria established for qualitative screening methods.

Hapten Design and Immunoreagents

A group of novel haptens were synthesized maximizing different specific epitopes from CAP, and conjugated to different proteins. Different antisera were raised against different immunizing haptens. Using the prepared competitors, several combinations with good detectability were obtained.

ELISA Assay in Milk

Chloramphenicol standard curves prepared directly in milk, were tested. It can be observed that it’s possible to work directly with milk keeping good performances.

Selectivity Studies

The cross-reactivity was evaluated against different families of antibiotics being negligible for all the tested compounds (≤0.01%). In the case of thiamphenicol and florfenicol, the assay shows more selectivity, but is not important due to the sensitivity of the assay toward chloramphenicol.

Validation Assays

Validation assays were carried out in milk. CCa and CCβ parameters were calculated. β-error parameter was lower or equal than to 5% (percentage of false positives). Assays were performed on different three days using 20 blank milk samples spiked at the CCβ level for chloramphenicol.

Conclusions

• Selective ELISA assay for detection of CAP in milk samples was developed.
• Analytical performance shows good parameters of ICSi and detectability due the MRPL stablished by the EFSA.
• No significant reactivity with other family antibiotics was observed.
• Immunoassay developed was validated successfully according to Council Directive 96/22/EC and Council Regulation (EEC) No 2377/90, without giving non-false complans.

Acknowledgements

Ministerio de Innovación y Ciencia (MICINN): DETECTA (AGL2008-05578-C05-01). This work has been supported by CIBER-BBN. Nb4D is a consolidated research group of the Generalitat de Catalunya (expedient 2009SGR 1343). AH likes to thanks to ‘SAI’ (I4E-CSIC) for the I4E-preDoc grant (BOE 20/06/2011). Nb4D group, formerly NB4D, is a consolidated group of the Generalitat de Catalunya (expedient 2009 SGR 1343).

Literature