



## Work Package 8: Therapeutics

### Introduction

**B**ioCop addresses the issue of veterinary drug residues in food by developing novel screening tools for two chemical classes of concern: fluoroquinolone antibiotics and hormone growth promoters.

The overuse and/or illegal use of fluoroquinolone antibiotics in animal production is of particular concern to humans. Repeated exposure to these antibiotics *via* the food chain will limit the future effectiveness of these drugs, this is due to the development of antimicrobial resistance. BioCop have developed improved rapid diagnostic methods to screen for the presence of low concentrations of the entire family of fluoroquinolone antibiotics in animal products, such as, chicken muscle, eggs and fish.

Hormone growth promoters are banned for use in cattle fattening in the EU. Hormone abuse is a concern in the EU from a law enforcement, food safety, and an animal welfare perspective. Residues of hormones in meat are a potential health threat, especially for vulnerable populations such as preadolescents. Drawbacks of the current analytical methods is their restriction to a limited number of known substances and the relative high cost of instrumental methods. As a result of this, unexpected compounds, such as new designer hormones will be overlooked and the number of investigated samples is limited by the analysis costs. BioCop has addressed this problem by developing a cost-efficient protein biomarker-based biosensor assay for blood samples. Therefore allowing large sample series to be screened, not only for single compounds but for the biological effects of the use of any growth promoting hormones.

### Work Package Progress and Result

Residues of fluoroquinolone antibiotics can be analysed with the newly developed surface plasmon resonance (SPR) biosensor method in chicken, fish and eggs.

A prototype diagnostic food test kit has been produced, the kit allows for the reliable detection of very low levels of these compounds, including the most frequently used substances such as flumequine, oxolinic acid, cipro/enrofloxacin and norfloxacin.

The applied biosensor technology has the advantage of automated high-throughput and cost-efficient analysis. The performance of the method was verified in accordance with current EC legislation for the validation of analytical methods. In addition to this, it was shown in direct comparison with established methods such as liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and microbiological inhibition tests that the new assay meets and in many cases exceeds the performance of the existing methods. The practical applicability, reproducibility, ruggedness and easy transferability of the biosensor method was impressively demonstrated by an international interlaboratory method performance study (Nine laboratories from Europe and North America participated in this study). For a set of 42 naturally contaminated as well as residue-free chicken, trout and egg samples, all of the laboratories obtained almost identical findings with very low rates of false positive and false negatives (0% - 1%) results.

With view to a biomarker assay for the detection of the effects of illegal hormone growth promoter application, a set of candidate protein biomarkers was identified in Work Package 2. For the most suitable biomarkers, separate SPR biosensor methods were developed and optimised. These were then combined into a multi-analyte high-throughput assay for eight significant biomarkers in cattle blood. The developed testing procedure is currently awaiting its validation, both *via* a single laboratory validation and a small-scale interlaboratory method performance study.





The protein biomarker biosensor approach was complemented by targeted and non-targeted mass spectrometric profiling in associated tissue, hair and urine samples. As a result of the following activities a number of promising markers of hormone abuse have been identified so far.

### Benefits of the BioCop Project

#### Consumer

Novel technologies for the cost-efficient and high-throughput detection of residues of veterinary drugs will increase the frequency and volume of testing. As a result of this, food safety will increase, bringing benefits for the European consumer. In addition, lower expenses for food testing in the food industry may be reflected in food prices.

#### Food Industry

The Food industry will directly benefit from the developed screening technologies for residues of antibiotics. Profit margins in this sector are considerably lower in comparison to other areas, e.g. pharmaceutical industry.

The reduction of expenses for the quality control (in this case residue testing) of raw materials, e.g. eggs will have a direct impact on the production costs. The provision of easy to use high throughput screening technology to the European food industry will therefore contribute to increasing their competitiveness.

#### Scientist

From a scientific point of view, WP8 has generated a number of interesting results. One of the most sensitive screening assays for fluoroquinolone antibiotics has been developed. And an innovative approach was invented within the project to improve the cross-reactivity profile *via* a so-called dual antibody.

This antibody was generated by using an immunogen carrying two different target structures. The target steroid profiling of samples from treated animals revealed new insights in the transformation of the administered substances.

The non-targeted profiling of urine samples by sophisticated high-resolution mass spectrometry coupled to innovative data evaluation software proved to be a powerful tool for fingerprinting of suspect samples as well as for the identification of potential biomarkers.

### Training/Workshops

A major objective of the project is to disseminate the developed methodology and results to potential stakeholders and end-users. A number of technology transfers, training and demonstration activities were organised for both the BioCop consortia and external stakeholders, including that of a hands-on training workshop for the use of the developed SPR biosensor methods. A training workshop for the new fluoroquinolone residue method was held at the annual meeting of the European Community Reference Laboratory for Residues of Antibiotics. The event was attended by 25 National Reference Laboratories of member states and candidate countries. Further to this, a partially interactive demonstration of the newly developed methods was given at the 2nd North American Workshop on Biosensor Applications for Food Safety and Analysis in Canada, and at the BioCop Open Day held in York, UK on the 3rd September 2008.

### Future Activity

The SPR biosensor kit for the analysis of fluoroquinolone antibiotics in poultry, egg and fish is fully developed and validated. It now further awaits its commercialisation by the industrial partners involved in the project.

The protein biomarker biosensor assay for the detection of illegal growth promoter application will be fully validated in the near future. Upon successful validation, the outcome will be disseminated *via* training workshops and demonstration events.

