SCREENING FOR VETERINARY DRUG RESIDUES USING BIOCHIP ARRAY TECHNOLOGY

Randox Food Diagnostics develop and manufacture a range of cost effective screening solutions for drug residue diagnostics offering excellent tools for screening of antimicrobials, growth promoting hormones and drugs of abuse in animals and foodstuff. Our extensive range consists of 33 ELISA test kits and 12 multiplex biochip screening platforms.

Introduction: the importance of drug residue testing in food products

Veterinary drugs are an important tool for food manufacturers worldwide. When properly used, they increase the overall health of food producing animals, therefore increasing food production and quality.

However, when used at levels above the permitted or for longer periods than recommended, drug residues can accumulate in the animal tissue and enter the food chain, posing a risk for the consumers. The Codex Alimentarius defines drug residues as “parent compounds and/or their metabolites in any edible portion of the animal product” [1]. Common drugs that are administered to food producing animals are antimicrobial drugs, anti-parasitic compounds and growth promoters [2], [3].

Antimicrobial drugs are widely used for the treatment of infections and their prevention in food producing animals [4], [5]. When used responsibly, they are great tools to treat or prevent bacterial infection. Increasing the overall health of the herd reduces mortality, therefore increasing production.

However, if administered in incorrect amounts, or without respecting a withdrawal period, they can enter the food chain. Also, they can be used as growth promoting agents [4]. Some examples of antimicrobials used in animals are Sulphonamides, Quinolones, Tetracyclines, Beta-lactams, Macrolides and Aminoglycosides [5]. The use of these drugs is either prohibited or regulated in accordance to their relative risk. In recent years, with the rise of aquaculture as one of the fastest-growing industries in developing countries, the use of prohibited antimicrobial drugs such as Chloramphenicol has raised public health concerns [6].
Antimicrobial compounds, when present in edible produce, can have negative health effects even when they are present at very low concentrations. Allergic reactions and carcinogenic effects are examples of concerning secondary effects of antimicrobials, but the main issue with the abuse of antimicrobial treatment in animals is the development of antimicrobial resistant bacteria.

Growth promoting hormones have also been a major veterinary group of medicines used in food producing animals, especially cattle [4]. The use of these types of drugs will increase meat quality (protein to fat ratio), weight gain, feed to gain ratio and meat production overall. Adverse health effects related to growth promoters intake can be severe migraines, increased heart rate and carcinogenic effects. Due to these issues, the use of growth promoters in animal production is either strictly controlled or banned in most countries [5]. Examples of drugs within this group are Beta-agonists, Corticosteroids and Anabolic Steroids.

In the European Union, the legislative framework for the control of drug residues is laid out in the European Directive 96/23/EC. The rules for monitoring and controlling drug residues and other substances in products for human consumption are developed in that directive, with the aid of the Regulation 2377/90 which details the MRLs (Maximum Residue Limits) for approved substances, and also the Decision 2002/657/EC which gives technical guidance on the developing of analysis and methods to monitor drug residues in food.

**BAT principle and capabilities**

Biochip Array Technology (BAT) is a novel method based on ELISA principles and can be used as a powerful tool for the rapid screening of veterinary drug residues in food products. The ELISA (Enzyme Linked ImmunoSorbent Assay) is one of the most well-known and widely-used immunoassays in the market. ELISA tests can usually be classified under two broad terms: “sandwich” and “competitive” ELISA, the latter being the one of interest in this article.

Immunoassays are based in the highly specific interaction between antibodies and antigens. In competitive ELISA, both the antigen and an enzyme conjugated molecule will compete for the same active sites in the antibody. The enzyme in the conjugate will catalyse a colour reaction that can be measured. There is an inverse relationship between the colour development and the concentration of antigen in the sample.
The high sensitivity, specificity and accuracy, with the added advantage of being inexpensive and the possibility of analysing a large number of samples within a short period of time has made ELISA tests one of the most popular choices for screening drug residues in food.

Conventional ELISAs have the capability to detect and quantify a single antigen or a group of antigens of similar chemical structure. Biochip Array Technology brings this capability to the next level, by allowing the simultaneous and parallel detection and quantification of multiple antigens, or groups of antigens, from a single sample. This upgrade in the technology has been accomplished by grouping various antibodies together in a single reaction well. Several panels have been developed; each one of them allows testing for a group of interrelated analytes. Analysing multiple compounds simultaneously reduces assay time and labour costs greatly, resulting in improved laboratory performance. Another advantage of parallel testing is assay consolidation, since many tests are done by the same laboratory operator, from the same sample using a single sample preparation. In laboratories with a high sample throughput, Biochip Array Technology can be the tool of choice for internal monitoring of drug residues.

The biochip itself is a ceramic square, 9x9 mm which is divided in 5x5 discrete test regions (DTRs), and different antibodies can be covalently bonded into each of these DTRs, having a capability of testing for 25 drug residues simultaneously. Each DTR acts as a single analyte ELISA cuvette. Due to the design of the biochip, 3 of the DTRs need to be used as quality control spots, leaving a capability of testing for 22 drug residues simultaneously. Biochips are arranged in a 3x3 format (see figure 1) called biochip carrier, for their routine use.

The biochip immunoassay format is based on the competitive ELISA explained before (see figure 2). First, an assay diluent will be added to the surface of the biochips. This reagent has two functions: provides a liquid medium for all reactions to take step a set of multi-analyte standards, samples and
place and activate the bound antibodies. After this step a set of multi-analyte standards, samples and controls are added to the biochips. The multi-analyte standards contain all of the analytes of interest for the panel.

The biochips are incubated for 30 minutes under agitation at a controlled temperature, protected from the light. During this first incubation period, the antigens present in the standards, samples and controls will specifically bind with the antibodies on the surface of the biochips.

Following this, an enzyme labelled conjugate, containing conjugated versions of the antigens, will be added to each of the reaction wells. These conjugated antigens will compete for the binding sites in the antibodies as in a normal competitive ELISA. Higher binding will occur if there is a lower initial concentration of antigen in the sample, and vice versa. A further 60 minutes incubation period is then required.

Afterwards, the biochips will be washed with a mild detergent solution that will remove any unwanted proteins and other interferents; and the unbound conjugate. A chemiluminescent reagent, called signal reagent, is added to the biochips and incubated for 2 minutes. The signal reagent has the capability of generating a light reaction that is catalysed by the HRP present in the conjugate. Where there is a low initial concentration of antigen, more conjugate will be present, and a more intense light signal will be generated. After the incubation the biochips are imaged using the Evidence Investigator analyser (see figure 3).

The Evidence Investigator is equipped with a charged couple device (CCD) camera, thermoelectrically cooled down to -50 °C to reduce background noise, and can simultaneously record the light emission for all of the DTRs of the 9 biochips present in a carrier.
The images from the Evidence Investigator are processed and analysed by dedicated imaging software, which will differentiate and calculate the light emission from each of the DTRs in each of the biochips. The programme will generate calibration curves for all of the analytes in the panel, and also will calculate the sample results. The user can then print all the calibration curves and the results for all of the samples in an easy-to-read report format, or export the data for further treatment using a spreadsheet programme. Despite the complexity behind the technology, the Evidence Investigator and Biochip Array Technology requires minimal training, and can be easily implemented in many different food laboratories.

The applicability of the Biochip Array Technology as a screening method has been studied by John O’ Mahony et al [7] and Valerie Gaudin et al [8], and comparison studies with confirmatory methods like HPLC has been carried out by A. Carneiro et al [9].

The portfolio of analysis for food using Biochip Array Technology includes, but is not limited to, Tetracyclines, Sulphonamides, Beta-lactams, Quinolones, Aminoglycosides, Macrolides, Nitrofurans, Chloramphenicol, Anthelmintics, Coccidiostats, Beta-agonists, Corticosteroids, Oestrogenic Hormones and Synthetic Steroids. These analytes can be analysed over a range of matrices including honey, animal tissue (muscle, liver and kidneys, fish and shrimp), egg, milk, feed and urine.

Covering such a range of analytes in most of food types makes Biochip Array Technology and the Evidence Investigator a great tool for many different types of laboratories from small food producers to large supermarket chains or governmental laboratories.

**Summary**

As a measure to grant the safety of consumers of animal produce, intensive monitoring of food “from stable to table” is the approach followed by the European Union. Food producers, manufacturers, distribution chains and governmental bodies are in charge of assuring that the final consumer animal produce is safe. Due to the size of the food producing market, the expected amount of tests needed to ensure food safety is likely to be high, and with recent food scandals, alerts and product recalls, the public demands a more proactive approach to the food safety issue.
Confirmatory methods are highly effective, but also long, tedious, expensive and they require highly trained personnel. Initial investment in such methods can only be undergone by heavily granted laboratories. Food producers with limited laboratory installations, usually have to subsidize these tests to bigger laboratories, at a high cost per sample. Screening methods are a cheaper alternative to this. Internal monitoring of samples using an inexpensive, fast and easy-to-use method allow for the laboratories to implement a faster response. Compliant samples can continue with processing without the necessity of waiting for the external laboratory results.

Biochip Array Technology is an upgrade of the most popular screening method in the market: ELISA. Having the capability of testing a high number of samples for multiple drug residues simultaneously in a short period of time improves laboratory efficiency. This technology is a good option for those laboratories with big sample throughput that have an interest in saving in confirmatory tests.
References


