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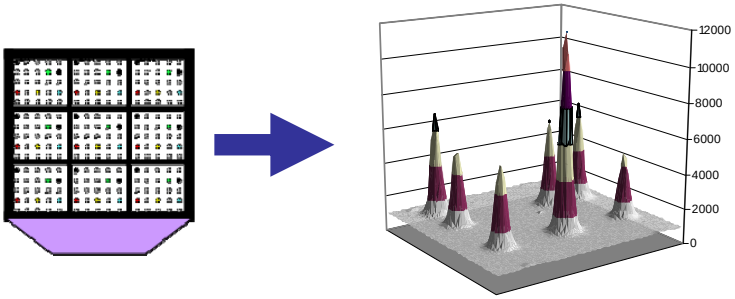
Advanced systems for the rapid detection of anti-parasitic drugs in food



Key external stakeholders:

Dairy, beef and sheep farmers; primary meat and milk processors; regulatory agencies: DAFF, FSAI, IMB.

Practical implications for stakeholders:

Excellent progress has been made in the development of screening assays for drug residues in food. Immunochemical screening assays were developed in this project as a rapid low cost means of detecting benzimidazole residues in food, as an alternative to chemical assays with a number of assays successfully validated. A biochip array assay was successfully developed to detect four different drug classes and shows good potential for application in specialist laboratories or at an industry level.

The milk industry is the only industry likely to apply this technology because they are the only industry that carries out monitoring at a factory level. However, the scope of assays needs to be extended to key flukicide residues (nitroxynil, closantel, rafoxanide, clorsulon and triclabendazole) to meet industry demands if they are to be used.

With benzimidazole drugs widely used in the treatment of worm and fluke infections in food producing animals, these novel immunochemical assays are proposed as an alternative low cost means of detecting benzimidazole residues in food. These assays are applicable in specialised laboratories or at a factory level to prevent contaminated produce entering the food chain.

Main results:

- Three working immunobiosensor assays were developed and validated to detect 17 benzimidazole residues in milk and meat.
- A novel multiplex immunoassay was developed for detecting benzimidazole and macrocyclic lactone residues in fruit juice.
- The new technologies developed were validated to meet EC 2002/657 criteria.
- These represent a rapid, low-cost, effective means of screening drug residues, and a viable alternative to chemical assays, applicable in specialised laboratories or at factory level.

Opportunity / Benefit:

Teagasc can be at the forefront of engaging with food producers with low-cost screening techniques, through our extensive expertise in the field.

Collaborating Institutions:

Dublin City University

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Teagasc project team: Dr. Martin Danaher (PI)
Dr. Jemma Keegan
External collaborators: Prof. Richard O'Kennedy, DCU
Miss Elaine Darcy, DCU

1. Project background:

In this project, Teagasc and DCU embarked on a joint collaborative research project to develop rapid immunochemical screening assays for a range of drug residues in food. The projected combined the expertise of Teagasc in residue analysis and DCUs capabilities in antibody production. Immunochemical assays in ELISA and immunobiosensor assay formats are proposed rapid low cost diagnostic procedures for detecting benzimidazole and levamisole residues in foodstuffs of animal origin. Rapid systems includes ELISAs (for application at a plant level) and Biacore assays (for application at a laboratory level or for on-line process monitoring at large food production facilities).

The rapid methods developed on this project can potentially be implemented at a factory level allowing rapid detection of residues in food. This can potentially enable industry to self-monitoring and improving the safety of food through improved surveillance of chemical residues. The rapid methods developed on the project will provide faster analytical results enabling end-users to rapidly identify contaminated produce and take prompt follow-up action. The safety of food will be enhanced through improved residue surveillance and will boost consumer confidence in food.

2. Questions addressed by the project:

Can immunochemical assays be applied to residues in food?

3. The experimental studies:

A range of chemistries and antibodies were procured in the early stage of the project. These reagents were used in subsequent tasks to develop rapid assays for benzimidazoles, amino benzimidazoles, triclabendazole, thiabendazole and avermectin residues. Some of these chemistries were used as immunogens to produce antibodies to benzimidazole carbamate, levamisole, thiabendazole and triclabendazole by project partners DCU. In addition, researchers at DCU attempted to produce recombinant antibodies by screening of commercially available and immune libraries by panning and scFv antibody fragments isolated. mRNA from selected hybridomas were used to generate cDNA and this in turn used to generate specific and sensitive scFv's. DCU subsequently optimised the performance of antibodies through genetic engineering to give improved sensitivity, cross-reactivity and stability. Teagasc were also responsible for the development of extraction and clean-up procedures used on the project. Teagasc were responsible for the development and validation of biosensor assays.

4. Main results:

DCU produced polyclonal antibodies to benzimidazole carbamate and triclabendazole residues but were unable to produce recombinant antibodies. Insufficient quantities of polyclonal antibodies were produced to develop working assays. A recombinant antibody was developed by DCU to thiabendazole but in low quantities. Attempts to produce antibodies to levamisole were unsuccessful.

Teagasc successfully developed a range of assays for benzimidazole residues of the course of the project:

1. A multi-residue biosensor assay was developed to detect **11 benzimidazole carbamates residues** in milk during the early part of the project. The limit of detection of the assay was <5 μg kg^{-1}. The suitability of the method was verified through application to incurred milk samples from animals treated with albendazole, fenbendazole and mebendazole actives. The performance compared favourably against LC-MS/MS. Much of this work was achieved through collaboration developed with Teagasc, Moorepark; University of Naples and the European Community Reference Laboratory, in Berlin. This work has been published in Analytica Chimica Acta in 2009.
2. In subsequent work, a multi-residue assay was developed to detect **11 benzimidazole** and **four amino-benzimidazole residues** in ovine liver tissue. This work was particularly novel because it was the first benzimidazole screening assay that allowed detection of benzimidazole carbamate and amino benzimidazole residues. The assays developed for benzimidazole carbamates and amino-benzimidazole residues were developed using antibodies sourced from Agri-food and Biosciences Institute and RANDOX laboratories, respectively. This work has been published in Analytica

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Chimica Acta.

3. An assay was developed to detect **thiabendazole** residues in liver tissue. This assay was developed using a recombinant antibody developed by researchers at DCU.
4. An assay was developed to detect **triclabendazole** residues in liver tissue.
5. An biochip array assay was developed to simultaneously detect **thiabendazole, carbendazim, amino-benzimidazole and avermectin** residues orange juice samples.

One of the most significant developments from this work has been the development of multiplex biochip array assays for drug residues. This technology has the potential to compete with LC-MS/MS assays, which can currently detect a wider range of drug residues. This project has resulted in the development of assays to the 21 benzimidazole marker residues. Improved triclabendazole antibodies need to be developed to allow sensitive detection of residues in milk. Antibodies to levamisole were available during the course of this work but showed low tolerance for organic solvent and could not be include into multiplex assays. Some good quality antibodies are available to the avermectins but only a limited number are available to the milbemycins. A major obstacle faced in developing a successful working assay was the lack of suitable antibodies to flukicide drug residues, namely, nitroxynil, closantel, rafoxanide, oxyclozanide and clorsulon. These residues were not a target of the project but became have become an important group of residues for the food industry in 2010.

5. Opportunity/Benefit:

The technology developed on this project can be used a tool in specialist laboratories for the routine surveillance of residues in food.
The assays have also potential to be exploited in the form of commercial kits.

6. Dissemination:

Main publications:

1. Keegan, J., Whelan, M., Danaher, M., Crooks, S., Sayers, R., Anastasio, A., Elliott, C., Brandon, D., Furey, A., and R. O'Kennedy, Benzimidazole carbamate residues in milk: Detection by Surface Plasmon Resonance-biosensor, using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method for extraction.Analytica Chimica Acta, 654 (2009) 111.
2. Danaher, M., Radeck, W., Kolar, L., Keegan, J., Cerkvenik-Flajs, V. Recent developments in the analysis of avermectin and milbemycin residues in food safety and the environment. Current Pharmaceutical Biotechnology 2012 (in press).
3. Keegan, J., Crooks, S., Elliott, C., Brandon, D., and O'Kennedy, R., and Danaher. M. Detection of benzimidazole carbamates and their amino metabolites in ovine liver by Surface Plasmon Resonance-biosensor Analytica Chimica Acta 700 (2011) 41-48.

Popular publications:

1. Darcy, E. Leonard, P., Fitzgerald, J., Danaher, M., Brandon, D. and R. O'Kennedy. Recombinant Thiabendazole (TBZ) Antibody Production. 6th International Symposium on Hormone and Veterinary Drug Residue Analysis, University Forum, Ghent Belgium, 1-4th June 2010.
2. Keegan, J., Danaher, M. O'Kennedy, R. (2007). Development of a biosensor method for the detection of benzimidazole residues in food of animal origin. In: Proceedings of the Berlin Community Reference Laboratory Workshop, Berlin, Germany, 24 - 27th April 2007.
3. Keegan, J., Crooks, S., Elliott, C., Brandon, D., O'Kennedy, R., Danaher, M. (2010). SPR Biosensor Detection of Benzimidazoles in Ovine Liver. In: proceedings of sixth international symposium on hormone and veterinary drug residue analyses, Ghent, Belgium, June 1-4 2010.

7. Compiled by: Martin Danaher

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