Multiplex flow cytometric immunoassay for the simultaneous detection of 6 coccidiostats in feed and egg.

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Introduction

Coccidiosis is an infection of the intestinal tract which especially affects poultry and results in economic losses. To control this disease 11 different coccidiostats are allowed to be used as feed additives. These coccidiostats should be monitored for potential cross-contamination to non-targeted feeds. To protect the consumers, maximum residue levels (MRL's) in eggs and maximum levels (ML's) in feed have been set by the European Union (regulation 124/2009 and Commission Directive 2009/8/EC)

Technology

For the simultaneous detection of 6 coccidiostats, a flow cytometry-based immunoassay (FCI) is developed using the Luminex flow cytometer in combination with the MultiAnalyte Profiling (xMAP) technology. Five antigens (protein conjugates) were covalently coupled on the carboxylated polystyrene microspheres (beads) internally dyed with a red and orange fluorophore and were combined with five polyclonal antisera. The flow cytometer contains a red laser for identification of the bead set by its characteristic colour and a green laser for the quantification of the amount of anti-rabbit-PE corresponding with the amount of antibodies bound to the beads. This combination makes it possible to simultaneously measure the five different interactions in a single well.

Extraction method

A universal extraction method was developed for coccidiostats in egg and feed (Figure 1).

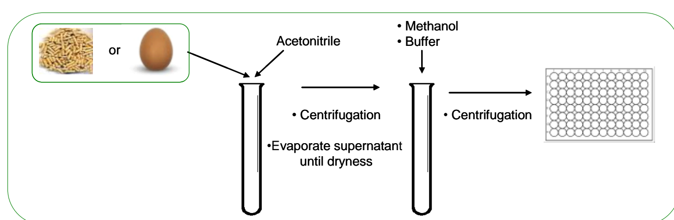

Figure 1: The extraction method for feed and egg.

Conclusions

- One multiplex assay made it possible to detect six coccidiostats in egg (at MRL) and feed (at ML) extract.
- This multiplex method has a high potential to be used in food and feed analysis.
- One extraction method for egg and feed could be applied.

Flow cytometry-based immunoassay (FCI)

This FCI is based on the competion for the antibody binding site between bound coccidiostat (conjugates on beads) and free coccidiostas in the sample (Figue 2).

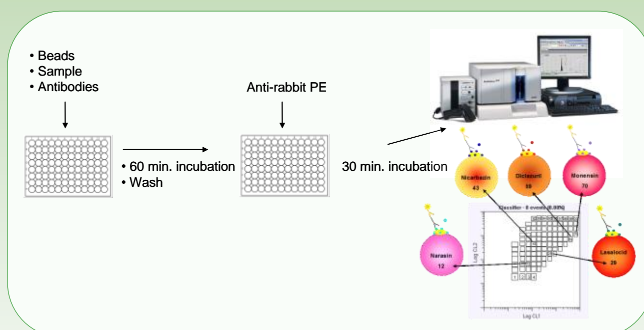

Figure 2: The assay procedure.

Results

For the detection of nicarbazin, diclazuril and salinomycin, narasin, lasalocid and monensin five different single-plex assays were combined to a five-plex. The molecular structures of narasin and salinomycin are comparable and both coccidiostats could be detected with one assay (narasin equivalents).

Blank eggs and feed were spiked at MRL or ML and a reduction of the maximum signal was measured for all 6 coccidiostats (Figure 3).

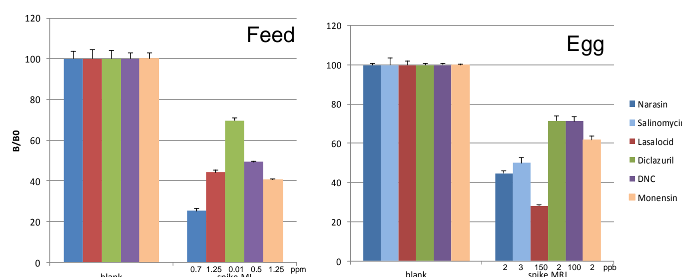

Figure 3: Left. Results of blank and spiked (nicarbazin, diclazuril, monensin, lasalocid and narasin) feed samples. Right. Results of blank and spiked (nicarbazin, diclazuril, monensin, lasalocid, salinomycin and narasin) egg samples.

The next challenges are the stabilization of the lasalocid beads and the single-laboratory validation of the method.

The CONffIDENCE project is funded by the European Commission, call identifier FP7-KBBE-2007-1, Grant Agreement number 211326.

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