Multiplex flow cytometric immunoassay for the simultaneous detection of coccidiostats.

Monique Bienenmann-Ploum[1], Mirjam van Aalderen[1], Anne-Catherine Huet[2], Katrina Campbell[3], Terence Fodey[3], Willem Haasnoot[1], Philippe Delahaut[2], Chris Elliott[3] and Michel Nielen[1,4].

Introduction

Coccidiosis is an infection of the intestinal tract which especially affects poultry and results in economic losses. To control this disease, different coccidiostats were developed as feed additive. The correct use of these feed additives should be monitored in the frame of a potential cross-contamination to non-targeted feeds and, to protect the consumers, MRL's in eggs have been set by the European Union (regulation 124/2009).

Technology

For the simultaneous detection of coccidiostats, a flow cytometry-based immunoassay (FCI) is under development using the Luminex flow cytometer in combination with the MultiAnalyte Profiling (xMAP) technology. The antigens (drugs or drug-protein conjugates) were covalently coupled on the carboxylated polystyrene microspheres (beads) internally dyed with a red and orange fluorophore. The Luminex (Figure 1) 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 fluorescent dye corresponding with the amount of antibodies bound to the beads. Thus, this combination makes it possible to simultaneously measure up to 100 different biomolecular reactions in a single well.

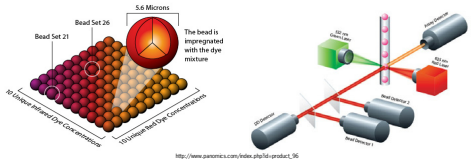

Figure 1: Principle of the Luminex technology

Method

Beads were coupled with conjugates (QUB) as described by Luminex. l-Glutamic acid gamma-(p-)nitroanilide was directly immobilized on the beads.

In a well of a 96 well filter plate, 100 µl sample, 10 µl diluted polyclonal antibody (CER) and 10 µl bead suspension were added. After 1 hour incubation, the filter plate was washed 3 times with buffer and 100 µl anti-rabbit-phycoerythrin (Prozyme) was added to each well. Samples were analysed in the Luminex 100, after a 30 minutes incubation step. For each assay, the median fluorescence intensity (MFI) on 100 beads was counted per well.

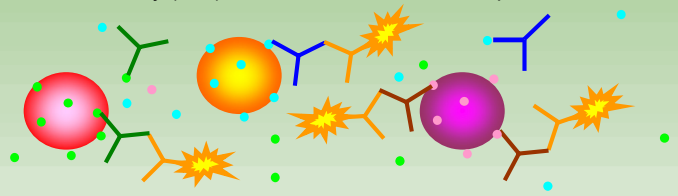

Figure 2: Principle of the multiplex inhibition assay in a well. The 3 different antibodies are represented in green, blue and red.

Results

For the detection of nicarbazin, diclazuril and salinomycin three different assays were developed. The molecular structure of narasin is almost comparable with salinomycin and a high cross-reaction was expected.

- Calibration curves of nicarbazin, diclazuril and salinomycin showed IC_{50} values of 7.8, 0.6 and 0.3 ng/ml in buffer, respectively.
- The narasin cross-reaction of 2% was disappointing and resulted in an IC_{50} of only 19 ng/ml in buffer.

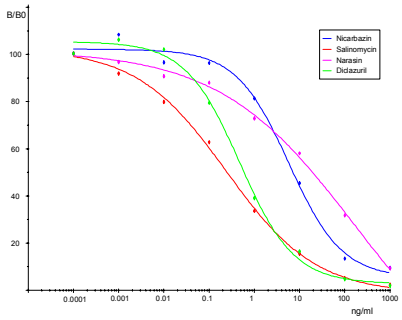

Figure 3: Calibration curves of nicarbazin, diclazuril, salinomycin and narasin in buffer.

Conclusions

- The sensitivities of the nicarbazin, diclazuril and salinomycin assays are promising, in comparison to the MRL of 75, 2 and 3 µg/kg egg, respectively. The performance in egg and feed extracts have to be tested.
- The narasin assay needs further optimization.

The CONffIDENCE project is funded by the European Commission under FP7 grant 211326.

[1]RIKILT-Institute of Food Safety, Wageningen UR, P.O. Box 230, 6700 AE Wageningen, the Netherlands.
[2]Centre d'Economie Rurale (CER), Santés animale et humaine, rue Point du Jour 8, 6900 Marloie, Belgium.
[3]Queen's University Belfast (QUB), Institute of Agri-Food and Land Use, Belfast, United Kingdom.
[4]Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703 HB Wageningen, the Netherlands.