Multiplex flow cytometric immunoassay for the simultaneous detection of coccidiostats in eggs.

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Introduction

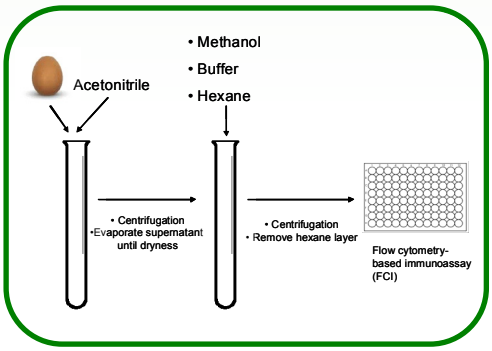
Coccidiosis is an infection of the intestinal tract which especially affects poultry and results in economic losses. To control this disease 11 different coccidiostats are allowed to be used as feed additives. These coccidiostats should be monitored for potential cross-contamination to non-targeted feeds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

Technology

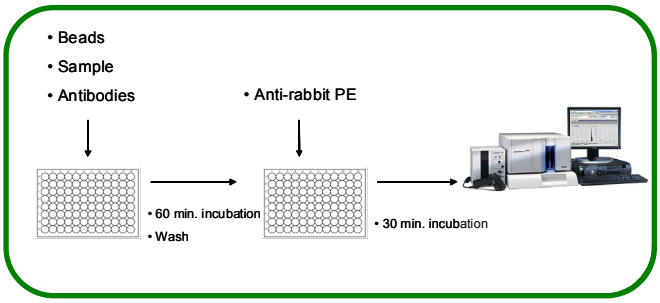
For the simultaneous detection of 6 coccidiostats, a flow cytometry-based immunoassay (FCI) is under development using the Luminex flow cytometer in combination with the MultiAnalyte Profiling (xMAP) technology. The antigens (coccidiostat or the protein conjugates) were covalently coupled on the carboxylated polystyrene microspheres (beads) internally dyed with a red and orange fluorophore. 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 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.

Method

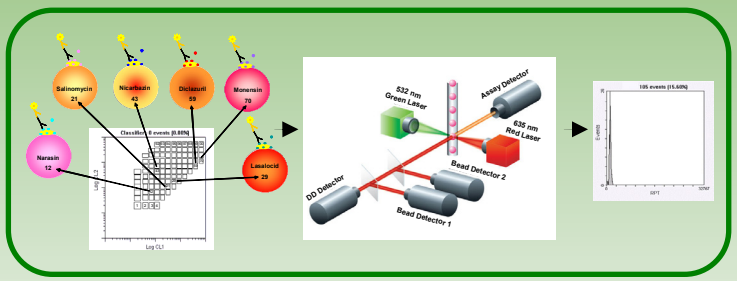
Egg extration:



Flow cytometry-based immunoassay (FCI):



Measurement:



Results

For the detection of nicarbazin, diclazuril and salinomycin, narasin, lasalocid and monensin five different single plex assays were developed. The molecular structure of narasin is comparable with salinomycin and both coccidiostat could be detected within one assay.

Due to assay interference two complementary multiplexassays were used, a 3- and 2-plex assay.

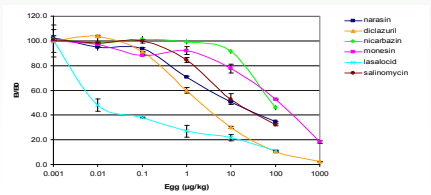


Figure 1:
Calibration curves of nicarbazin, diclazuril, salinomycin, monensin, lasalocid and narasin in egg.

The next challenges are to stabilize the narasin beads, the sensitivity improvement of the monesin assay and the optimization of the extraction method in order to improve the recovery of all 6 coccidiostats.

Conclusions

- Two complementary multiplex assays, a 3- and 2-plex assay, made it possible to detect six coccidiostats in egg extract.
- Calibration curves of nicarbazin, diclazuril, salinomycin, narasin, lasalocid and monensin showed IC_{50} values of 40, 2, 2, 4, 0.1 and 36 µg/kg of egg extract, respectively.
- This multiplex method has a high potential to be used in food and feed analysis.

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

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