Validation of a multiplex flow cytometric immunoassay for the simultaneous detection of six 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, different coccidiostats are allowed to be used as feed additives. These coccidiostats should be monitored for potential cross-contamination into 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 (FCIA) is developed. Five coccidiostat protein conjugates were covalently coupled on the beads and were combined with five polyclonal antisera.

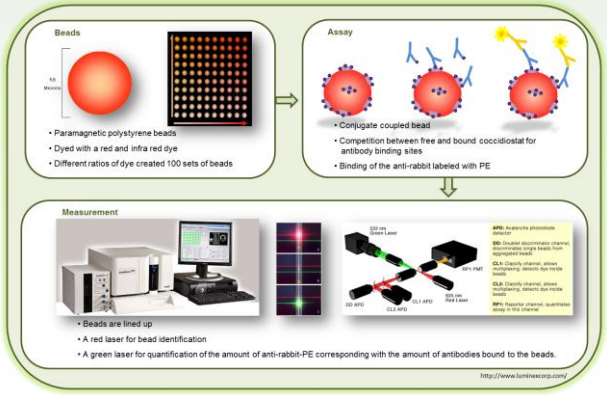

Figure 1: Flow cytometry-based immunoassay (FCIA) technology.

Method

A five-plex assay was used for the detection of nicarbazin, diclazuril, salinomycin, narasin, lasalocid and monensin.

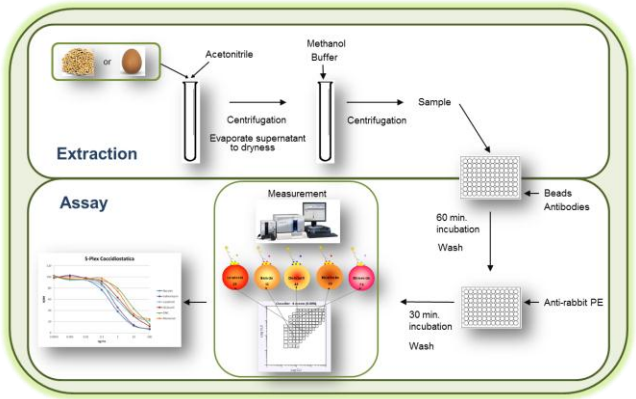

Figure 2: The extraction method and assay procedure for feed and egg.

The assay was combined with a common extraction method for egg and feed (Figure 2).

Validation

For the validation of the method [(1)], 20 different blank egg and feed samples were spiked with 6 coccidiostats at the corresponding ½ M(R)L and M(R)L for egg or feed, respectively. The samples were measured over 3 different days. The Cut-Off level for the assay will be set at the highest level of the ½ M(R)L [(1)].

Results

Validation results are shown in Figure 3 and Table 1. There is no overlap between the highest ½ M(R)L and the lowest blank value. Therefore the CCβ of the assay is less than or equal to the ½ M(R)L[(1)].

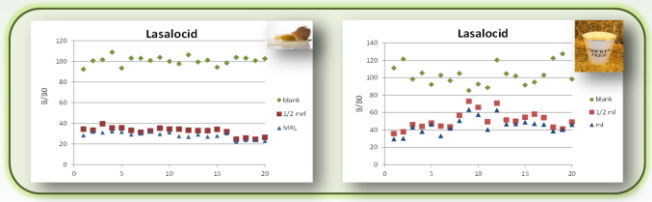

Figure 3: Validation of the lasalocid assay in eggs (left), in feed (right)

	Cut-off (B/B0)		CCβ		Interassay CV%			Cut-off (B/B0)		CCβ		Interassay CV%	
	Egg	Feed	Egg	Feed	Egg	Feed		Egg	Feed	Egg	Feed	Egg	Feed
Narasin	59	72	1	350	5.2	23.5	Diclazuril	87	-	1	-	4.5	8.2
Salinomycin	78	82	1,5	350	4.8	30.4	DNC	88	80	50	625	3.9	10.4
Lacalocid	40	73	75	625	11.3	17.2	Monensin	82	80	1	625	7.2	20.4

Table 1: Assay performance.

The complete validation results will be published in the near future.

Conclusions

- One common extraction method for egg and feed could be applied.
- One multiplex assay made it possible to simultaneously detect six and five coccidiostats in egg and feed extracts respectively at their M(R)L.
- Narasin and salinomycin are detected with the same assay, providing results in narasin equivalents.
- The diclazuril assay did not pass the validation criteria in feed.
- A ring test is performed at the moment.
- This multiplex method has a high potential to be used in food and feed analysis.

Literature

1. Community Reference Laboratories Residues (CRLs) (2010) Guidelines for the validaton of screening methods for residues of veterinaty medicines (initial validation and transfer).

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