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DIAGNOSTIC ENGINEERING

ACEA Biosciences, Inc.

Using NovoCyte® Benchtop Flow Cytometer for Multiplexing Antibiotic Screening in Food Commodities with BEADYPLEX™ Assay

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Introduction

The misuse of antibiotics in animal farming may lead to the presence of residues in edible products, thus compromising consumer health (toxicological and allergic reactions, as well as bacterial antibiotic resistance development) and industrial food processes. Rapid and efficient screening analytical methods for the early detection of antibiotic residues are therefore essential to guarantee food safety and to reduce the negative impact on industrial fermentation processes.

The current trend in food analysis entails the implementation of multi-residue technologies which considerably improve analysis time and overall cost. **BEADYPLEX™** is a competitive suspension bead-based Flow Cytometric ImmunoAssay (FCIA) for the simultaneous screening of more than 80 residues from the 10 most relevant antibiotic families, namely tetracyclines, sulfonamides, β-lactams, aminoglycosides, macrolides, fluoroquinolones, lincosamides, phenicols, polymyxines and pleuromutilins, providing the family identification in one single test. The method has been validated in muscle (porcine, bovine and poultry) and fish (salmon and coley) following the 2010 CRL Guidelines supplementing European Decision 2002/657/EC. Preliminary results show the potential applicability of BEADYPLEX™ to other matrices, such as milk or eggs.

BEADYPLEX™ has been validated using **NovoCyte® 2000 flow cytometer** (with 488 nm and 640 nm lasers and 4 fluorescence channels) and the **BeadyExpress™ software** developed by **ACEA Biosciences**. NovoCyte® is a high performance bench flow cytometer featured with wide dynamic range (7 logs), high sensitivity, high speed, and ease of use. BeadyExpress™ software, developed for BEADYPLEX™ FCIA multiplexing assay, allows the straightforward and automatic data acquisition and analysis. With a few clicks of button, the software automatically sets the gates for each population of the BEADYPLEX™ beads, acquires and analyses the external fluorescence signal, and confirms the presence/absence of antibiotics, using the proprietarily developed algorithm. The software generates a report indicating the validity of the assay and identifying each analyzed sample as Positive or Negative for the 10 antibiotic families.

Assay Principle and Methodology

A **twelve-plex competitive Flow Cytometry Immunoassay** has been developed for the detection of **10 antibiotic families** in food commodities (more than 80 residues included).

Antibiotic-mimicking molecules are individually coated on fluorescent beads encoded by their size and internal fluorescence. First, a mixture of primary binders directed to the different antibiotic families are incubated with the sample extracts and the antibiotic-conjugated beads. Then, secondary binders labeled with a fluorescent reporter are added for incubation. A washing step is required after every incubation step.

Upon reading with the NovoCyte®, the beads are classified according to their size and internal fluorescence (excited by 640 nm laser and detected in APC channel). The external assay signal for every bead population is detected from the secondary binders in FITC channel by 488 nm laser excitation.

According to the competitive principle of the assay, the absence of antibiotics in the sample is translated into the maximum fluorescent assay signals, whereas a decrease in fluorescence is observed upon the presence of free antibiotics for the associated bead.

Flow Cytometry Immunoassay
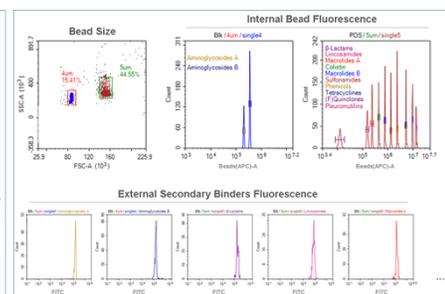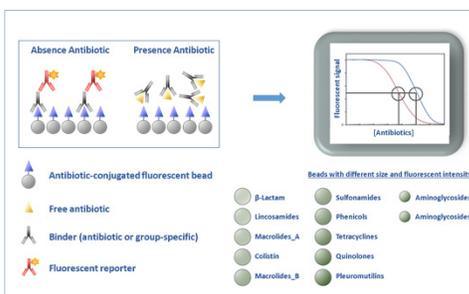
Results

Most of the antibiotics included on the scope of **BEADYPLEX™** are detected at concentrations equal to or below the European regulatory limits. LOD (Limit of Detection, Detection Capability).

Family	Antibiotic	LOD (µg/kg)	Family	Antibiotic	LOD (µg/kg)	Family	Antibiotic	LOD (µg/kg)
Aminoglycosides	Streptomycin	250	Sulfonamides	Sulfadimidine	100	Tetracyclines	Chlortetracycline	25
	Dihydrostreptomycin	125		Sulfadiazine	50		Doxycycline	25
	Gentamicin	50		Sulfamethoxazole	10		Oxytetracycline	50
	Neomycin B	100		Sulfadimethoxine	50		Tetracycline	50
	Kanamycin A	100		Sulfadoxine	10		Demeclocycline	50
	Paromomycin	250		Sulfachloropyridazine	1		Methacycline	25
β-Lactams	Cefquinome	50		Sulfapyridine	0,5	Quinolones	Marbofloxacin	20
	Ceftiofur	50		Sulfaguanidine	1		Flumequine	800
	Desfuroylceftiofur	2000		Sulfathiazole	0,5		Enrofloxacin	5
	Cefoperazone	3		Sulfamethoxypyridazine	0,25		Ciprofloxacin	10
	Cefalexin	3500		Sulfamerazine	20		Danofloxacin	250
	Cefalonium	5		Sulfamethizole	1		Oxolinic acid	800
	Cefapirin	75		Sulfamonomethoxine	10		Difloxacin	50
	Desacetylcefapirin	100		Sulfaquinoxaline	50		Norfloxacin	2,5
	Cefazolin	100		Sulfameter	5		Sarafloxacin	125
	Cefacetrile	50		Sulfanilamide	50		Pefloxacin	10
	Penicillin V	12,5		Dapsone	0,2		Enoxacin	50
	Penicillin G	5	Macrolides	Tilmicosin	50		Lomefloxacin	15
	Ampicillin	25		Tylosin A	10		Ofloxacin	15
	Amoxicillin	25		Tylvalosin	125		Cinoxacin	800
	Oxacillin	50		Tildipirosin	1200	Phenicols	Chloramphenicol	0,45
	Cloxacillin	50		Spiramycin	250		Florfenicol	2100
	Dicloxacillin	30	Lincosamides	Lincomycin	25		Thiamphenicol	100
	Nafcillin	300		Clindamycin	150	Pleuromutilins	Valnemulin	50
	Piperacillin	5	Polymyxin	Colistin	150		Tiamulin	25

Example of automatically generated report by the software **BeadyExpress™** upon plate reading with **NovoCyte®**.

Blank and Positive Control Samples
Validation of the test

Unknown Samples
Identification of Positive `POS` and Negative `NEG` results for each antibiotic family

Conclusions and Perspectives

BEADYPLEX™ is a user-friendly assay that provides sensitive and specific broad spectrum detection of antibiotics in multiple matrices in one single test with high-throughput capability.

NovoCyte® and BeadyExpress™ software highly simplify the data acquisition and the interpretation of results, by providing a direct identification of the antibiotic family from the sample, with significant reduction of analysis time and cost.

The work presented here illustrates the potential of using Flow Cytometry for food analysis. New FCIA kits are under development in order to expand the application of this versatile and flexible technology to other types of contaminants.