LABEL-FREE WAVEGUIDE SIMULTANEOUS DETECTION OF SULFONAMIDE, FLUOROQUINOLONE AND B-LACTAM AND TETRACYCLINE ANTIBIOTICS IN MILK USING A WAVELENGTH INTERROGATED OPTICAL SYSTEM (WIOS)

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The widespread use of antimicrobials outside human medicine is one cause of the alarming emergence in humans of bacteria, which have acquired resistance to antimicrobials. It has been reported that more than 70% of bacteria are insensitive against at least one antibiotic. This situation is causing a serious threat for the public health, as more and more infections can no longer be treated with the presently known antidotes[1]. However, although the amount of antimicrobials used in food animals is not known precisely, it is estimated that about half of the total amount of antimicrobials produced globally is used in food animals. Governmental agencies have set limitations on the residue levels in the majority animal tissues (2377/90/EC) destined for human consumption to control this situation.

Nowadays, non invasive target samples such as milk are considered to be used for control analysis of the misuse of antibiotics in animals. However, the need for detecting contaminants as early as possible in the food chain sometimes is not compatible with elaborated laboratory reference methods what stimulates the use of new easy-to-use label-free detections systems able to provide a direct rapid response in the presence of the contaminant. In this aspect, new era of biosensors combine the exceptional features of biomolecules such as antibodies or receptors with the latest advances in the investigation of new transducing principle based on singular electronic and optical properties integrated in miniaturized microelectronic devices[2].

In this manner, class-selective immunoreagents previously evaluated with a multianalyte ELISA test, have been implemented in an optical biosensor developed by CSEM (WIOS: wavelength interrogated optical sensor[3]) to perform simultaneous detection of sulfonamide (SA), fluoroquinolone (FQ) and ß-lactam (BL) and Tetracycline (TC) compounds in milk. The technique uses the evanescent field of light to probe changes in the refractive index at the surface of a waveguide[3]. Monitoring of the resonance wavelength allows real-time binding analysis of non-labeled molecules on the waveguide grating surface. Combining the reduced size of the optical system with a fluidic cell, *in situ* measurements can be performed being an interesting future option for field assays. SA and FQ immunoreagents used were developed by our group (AMRg), BL and TC ones were provided by UNISENSOR while blind spiked milk samples supplied by Nestlé were used to evaluate this technique.

References:

(1) Wallmann, J.; Schroter, K.; Wieler, L. H.; Kroker, R. *Int. J. Antimicrob. Ag.* **2003**, *22*, 420-428.
(2) Comley, J. *J. Drug Discovery World Winter* **2004**, *5*, 63-74.
(3) Cottier, K.; Wiki, M.; Voirin, G.; Gao, H.; Kunz, R. E. *Sensors and Actuators B: Chemical* **2003**, *91*, 241-251.

Figures:

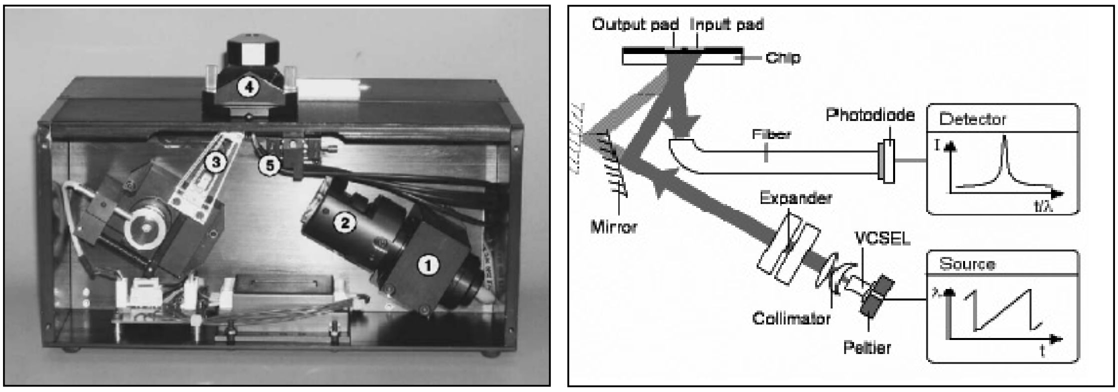

Figure 1. Left: Compact WIOS instrument. (1) Laser source, (2) beam expanding optics, (3) deflection mirror, (4) chip support with fluidic cell, and (5) array of plastic optical fibres. Right: Scheme of WIOS principle.

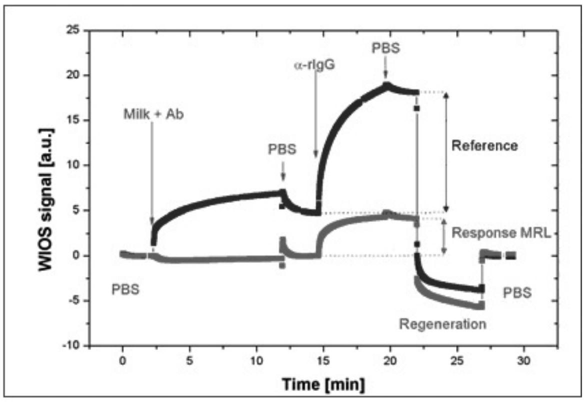

Figure 2. WIOS response of a whole measurement cycle in milk. Typical adsorption curve showing the competition between the immobilized antigen and the antibiotic (or nothing) in solution with the specific antibody. Adsorption of the secondary antibody (antiIgG) provides an amplified response signal, which is inversely proportional to the concentration of antibiotic in solution. Finally, regeneration of the active surface allows new measurements with the same chip.

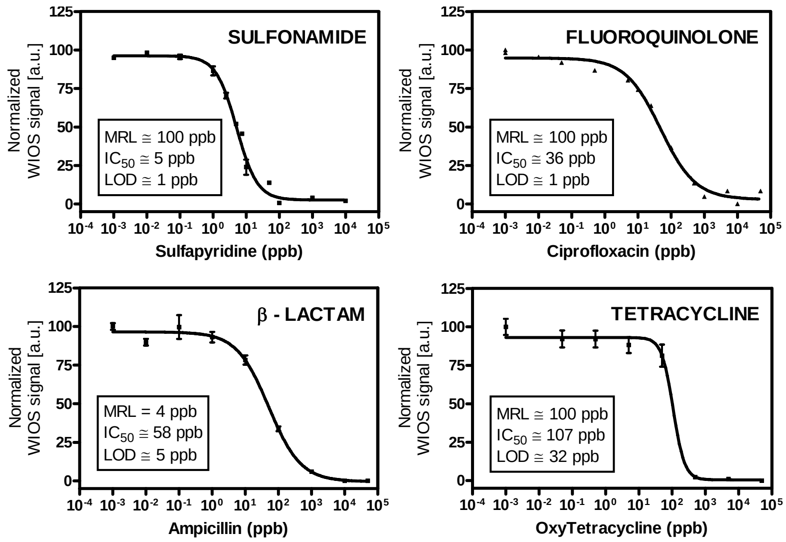

Figure 3: Standard calibration curves for all the antibiotic families using the WIOS.