

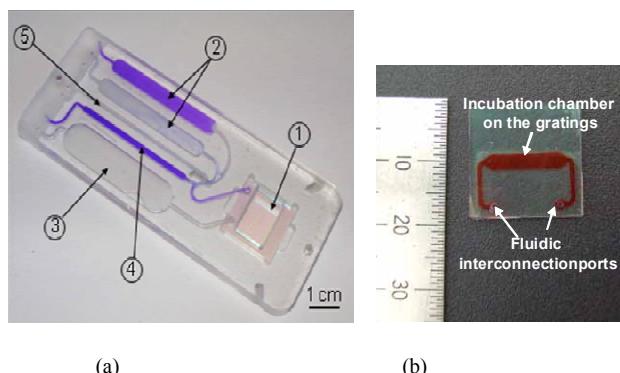
Supporting Information

I- Immunoassay formats :

Reagents: Phosphate buffer saline (PBS) tablets were purchased from Sigma-Aldrich. Sulfonamide and fluoroquinolone haptenized protein conjugates and corresponding antiseraums were prepared and kindly provided by Applied Molecular Receptors group (CSIC-IIQAB Barcelona, Spain). Sulfapyridine (sulfonamide) and ciprofloxacin (fluoroquinolone) were purchased from Riedel-de-Haën and Aldrich Chemical Co (Milwaukee, WI, USA). Biotin-DNA, DNA-modified bioreceptor, the corresponding antibody and oxytetracycline (tetracycline) were developed and kindly provided by Unisensor SA (Wandre, Belgium).

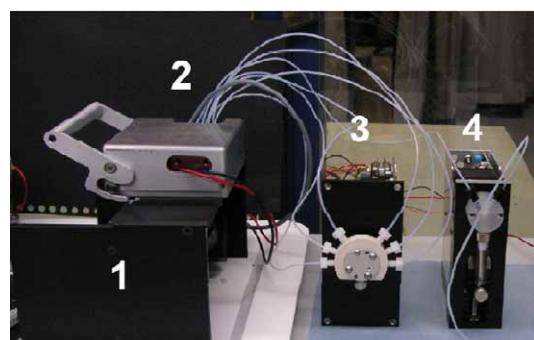
II- Microfluidic cartridge fabrication:

Reagents: acrylate third generation polyether core HBP was provided by Perstorp AB (Sweden) and SU-8 was purchased from Gersteltec (Switzerland). The photoinitiator used for sensor chip gasket molding was IrgaCure 184 (1 wt.%) was provided by Ciba Specialty Chemicals (Switzerland). Propylene glycol monomethyl ether acetate (PGMEA) and UV adhesive were purchased from Aldrich Chemical Co (Milwaukee, WI, USA) and Dymax (Torrington, CT, USA), respectively.

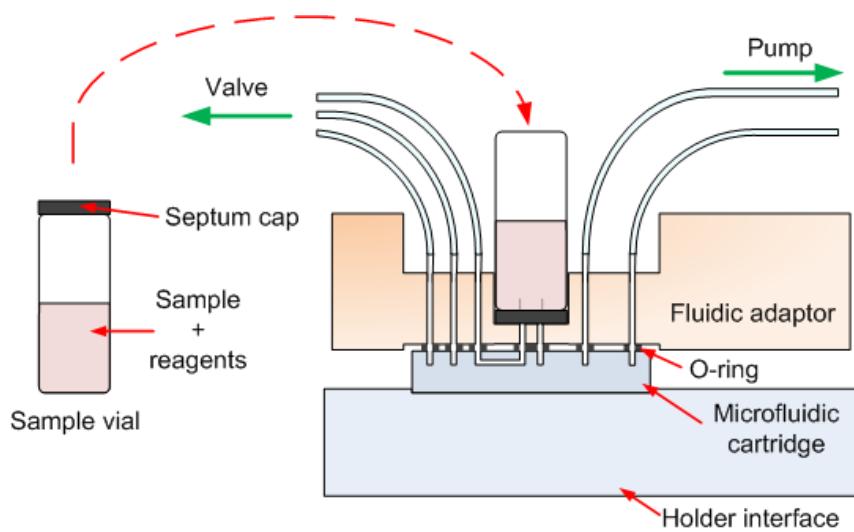


**Fig. S1** Photograph of the lab-on-a-chip (LOC) cartridge for simultaneous detection of three antibiotics in raw milk: (a) micromilled PMMA cartridge combined with sensor chip: (1) sensor chip (optical transducer with incubation chamber); (2) reservoirs for immunoassay solutions; (3) main waste; (4) loading waste; (5) sample introduction channel; (b) enlarged view of sensor chip (HBP incubation chamber bonded on the grating chip).

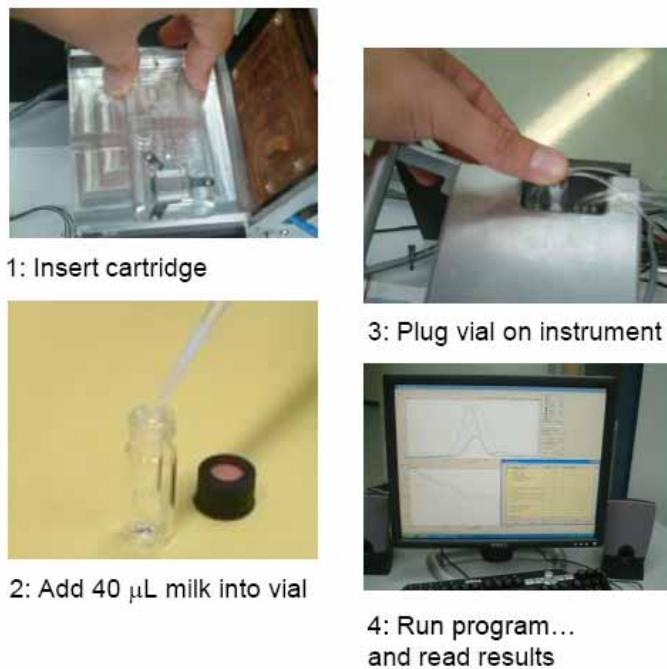
III- Fluidic setup and test procedure: Fluidics are an essential part of the whole multianalyte detection system depicted in Figure S2. Fluidic connections between the cartridge and the valve/pump system are ensured through a so called “holder interface” located on the WIOS instrument. Once the cartridge is positioned into the fluidic holder box the cover is closed applying some pressure on rubber o-rings that seal the connection between the inlets/outlets flat ports of cartridge and external tubings. Small volume of milk sample is introduced by pipetting (< 40 µL) into a septum-vial pre-filled with assay reagents (competitive antibodies) and then plugged on the specific location of the “holder interface”, as depicted in Fig. S3. By this way, the sample is directly connected to the fluidic system and can be delivered to the lab-on-a-chip through external pump/valve actuation. A general test protocol sequence is illustrated in Fig. S4.



**Fig. S2** Photograph of automated multi-detection system: 1) WIOS detection instrument; 2) Cartridge inserted into “holder interface” box; 3) multiposition valve; 4) syringe pump.



**Fig. S3** Schematic cross-section representation of fluidic setup and milk sample introduction on detection system.



**Fig. S4** Sequence of pictures illustrating test protocol for lab-on-a-chip multiplexed detection of antibiotics in milk.