La citofluorimetria per la salute pubblica: nuove applicazioni e prospettive future

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Abstract

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La versatilità della citofluorimetria (CF), che consente analisi multiparametriche, funzionali, rapide e quantitative su un elevato numero di eventi, ha permesso il suo impiego in diversi settori come quello dei processi industriali, dei controlli ambientali, agro-alimentare e veterinario. In quest'ultimo ambito, tralasciando le osservazioni di tipo clinico e patologico traslate dall'uomo agli animali, la CF sta suscitando un enorme interesse come possibile approccio metodologico per analisi di tutto ciò che possa compromettere la qualità e la sicurezza di un prodotto alimentare.

Nella relazione verranno presentati alcuni esempi di applicazione in questi settori. In particolare, la produzione di latte e dei suoi derivati rappresenta un valido esempio di una filiera con diversi punti critici che necessitano di continui accertamenti a tutela sia della salute degli animali che della sicurezza e della qualità del prodotto finale. Sebbene la CF è in parte già utilizzata, risulterebbe di enorme beneficio estendere il campo di applicazione per coprire il maggior numero di stadi sensibili lungo tutta la filiera di produzione.

Partendo da questo modello valuteremo, laddove possibile, l'idea di intraprendere percorsi di valutazione intra- e inter-laboratorio che possano consolidare nuovi metodi di controllo basati sulla CF e quello più complesso del riconoscimento come metodi di riferimento alternativi dando così un importante contributo nel controllo dei rischi per la salute degli animali e dell'uomo.

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Cell Factory FaBioCell
Carmela Rozera

Citometry
Massimo Sanchez

EPR
Donatella Pietraforte

NMR
Rossella Canese

Proteomics
Marta Ponzi

Microscopy
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Sequencing
Fiorella Ciaffoni

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Technical-scientific areas

Scientific Computing

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High-Performance Computing (HPC), development and implementation of parallel algorithms. Design, optimization and control of methods, tools and systems aimed at processing data flows generated by scientific biomedical instruments, digital sources and databases. Algorithm implementation on multi-core, multi-CPU, multi-GPU architectures. Applications and libraries of machine learning, artificial intelligence and Bayesian networks.

Cell Factory FaBioCell

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FaBioCell is a cell factory authorized by the Italian Medicines Agency (AIFA) for the production of cell therapy products. It works in conformity with the "current Good Manufacturing Practices" (cGMP), the European guidelines regulating the activities related to the production of pharmaceutical drugs, including experimental drugs. The cell factory offers services for GMP validation of experimental protocols, and for the production and quality control of cell drugs. The facility also offers support for the preparation of documents to be submitted to the regulatory agencies for the authorization of clinical trial protocols.

EPR

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The Electronic Paramagnetic Resonance (EPR) Area is equipped with four spectrometers operating in continuous wave X-band, one being an ultra-high sensitivity instrument. EPR is applied, in vitro/ex vivo, to the study of free radicals, oxidative stress, antioxidants, protein structures, membranes, metalloproteins, radiation damage, pre-clinical research into degenerative diseases and tumors. As for radiation damage, the Area has expertise and instrumental equipment for ionizing radiation detection and dosimetry; for the study of oxidative stress, biochemical and immunochemical techniques are applied.

NMR

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The high-resolution Nuclear Magnetic Resonance (HR-NMR) Unit is equipped with a spectrometer operating at 9.4 T for metabolism and metabolomics studies in cells, biological fluids and tissues.
The preclinical Magnetic Resonance Imaging (MRI) Unit is equipped with an MRI system operating at 4.7 T for morphological, molecular and functional imaging and spectroscopy studies on rodents in vivo.
The area has developed multidisciplinary skills in in oncology, neuroscience, aging and metabolic diseases.

Technical-scientific areas

Proteomics

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The Proteomics Area uses mass spectrometry (LC-MS/MS), immunometric techniques (Luminex), and Reverse-Phase Protein Microarrays (RPPA). LC-MS/MS analysis can be applied to identify and characterize semi-purified proteins, detect post-translational modifications and adducts, and to thoroughly analyze complex protein mixtures. It can also be used for qualitative and quantitative investigations of total proteomes and subcellular compartments, biomarker discovery, and analysis of protein complexes. The area relies on its expertise in biological sample preparation and bioinformatics. RPPA is suited for in-depth analysis of signal transduction pathways. The relative measurement of pathway activation levels is performed simultaneously on hundreds of protein extracts, via immunostaining with up to 400 validated antibodies.

Microscopy

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Within the Microscopy Area, the Confocal Microscopy Unit is equipped with a laser scanning confocal microscope for a wide range of applications: from cells to tissues, from intact organisms to nanovesicles. The staff have long-term experience with pre-clinical research in oncology, immunology, neurosciences and genetic diseases. The Electron Microscopy Unit is equipped with a field emission scanning electron microscope (INSPECT F-FEI) and a transmission electron microscope (PHILIPS 208S EM-FEI) for ultrastructural analyses in the life sciences. The expertise of the unit spans from microbiology and parasitology to cell biology. Standard electron microscopy methods are used for morphological studies, and immunolabelling studies for antigen localization by both TEM and SEM.

Sequencing

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The Sequencing area is equipped with an Ion Torrent PGM system for Next Generation Sequencing. The Ion Torrent Technology represents a fast, simple and accessible sequencing solution that ensures robust and reliable results.
The Ion Torrent PGM technology is mainly indicated for the following applications:

- Microbial sequencing (DNA, RNA , also de novo).
- Targeted DNA and RNA sequencing based on the Ampliseq technology.
- Small RNA and miRNA sequencing.

Citometry

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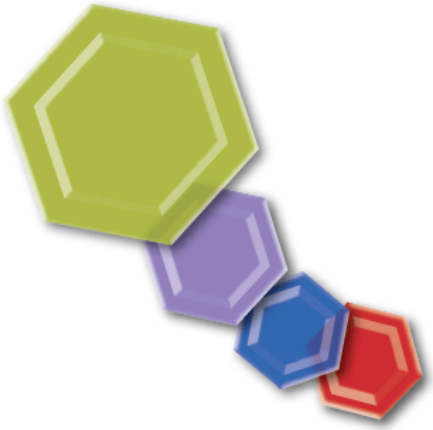
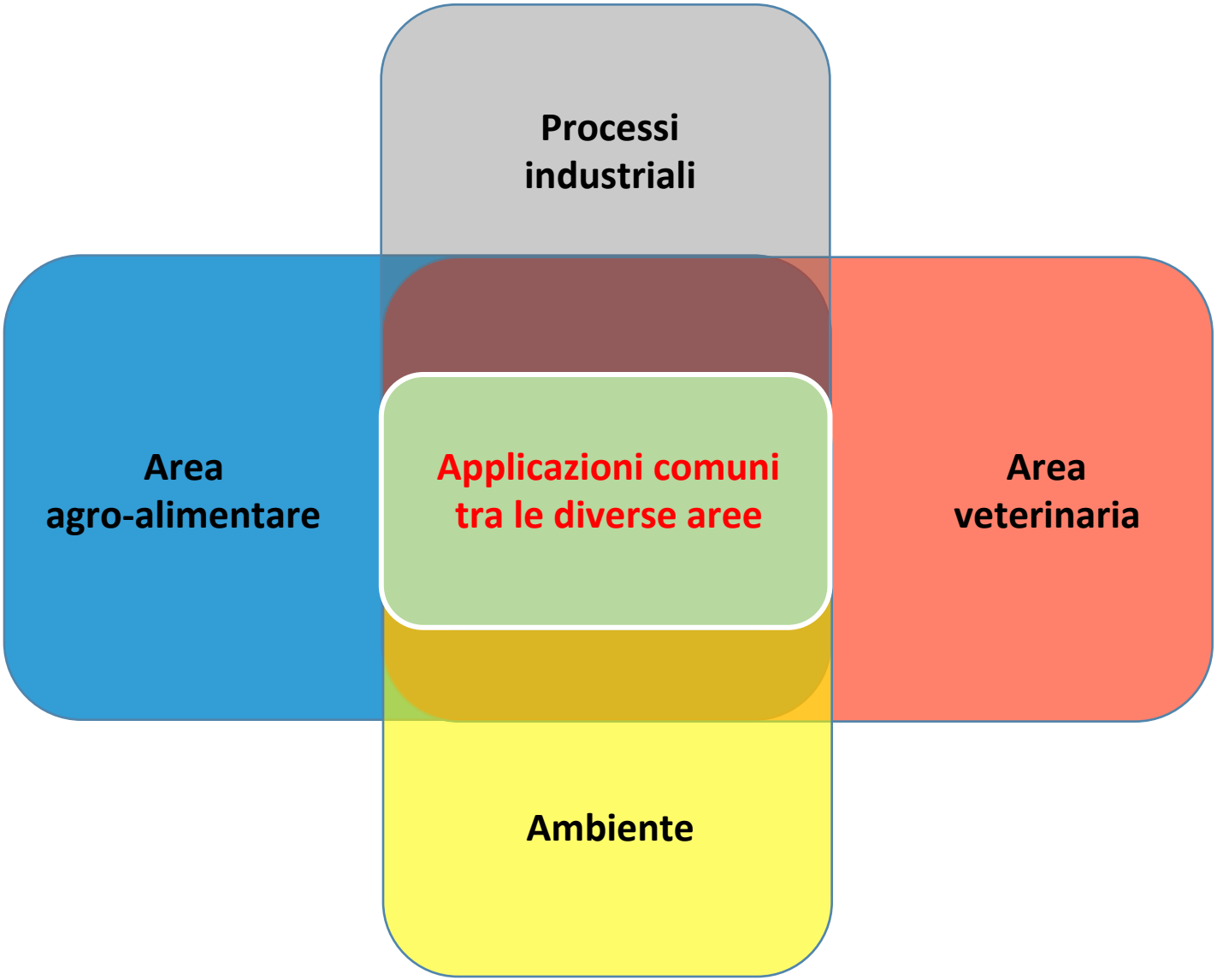
The Cytometry Area is equipped with three analyzers, two cell-sorters and Single-Cell Mass Cytometry (CyTOF). The staff have expertise in a wide range of cytometric techniques and offer qualified support in the design of the most appropriate multiparametric labelling panels, data processing and interpretation of analyses. Besides a Cell Sorting service, the facility provides support to the conception, planning, and data analysis to develop new experimental approaches. High-throughput multi-parameter cytometric analyses can be run in the facility's laboratory.



L’impiego della citofluorimetria in campi non strettamente biomedici
Possibili interessi comuni e risparmio economico



l'impiego della citofluorimetria in campi non strettamente biomedici
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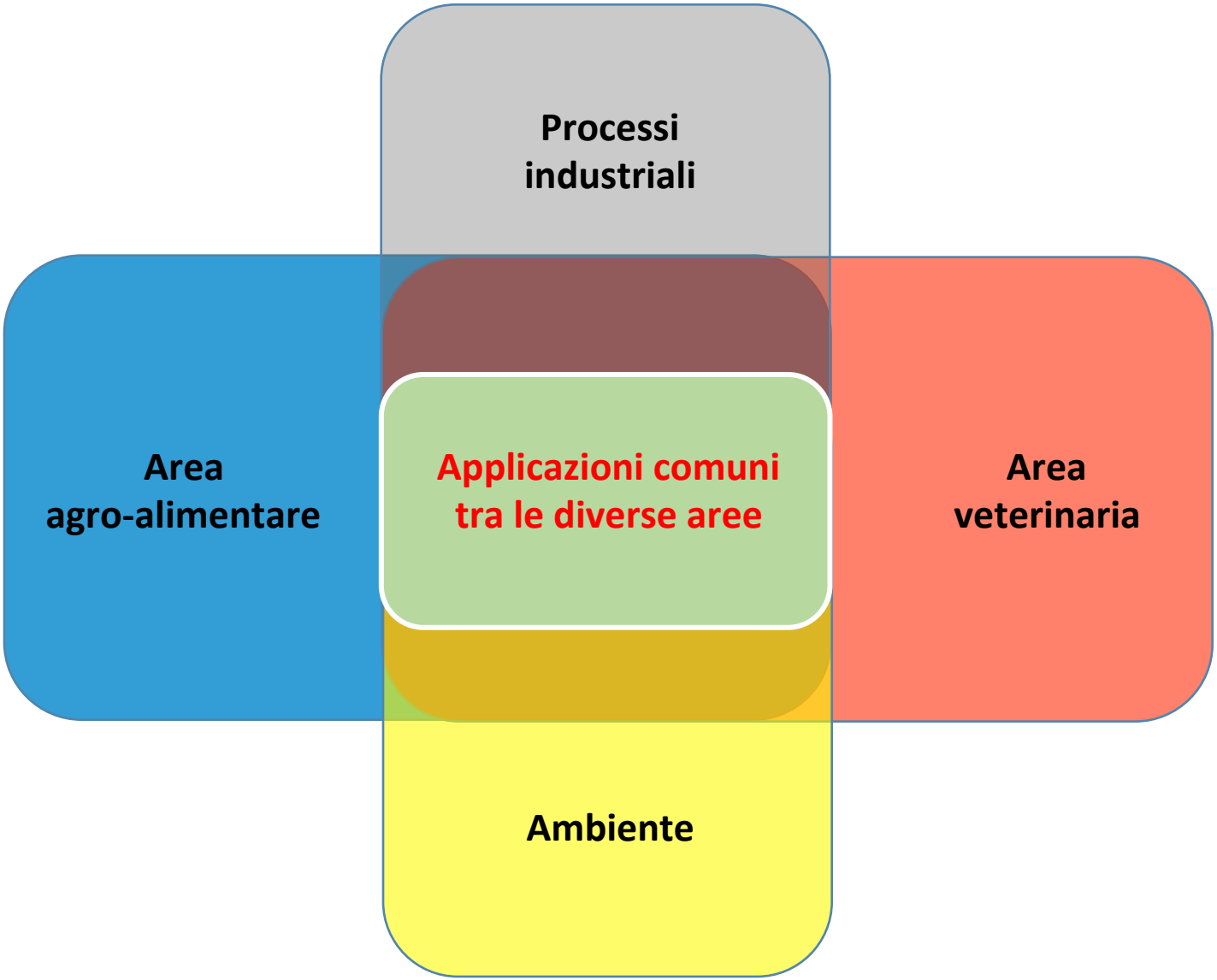

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Review

Application of flow cytometry to industrial microbial bioprocesses

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Table 2
Pharmaceutical and clinical FC applications.

Application	Cell type	Functional or structural parameter	Reference
Antibacterial effect and mechanism of action of silver solutions	*Staphylococcus aureus, Escherichia coli*	Membrane integrity and esterase activity	[32]
Evaluation of antibiotics effects on cell viability	*Staphylococcus aureus Micrococcus luteus* *Streptococcus pyogenes*	Membrane integrity and potential Membrane integrity	[130] [149]
Diagnosis assays	*Candida* species	Acid nucleic	[195]
Research in mycoplasmology	Mycoplasmas	Acid nucleic	[197]
Evaluation of antifungical activity	*Candida, Aspergillus* dermatophytes	Metabolic activity	[199,201]
Cell susceptibility against antimycobacterial drugs	*Mycobacterium tuberculosis*	Membrane integrity	[200]
Persistence of cultures in stationary-phase	*Streptococcus pyogenes*		[202]
Screening of mutants for improved heterologous protein secretion	*Saccharomyces cerevisiae*	Detection of secreted proteins	[207]

Table 4
FC applications to alcoholic beverage production.

Application	Microorganism	Functional or structural parameter	Reference
Monitoring cider fermentation	*Saccharomyces cerevisiae, Lactobacillus hilgardii*	Membrane integrity and esterase activity	[23]
Viable yeast and bacteria detection in wines	*Saccharomyces cerevisiae, Saccharomyces bayanus, Oenococcus oeni*	Membrane potential, esterase activity	[114]
Early detection of alcoholic fermentation arrest	*Saccharomyces cerevisiae*	Esterase activity	[118]
Yeast viability throughout cider fermentation	*Saccharomyces cerevisiae*	Membrane potential	[127,236]
Optimization malolactic starters production	*Lactobacillus hilgardii*	Membrane integrity enzymatic activity	[144]
Optimization of high gravity and diet beer production	*Saccharomyces carlsbergensis*	DNA, lipids, sterol	[226]
Commercial yeast characterization in beer industry	*Saccharomyces cerevisiae*	DNA, lipids, sterol, FSC	[227]
Industrial beer production	*Saccharomyces cerevisiae*	Proteinases activity	[228]
High-density industrial wine fermentation	*Saccharomyces cerevisae*	DNA and membrane integrity	[229]
High-sugar Chardonnay fermentation	*Saccharomyces cerevisiae*	DNA and membrane integrity	[230]
Viability and cell count in a brewery	*Saccharomyces uvarum Saccharomyces cerevisiae*	Membrane potential	[233]
Cell cycle analysis during fermentation and beer quality	*Saccharomyces cerevisiae*	Nucleic acid	[234]
Assessment of brewing yeast age	*Saccharomyces cereviasiae*	Fluorescent labelled antibodies	[235]
Evaluation of yeast viability and concentration during wine fermentation	*Saccharomyces cerevisae*	Nucleic acid and membrane integrity	[237]
Cell count in oenology	*Saccharomyces cerevisae*	Enzymatic activity	[239]
Analysis of rehydrated yeasts populations for wine production	*Saccharomyces cerevisae*	Membrane integrity and permeability	[241]
Yeast viability and vitality in alcoholic fermentation	*Saccharomyces cerevisiae*	Esterase activity	[242]
Re-pitching of low malt beer fermentation	*Saccharomyces cerevisiae*	Membrane potential	[246]
Brewing yeast propagation	*Saccharomyces cerevisiae*	Glycogen, proteins and DNA	[247]
Monitoring of simultaneously inoculated alcoholic-malolactic Chardonnay fermentation	*Saccharomyces cerevisiae, Oenococcus oeni*	Fluorescent labelled antibodies	[248]

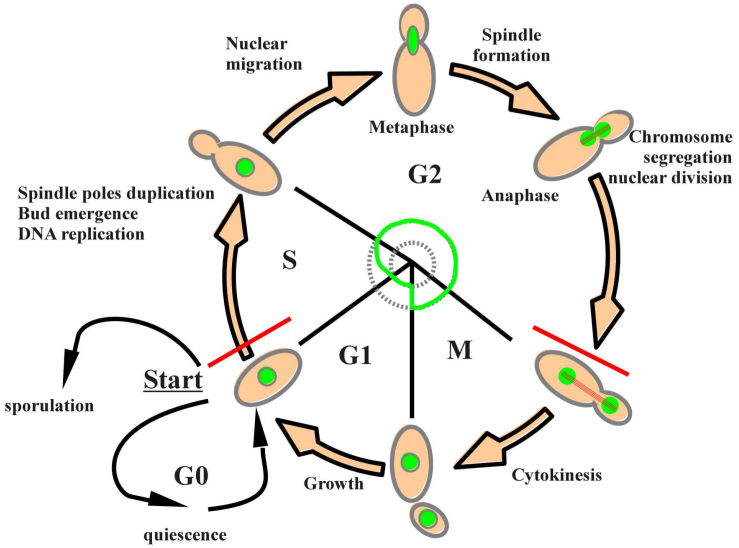
Table 1
Several applications of dyes to enological microorganisms.

Functions	Dyes	Applications	Matrix	References
Vitality	FDA	AF microorganisms	Pinot Noir grape must; Red wine	Malacrinò et al., 2001
			Synthetic wine	Salma et al., 2013
			White and Red wines	Gerbaux and Thomas, 2009
		MLF microorganisms	Growth medium	Bouix and Ghorbal, 2013
			White wine	Salma et al., 2012
			Red wine	Malacrinò et al., 2001
	cFDA	AF microorganisms	Synthetic must	Bouix and Leveau, 2001
				Bouchez et al., 2004
	FUN-1	AF microorganisms	Synthetic wine	Salma et al., 2013
Viability (membrane integrity)	PI	Sediment monitoring	Wine	Gerbaux and Thomas, 2009
		AF microorganisms	Synthetic must	Landolfo et al., 2008
				Mannazzu et al., 2008
				Branco et al., 2012
				Delobel et al., 2012
			Red and White must	Chaney et al., 2006
			White must	Farthing et al., 2007
		MLF microorganisms	Growth medium	Bouix and Ghorbal, 2013
			White and Red wines	Salma et al., 2012
Vitality and Viabilty	CV6/PI	AF microorganisms	Cider must	Herrero et al., 2006
	cFDA/PI		Growth medium	Monthéard et al., 2012
	CV6/PI	MLF microorganisms	Growth medium	Da Silveira et al., 2002
				Bouix and Ghorbal, 2013
			Cider	Herrero et al., 2006
VBNC State (Flow cytometry versus Petri dish)	Specific probe	AF microorganisms	Synthetic must	Andorra et al., 2011
	FDA	Spoilage yeast	Synthetic wine	Serpaggi et al., 2012
	CV6/PI	MLF microorganisms	Cider	Herrero et al., 2006
			Apple must and green cider	Quirós et al., 2009
Cell Cycle Analysis	SYBR Green	AF microorganisms	Synthetic must	Mendes-Ferreira et al., 2010
	PI		Synthetic wine	Salma et al., 2013
Membrane potential	$DiBAC_4(3)$	AF microorganisms	Synthetic "beer wort"	Kobayashi et al., 2007
		MLF microorganisms	Growth medium	Bouix and Ghorbal, 2013
			White and Red wines	Salma et al., 2012
Reactive Oxygen Species (ROS) presence	MitoTracker® Red CMXRos	AF microorganisms	Synthetic must	Mendes-Ferreira et al., 2010
	DHE			Mendes-Ferreira et al., 2010
	DHR		Synthetic "beer wort"	Kobayashi et al., 2007
Intracellular Lipid Content	Nile Red	AF microorganisms	Synthetic must	Mannazzu et al., 2008
Intracellular pH (pH_{in})	cFDA-SE and CDCF	MLF microorganisms	Growth medium and white wine	Bouix and Ghorbal, 2015

Dyes: FDA: Fluorescein diacetate; **cFDA**: Carboxy fluorescein diacetate; **FUN-1**: 2-chloro-4-(2,3-dihydro-3-methyl-(benzo-1,3-thiazol-2-yl)-methylidene)-1-phenylquinolinium iodide; **PI**: Propidium iodide; **CV6**: Chemchrom V6; **$DiBAC_4(3)$**: Bis-(1,3-dibutylbarbituric acid) trimethine oxonol; **DHE**: Dihydroethidium; **DHR**: Dehydrorhodamine; **cFDA-SE**: 5,6 carboxyfluorescein diacetate succimidyl ester; **CDCF**: 5–6 carboxy 2′7′-dichlorofluorescein.
Applications: AF: Alcoholic fermentation; **MLF**: Malolactic fermentation; **VBNC**: Viable but non culturable.

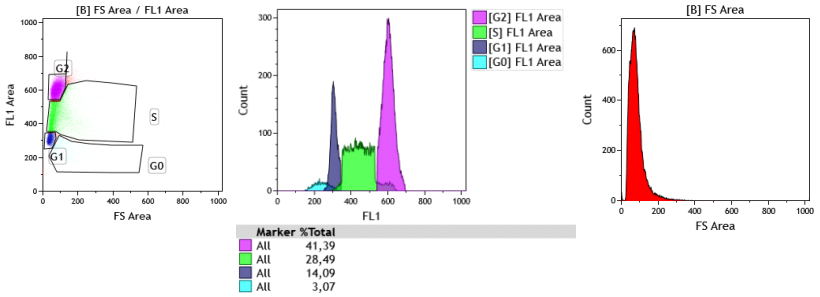
Ciclo cellulare di Saccharomyces Cerevisiae

Ecologia Applicata e Modelli Matematici di Sistemi Dinamici



SYTOX Green

Exponential



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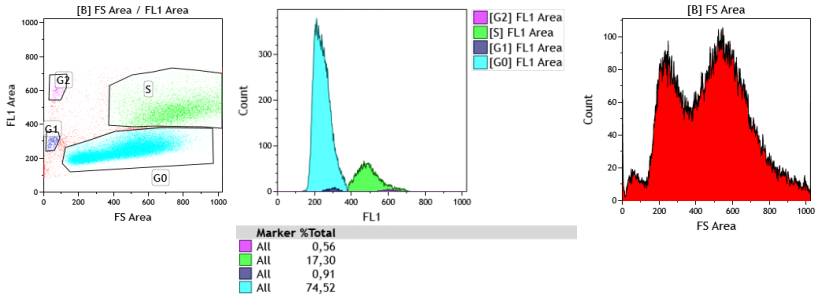

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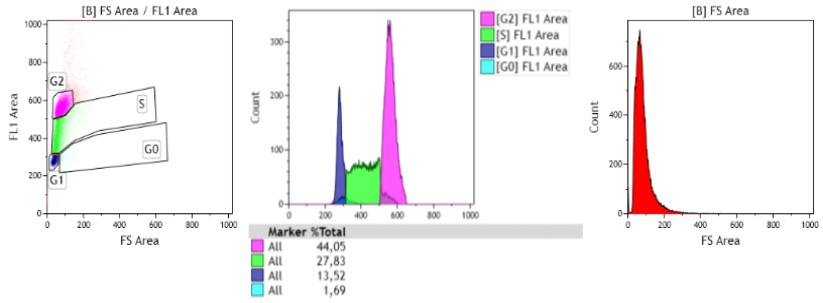

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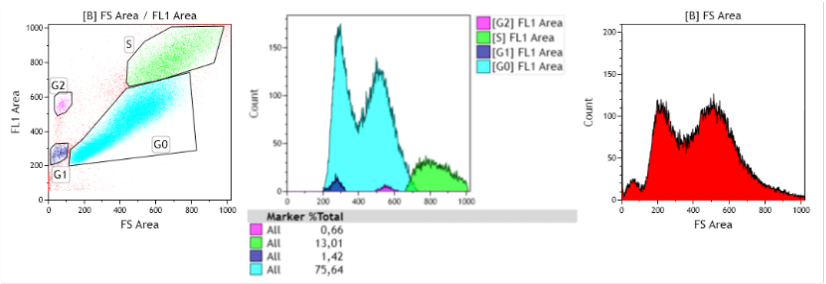

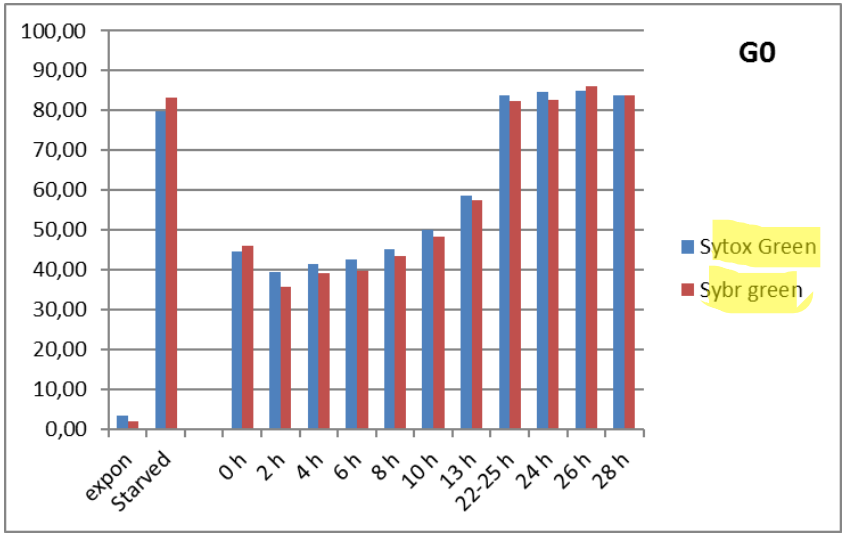
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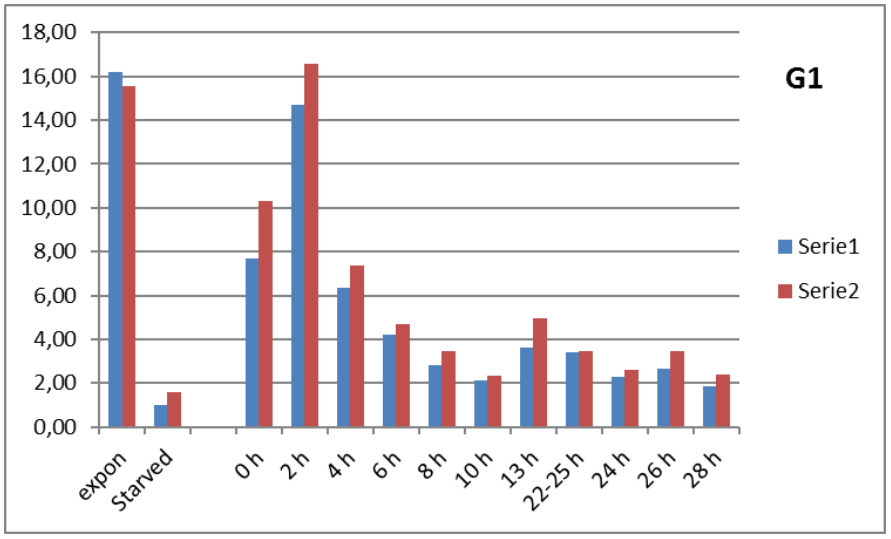
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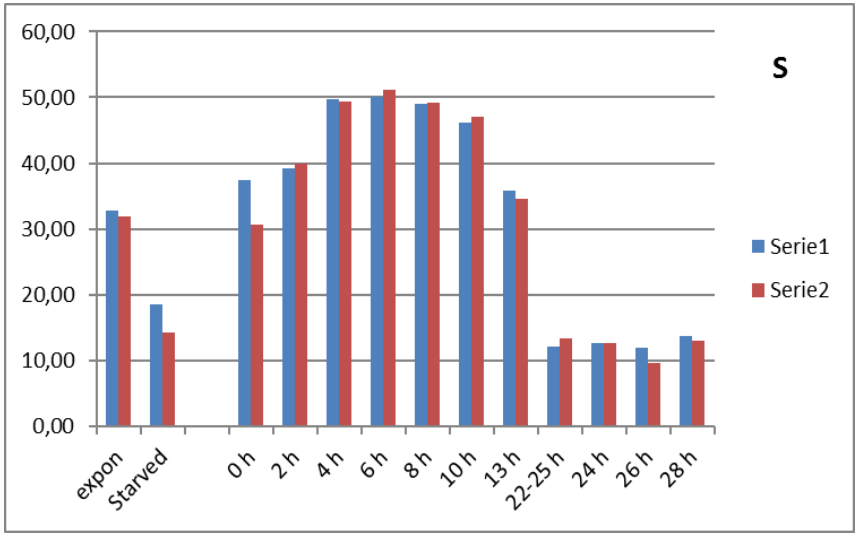
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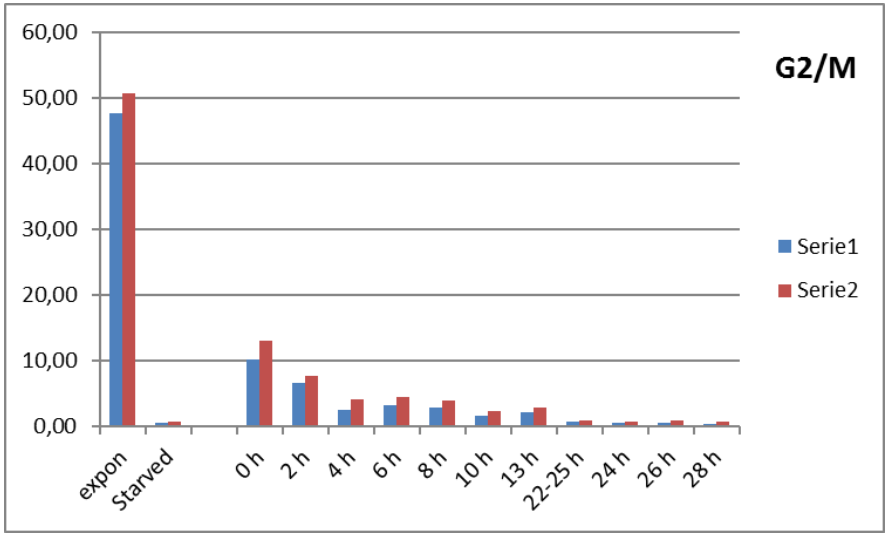
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PLOS ONE

A Simple FCM Method to Avoid Misinterpretation in *Saccharomyces cerevisiae* Cell Cycle Assessment between G0 and Sub-G1

Pierre Delobel[1,2,3], **Catherine Tesnière**[1,2,3]*

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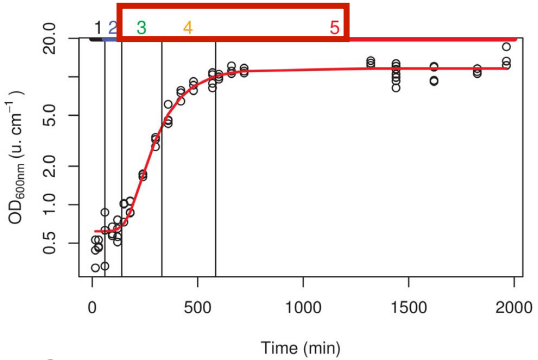
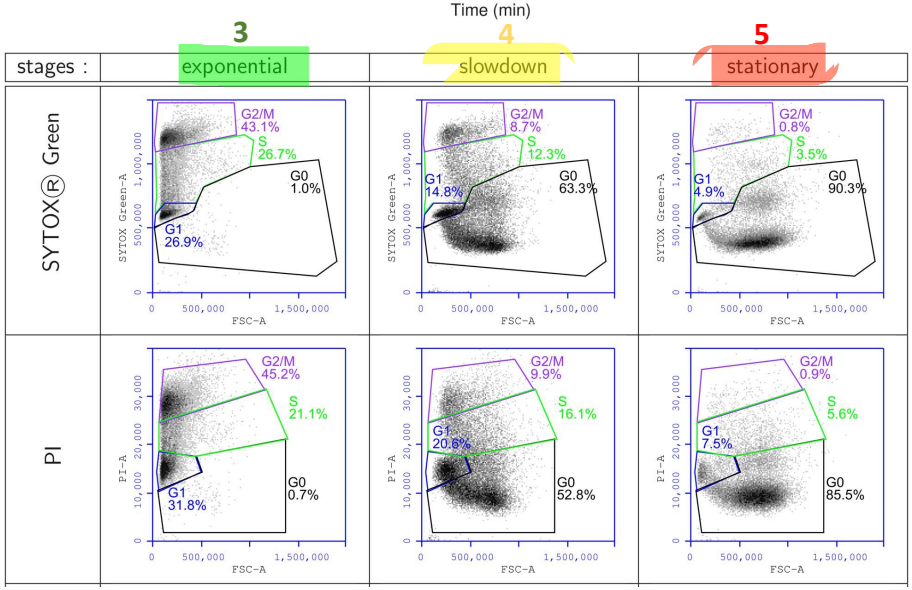
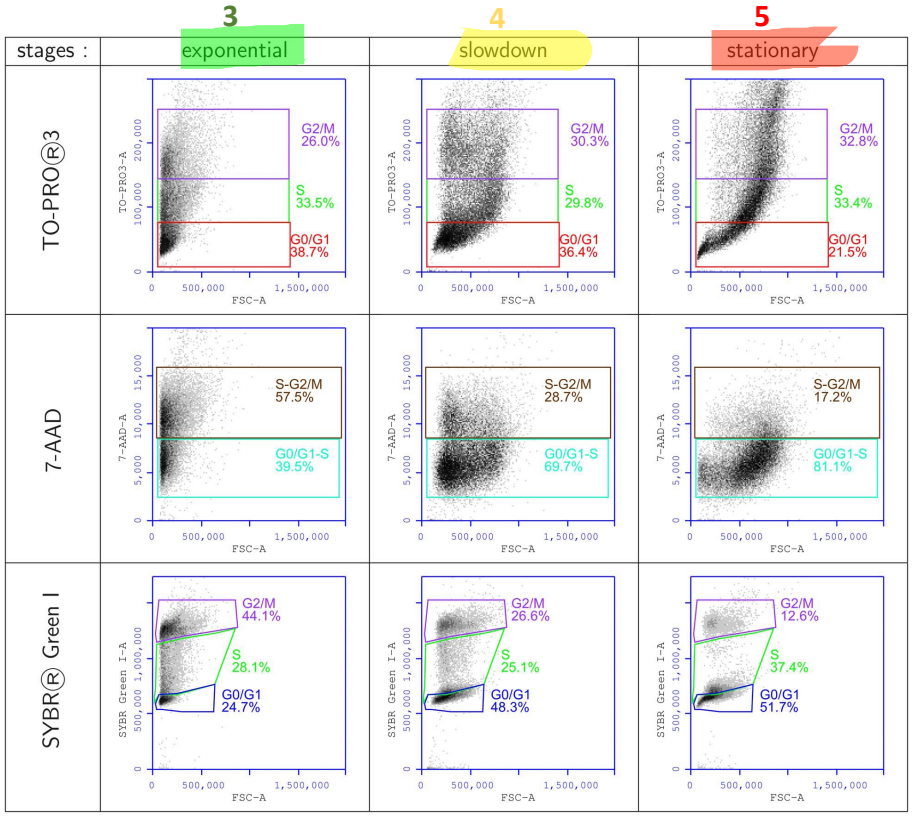






l’impiego della citofluorimetria in campi non strettamente biomedici
Possibili interessi comuni e risparmio economico

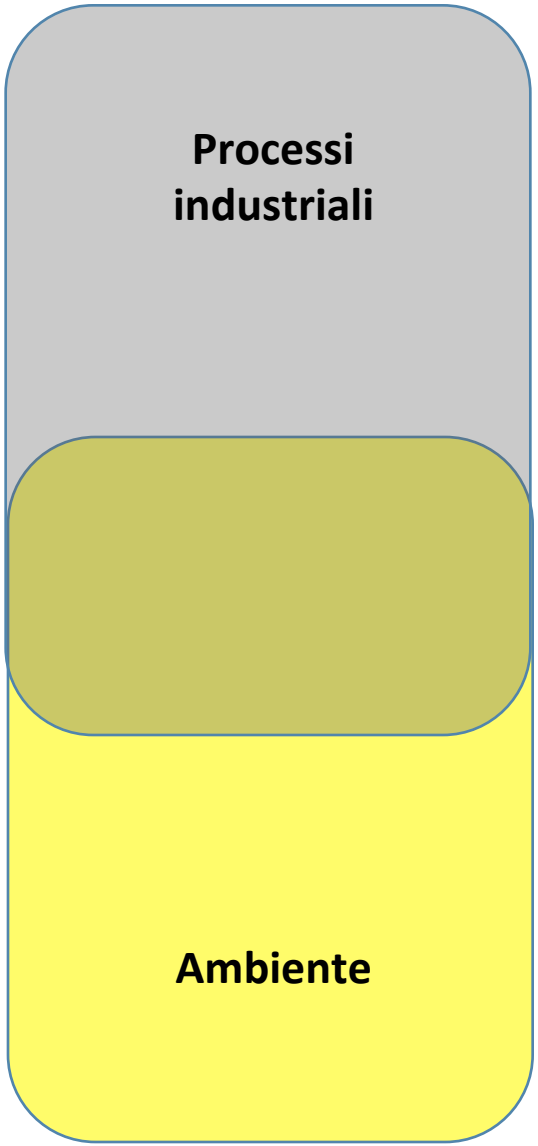


Table 5
Some FC applications to water and environmental microbiology.

Application	Microorganism	Functional or structural parameter	Reference
Characterization of freshwater and marine waters	*Bacterial cells*	DNA content, Membrane integrity and esterase activity	[16]
Cell functionality after peracetic acid treatments	*S. Typhimurium*	DNA	[43]
Cell enumeration in marine and ballast water samples	*Escherichia coli* and *Bacillus cereus*	Nucleic acid content	[92]
Characterization of activated sludge	*Comamonas testoteroni, Paracoccus pantotrophus, Escherichia coli*	Respiratory activity	[109]
Enumeration of viable cells in water after chlorination	*Legionella pneumophila*	Esterase activity	[120]
Cell survival in seawater during starvation	*Escherichia coli* and *S. Typhimurium*	Membrane integrity and potential	[134]
Effectiveness of disinfection treatments	*Escherichia coli, Salmonella Typhimurium, Shigella flexneri, Enterococcus faecalis*	Membrane integrity and potential, pump activity	[138,151]
Estimation of total bacteria in live and fixed samples from fresh and saline waters	*Salmonella typhimurium*	Nucleic acids	[252]
Cell characterization in tropical marine environmets	Bacterioplankton	DNA	[257]
Assessment of marine bacterial death	Natural bacterioplankton	Nucleic acids staining	[258]
Cell viability of in different types of water	*Aeromonas hydrophila*	DNA content and membrane integrity	[259]
Detection and viability assessment of water pathogens	*Giardia lamblia*	Fluorescent antibodies and membrane integrity	[260]
Chlorination treatment	*Nitrosospira* spp.	Membrane integrity and esterase activity	[262,263]
Evaluation of different environmental conditions and disinfection processes on VBNC cells in water	*Legionella pneumophila*	Esterase activity	[264]
Drinking water treatment processes	Heterotrophic bacteria	Nucleic acids detection	[266]
Determination of assimilable organic carbon in drinking water	*Pseudomonas fluorescens*	Nucleic acids	[267]
Assessment of water quality in papermaking	*Bacterial cells*	DNA content and membrane integrity	[268]

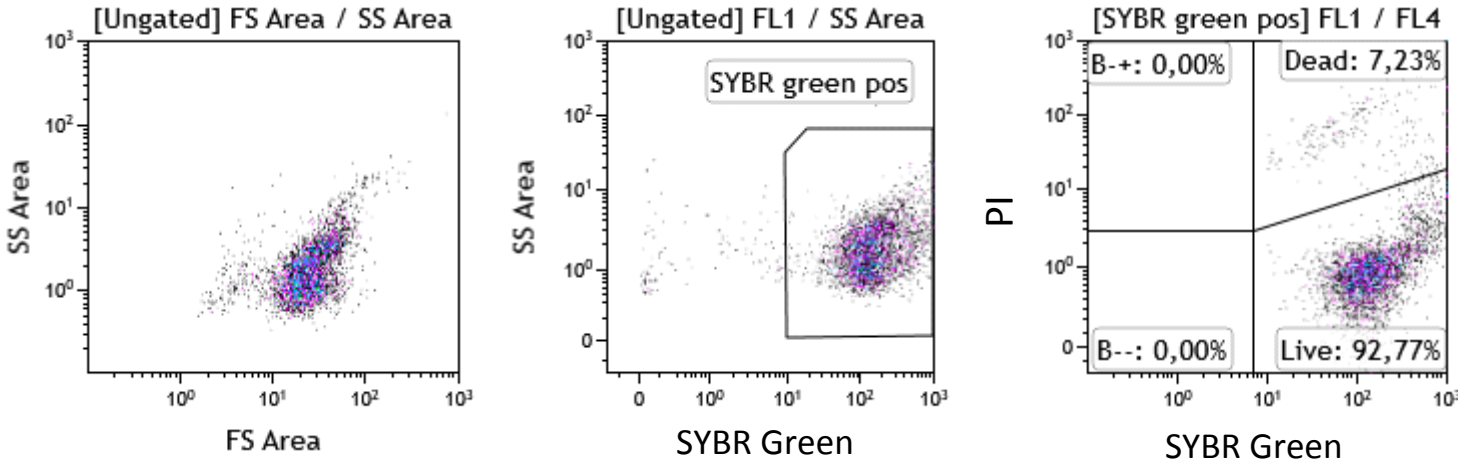
Validazione del citofluorimetro come strumento di controllo dei batteri contaminanti acque destinate al consumo umano

La Svizzera è il primo paese al mondo ad aver adottato questo metodo avanzato per la quantificazione delle cellule microbiche in acqua.

Source: ScienceDaily. Flow cytometry (FCM) can now be officially used for the quantification of microbial cells in drinking water. The new analytical method - developed at EAWAG (Swiss Federal Institute of Aquatic Science and Technology) and extensively tested both in Switzerland and abroad - has been incorporated into the Swiss Food Compendium (SLMB) by the Federal Office of Public Health (FOPH). FCM provides much more realistic results than the conventional method, in which bacterial colonies are grown on agar plates. The results demonstrate that even good-quality drinking water harbours 100 to 10,000 times more living cells than the conventional plate count method would suggest.

https://www.eawag.ch/en/consulting/consulting/competence-centre-for-drinking-water/projects/advances-in-the-microbiological-drinking-water-analysis/

Da colture di E. Coli



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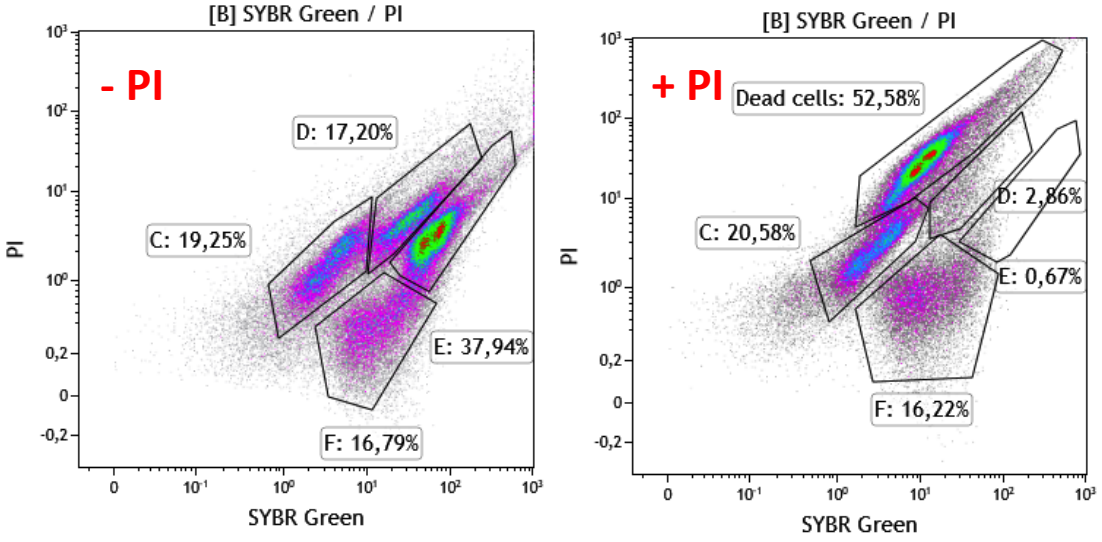

Microbiologia ambientale e controllo della qualità delle acque

- La proliferazione di alcuni cianobatteri in acque dolci, salmastre e marine costituisce un pericolo sia per la salvaguardia dell'ambiente che per la salute pubblica.

- Le Microcystis sp. Infatti sono in grado di rilasciare tossine microalgali (microcistine) durante la loro crescita e in seguito a morte cellulare.

- La citometria a flusso potrebbe diventare un valido strumento per studiare l'ecologia microbica della produzione di tossine Microcystis e contribuire ad un sistema di preallarme per le fioriture di Microcystis tossiche mediate rapidi controlli dei livelli di cianobatteri e delle relative tossine nelle acque.

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Multi-detection method for five common microalgal toxins based on the use of microspheres coupled to a flow-cytometry system

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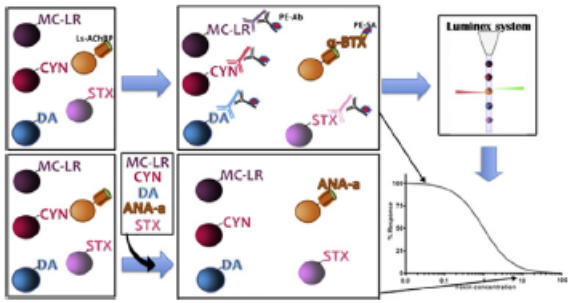
HIGHLIGHTS

- Multiplexed method for the detection of five microalgal toxin classes.
- Sensitive, easy-to-perform, rapid, semi-quantitative screening technique.
- Useful for the detection of freshwater toxins in cyanobacterial samples.

GRAPHICAL ABSTRACT



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Environmental Chemistry

MONITORING OF FRESHWATER TOXINS IN EUROPEAN ENVIRONMENTAL WATERS BY USING NOVEL MULTI-DETECTION METHODS

Ines Rodriguez,† Maria Fraga,† Amparo Alfonso,*† Delphine Guillebault,‡ Linda Medlin,‡§ Julia Baudart,§ Pauline Jacob,‖ Karim Helmi,‖ Thomas Meyer,# Ulrich Breitenbach,# Nicholas M. Holden,†† Bas Boots,†† Roberto Spurio,‡‡ Lucia Cimarelli,‡‡ Laura Mancini,§§ Stefania Marcheggiani,§§ Meric Albay,‖‖ Reyhan Akcaalan,‖‖ Latife Köker,‖‖ and Luis M. Botana*†

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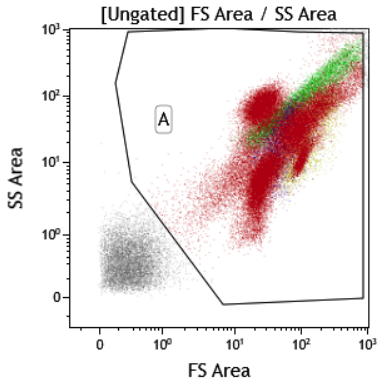
In questo lavoro è stata monitorata la presenza di microcistine, nodularina, acido domoico, cylindrospermopsin e diversi analoghi dell'anatossina-a (ATX-a).

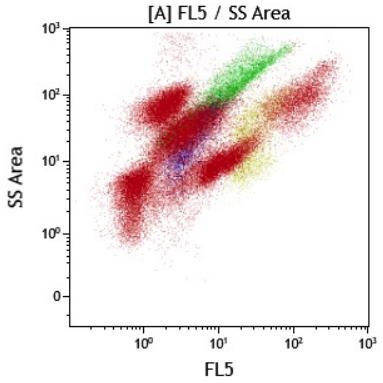
Comparazione tra Flow Cytometry e Ultraperformance Liquid Chromatography–tandem Mass Spectrometry, UPLC-MS/MS

La loro conclusione: …*the microsphere-based method as a semi-quantitative approximation to detect the toxins seems to require further refining of antibodies cross reactivity. This initial screening indicates presence or absence of toxins and reduces the number of samples to be analyzed by confirmatory analytical methods, which typically are more laborious. Therefore, the combination of methodologies described in the present study should reduce the number of samples and the time of analysis, especially since both methodologies are designed as multidetection assays.*

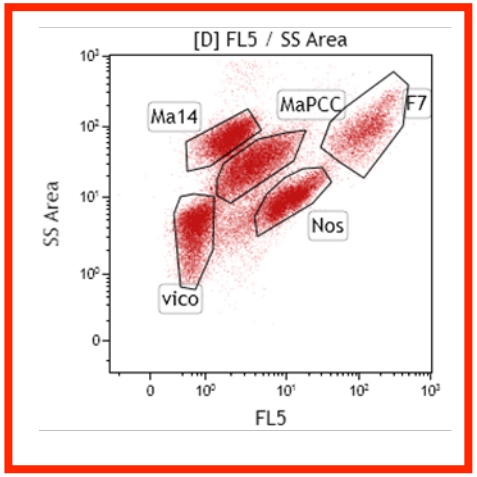
Profilo citofluorimetrico di popolazioni miste di cianobatteri realizzato sfruttando la fluorescenza naturale dei loro pigmenti

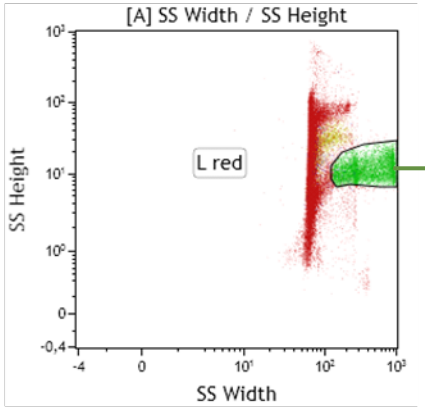
Merge dei campioni acquisiti

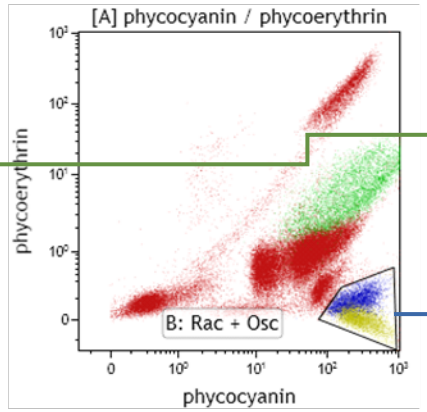


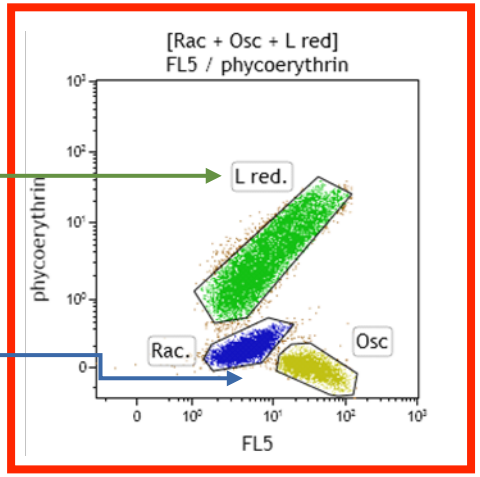


Ma14	**Microcystis Aeruginosa**
MaPCC	**Microcystis Aeruginosa**
Vico	**Synechococcus**
Nos	**Nostoc sp.**
F7	**Plaktothrix rubenscens**
L red	**Limnothrix**
Osc	**Oscillatoria**
Rac	**Cylindrospermopsis raciborskii**









Riassumendo…

Uno screening iniziale mediante Citofluorimetria può aiutare a individuare la presenza di cianobatteri e di tossine. Ciò porterebbe a una riduzione del numero di campioni da analizzare con metodi analitici di conferma, che risultano molto spesso più impegnativi, onerosi e *time-consuming*.

l'impiego della citofluorimetria in campi non strettamente biomedici
Possibili interessi comuni e risparmio economico

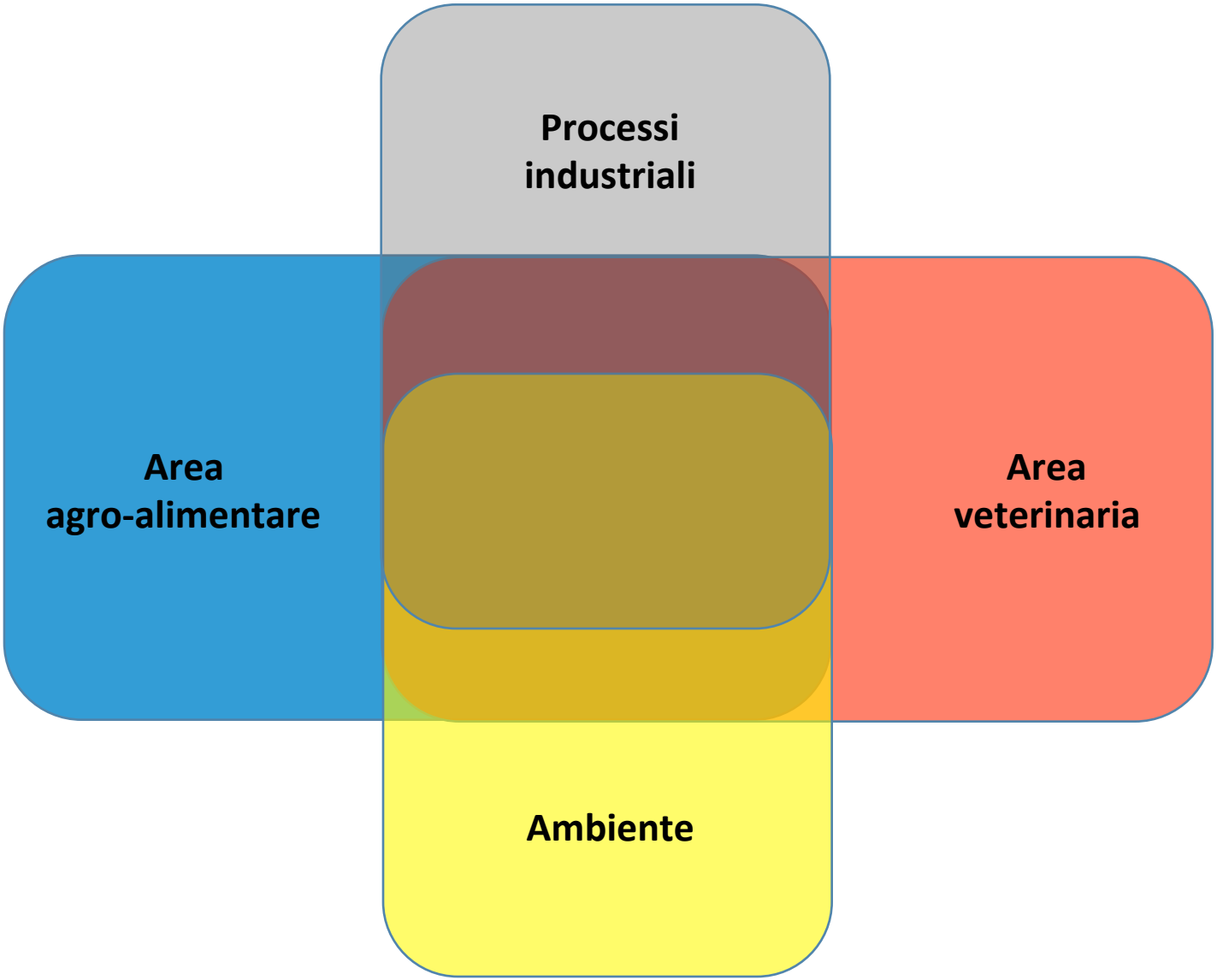


Table 3
Selected applications of FC technique to dairy industry.

Application	Microorganism	Functional or structural parameter	Reference
Evaluation of microbial and somatic cell load in milk	*Pseudomonas fluorescens*, *Escherichia coli*, *Staphylococcus aureus*	Membrane integrity and potential, DNA content, respiratory activity and specific fluorescent labelled oligonucleotides	[11,18,104]
Assessment of acid stress effect on cell physiology	*Streptococcus macedonicus*	Membrane integrity and potential, esterase activity, cell wall biosynthesis	[13,67]
Assessment of cell stress due to deconjugated bile salts	Probiotic bacteria *Bifidobacterium lactis* and *Bifidobacterium adolescentis*	Membrane potential, integrity and cell activity	[62]
Analysis of bacterial viability in dairy starters and probiotics	*Lactobacillus plantarum*	Membrane integrity and esterase activity	[141]
Viability evaluation after freezing and during storage	*Lactobacillus delbrueckii*	Esterase activity and membrane integrity	[209]
Assessment of different freezing methods	*Lactobacillus rhamnosus*	Esterase activity and membrane integrity	[210]
Monitoring cheese starter permeabilization and lysis	*Lactococcus lactis*	Membrane integrity	[216]
Enumeration of bacterial cells in fermented products	Probiotic bifidobacteria	Membrane integrity	[217]
Cell count in probiotics	*Lactobacillus rhamnosus Lactobacillus acidophilus*	Nucleic acids and esterase activity	[218]
Evaluation of cell response to osmotic stress	*Lactobacillus rhamnosus*	Esterase activity and membrane integrity	[219]
Enumeration of viable bacteria in milk powder and whey protein concentrate	Thermophilic bacteria	Esterase activity, DNA	[221,222]
Raw sheep milk quality evaluation	Bacteria	Bactoscan	[223]
Quantification and differentiation of bacteria in bulk tank milk	Pseudomonas, Bacillus, *Escherichia coli*, lactic acid bacteria	Specific labelled antigens FSC, SSC	[224]
Bacteriophages detection in dairy fermentation	*Lactococcus lactis*	Membrane integrity	[225]

La diffusione e della persistenza delle mastiti nelle mandrie da latte è causata soprattutto dalla condizione subclinica, una condizione in cui, sebbene la mammella e il latte appaiono normali, la ghiandola mammaria risulta infiammata, infetta o entrambe. Le mucche infette che mostrano un quadro subclinico costituiscono una riserva per i patogeni e la causa della diffusione della mastite negli animali sani

La mastite è causa di enormi perdite economiche all'industria lattiero-casearia. È indispensabile continuare a cercare nuovi biomarcatori che possano essere utili per rendere più efficiente e tempestiva la gestione delle mastiti nelle aziende lattiero-casearie.

The effective control of subclinical mastitis can clearly result in large economic profit for dairy farmers (van den Borne et al., 2010). However, the effectiveness of control is highly dependent on how fast these cows are detected, and hence the efficacy of the udder health monitoring program (van den Borne et al., 2010).

La determinazione rapida e simultanea della conta di cellule somatiche (SCC) e del loro conteggio differenziale (DSCC) in campioni di latte vaccino usando la citometria a flusso viene considerato un buon metodo per la valutazione predittiva di stadi infettivi preclinici, sebbene la complessità della mastite suggerisce ulteriori studi.

Ipotesi di sviluppo

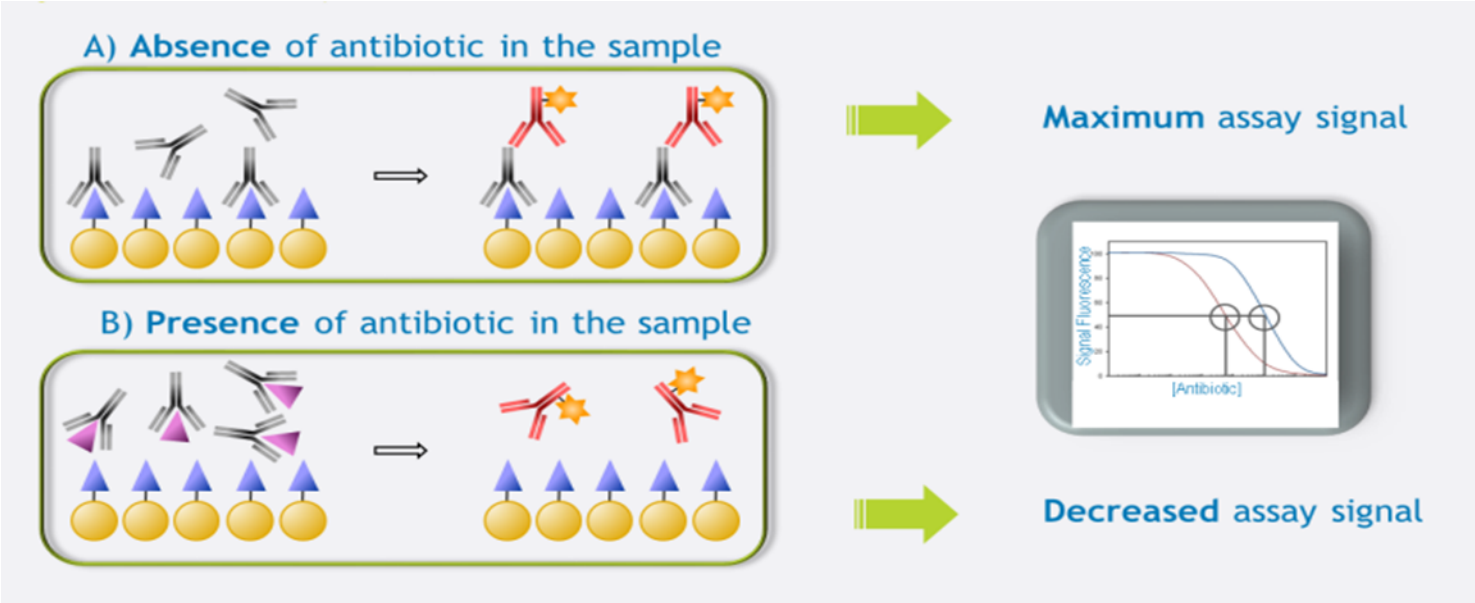
- Il citofluorimetro come strumento di controllo di alcuni parametri qualitativi lungo la filiera di produzione del latte e dei derivati caseari: dal foraggio destinato all'animale fino al consumatore

Filiera del latte: possibili applicazioni della citofluorimetria nei controlli intermedi per garantire la qualità del prodotto finale.

Controllo dell'uso di antibiotici

La resistenza agli antibiotici (ABR) è un fenomeno che desta particolare preoccupazione. L'OMS (Organizzazione Mondiale della Sanità) ritiene che il contenimento di questo fenomeno sia una priorità per la salute pubblica, tanto che nel 2015 ha lanciato un piano globale per contenere l'ABR. Un eccessivo uso di antibiotici può tradursi nello sviluppo di patogeni multiresistenti con conseguente perdita di efficacia terapeutica e gravi rischi per la salute pubblica

BEADYPLEX™ Assay



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.

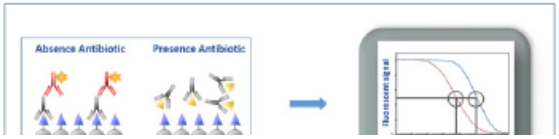
Upon reading with the NovoCyte®, the beads are clas
size and internal fluorescence (excited by 640 nm las
channel). The external assay signal for every bead
from the secondary binders in FITC channel by 488 nn

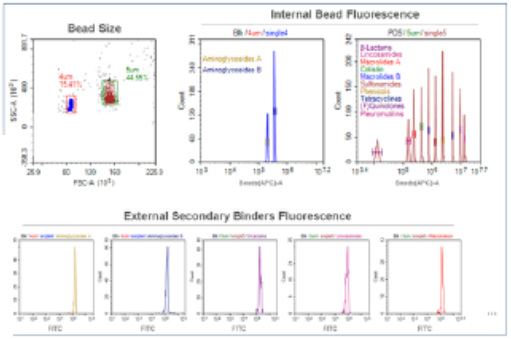
According to the competitive principle of the assay, th
in the sample is translated into the maximum fluo
whereas a decrease in fluorescence is observed upo
antibiotics for the associated bead.

Sample Extraction
1 g sample + 1 mL extraction buffer
(10 min) → centrifugation + filtration

1st Incubation
Sample extract + an
mix + primary antibo
30 min, RT, darknes

Flow Cytometry Immunoassay





Analysis Report

Test ID: 180710_Milk
Cytometer: NovoCyte 451160925722
BEADYPLEX Kit Lot ID: BYP Lot 170905 - Milk
Test Validation: VALID

Report Date: 10/15/2018
Software: BeadyExpress 1.2.8
Operator: administrator
Institution: N/A

Sample Name:	A1:ISS Blk, A2:ISS ...
Sample Type:	Blank
Assay Beads	Results
Tetracyclines	VALID
β-Lactams	VALID
Sulfonamides	VALID
(F)Quinolones	VALID
Aminoglycosides A	VALID
Aminoglycosides B	VALID
Colistin	VALID
Macrolides A	VALID
Macrolides B	VALID
Lincosamides	VALID
Pleuromutilins	VALID
Phenicols	VALID

Sample Name:	A3:ISS POS, A4:ISS...
Sample Type:	Positive Control
Assay Beads	Normalized Signal / Results
Tetracyclines	33 VALID
β-Lactams	9 VALID
Sulfonamides	16 VALID
(F)Quinolones	7 VALID
Aminoglycosides A	10 VALID
Aminoglycosides B	36 VALID
Colistin	18 VALID
Macrolides A	9 VALID
Macrolides B	5 VALID
Lincosamides	6 VALID
Pleuromutilins	7 VALID
Phenicols	54 VALID

Sample Name:	A5:ISS Sple4
Sample Type:	Unknown
Assay Beads	Normalized Signal / Results
Tetracyclines	103 NEG
β-Lactams	22 POS
Sulfonamides	111 NEG
(F)Quinolones	193 NEG
Aminoglycosides A	203 NEG
Aminoglycosides B	258 NEG
Colistin	135 NEG
Macrolides A	121 NEG
Macrolides B	167 NEG
Lincosamides	170 NEG
Pleuromutilins	298 NEG
Phenicols	298 NEG

Sample Name:	A6:ISS Sple4(1)
Sample Type:	Unknown
Assay Beads	Normalized Signal / Results
Tetracyclines	112 NEG
β-Lactams	20 POS
Sulfonamides	116 NEG
(F)Quinolones	178 NEG
Aminoglycosides A	206 NEG
Aminoglycosides B	256 NEG
Colistin	139 NEG
Macrolides A	113 NEG
Macrolides B	166 NEG
Lincosamides	164 NEG
Pleuromutilins	269 NEG
Phenicols	281 NEG

low Cytometry reading

Results

Most of the antibiotics included on the scope of BE
equal to or below the European regulatory limits. LOD

Family	Antibiotic	LOD (µg/kg)
Aminoglycosides	Streptomycin	250
	Dihydrostreptomycin	125
	Gentamicin	50
	Neomycin B	100
	Kanamycin A	100
	Paromomycin	250
β-Lactams	Cefquinome	50
	Ceftiofur	50
	Desfuroylceftiofur	2000
	Cefoperazone	3
	Cefalexin	3500
	Cefalonium	5
	Cefapirin	75
	Desacetylcefapirin	100
	Cefazolin	100
	Cefacetrile	50
	Penicillin V	12,5
	Penicillin G	5
	Ampicillin	25
	Amoxicillin	25
	Oxacillin	50
	Cloxacillin	50
	Dicloxacillin	30
	Nafcillin	300
	Piperacillin	5
Sulfonamides	Sulfadi	
	Sulfad	
	Sulfamet	
	Sulfadim	
	Sulfac	
	Sulfachlor	
	Sulfap	
	Sulfagu	
	Sulfat	
	Sulfametho	
	Sulfam	
	Sulfam	
	Sulfamono	
	Sulfaqu	
	Sulfa	
	Sulfam	
	Diap	
Macrolides	Tilmi	
	Tylo	
	Tylva	
	Tildip	
	Spira	
Lincosamides	Linco	
	Clinda	
Polymyxin	Col	

the software BeadyExpress™ upon plate reading with

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 TM kit è stato realizzato secondo le normative vigenti (le linee guida CRLs-COMMUNITY REFERENCE LABORATORIES RESIDUES**)**
per la convalida dei metodi di screening per i residui di medicinali veterinari (20/01/2010).

Results

The validation of this multi-class and multi-residue screening in meat, fish and milk using BEADYPLEX™ kit has been realized according to the CRLs Guidelines for the validation of screening methods for residues of veterinary medicines (20/01/2010); supplement to Commission Decision 2002/657/EC. A principal validation was performed in porcine meat and milk including the establishments of thresholds/cut-offs, check of specificity/selectivity, determination of detection capabilities (CCβ), robustness and applicability. For poultry and bovine meat as well as for high-fat and low-fat fish matrices (salmon and coley, respectively); a secondary validation was realized for the determination of CCβ in these matrices.
The table below summarizes the CCβ values obtained for the antibiotics detected in the mentioned matrices; for most compounds, they are at or below the corresponding European MRL value established under the Commission Regulation EU N°37/2010 supported by the Commission implementing regulation EU N°470/2018.

Sono stati determinati i valori soglia, la specificità/selettività, la capacità di rilevazione (CCβ), la robustezza e l'applicabilità.

I valori dei CCβ per la maggior parte dei composti, sono pari o inferiori ai corrispondenti valori MRL stabiliti dalla Commissione UE n. 37/2010, sostenuto dal regolamento di esecuzione della Commissione UE n. 470/2018..

Detection Capabilities (µg/kg) for bead-based FCIA method in porcine / bovine / poultry muscle + fat / lean fish + cow milk (non-exhaustive list)

		Porcine muscle		Bovine muscle		Poultry muscle		Fish		Milk	
Family	Antibiotic	MRL	CCβ	MRL	CCβ	MRL	CCβ	MRL	CCβ	MRL	CCβ
Aminoglycosides	Streptomycin	500	250	500	250	500	250	500	250	200	100
	Dihydrostreptomycin	500	125	500	125	500	125	500	125	200	50
	Gentamicin	50	50	50	50	50	50	50	50	100	100
	Neomycin B	500	100	500	100	500	100	500	100	1500	25
	Kanamycin A	100	100	100	100	100	100	100	100	150	75
	Paromomycin	500	250	500	500	500	500	500	500	*	250
β-Lactams	Cefquinome	50	50	50	50	50	50	50	50	20	20
	Ceftiofur	1000	50	1000	50	1000	50	1000	50	100	25
	Desfuroylceftiofur		2000		2000		2000		2000		50
	Cefoperazone	50	3	50	3	50	3	50	3	50	1
	Cefalexin	200	3500	200	3500	200	3500	200	3500	100	1600
	Cefalonium	20	5	20	5	20	5	20	5	20	2.5
	Cefspirin	50	75	50	50	50	50	50	50	60	5
	Desacetylcefspirin		100		100		100		100		30
	Cefazolin	50	100	50	100	50	100	50	100	50	20
	Cefacetrile	125	50	125	50	125	50	125	50	125	50
	Penicillin V	25	12.5	25	12.5	25	12.5	25	12.5	25	4
	Penicillin G	50	5	50	5	50	5	50	5	4	4
	Ampicillin	50	25	50	25	50	25	50	25	4	4
	Amoxicillin	50	25	50	25	50	25	50	25	4	4
	Oxacillin	300	50	300	50	300	50	300	50	30	10
	Cloxacillin	300	50	300	50	300	50	300	50	30	6
	Dicloxacillin	300	30	300	30	300	30	300	30	30	12
	Nafcillin	300	300	300	300	300	300	300	300	30	75
	Piperacillin	-	5	-	5	-	5	-	5	-	5

		Porcine muscle		Bovine muscle		Poultry muscle		Fish		Milk	
Family	Antibiotic	MRL	CCβ	MRL	CCβ	MRL	CCβ	MRL	CCβ	MRL	CCβ
Sulfonamides	Sulfadimidine		100		100		100		100		50
	Sulfadiazine		50		50		50		50		50
	Sulfamethoxazole		10		10		10		10		5
	Sulfadimethoxine		50		50		50		50		20
	Sulfadoxine		10		10		10		10		10
	Sulfachloropyridazine		1		1		1		1		1
	Sulfapyridine		0,5		0,5		0.5		0,5		0.5
	Sulfaguanidine		1		1		1		1		0.5
	Sulfathiazole	Sum sulfo = 100	0.5	Sum sulfo = 100	0.5	Sum sulfo = 100	0,5	Sum sulfo = 100	0.5	Sum sulfo = 100	0.25
	Sulfamethoxypyridazine		0.25		0.25		0.25		0.25		0.125
	Sulfamerazine		20		20		20		20		20
	Sulfaclozine		500		500		1000		500		150
	Sulfamethizole		1		1		1		1		1
	Sulfamonomethoxine		10		20		10		20		10
	Sulfaquinoxaline		50		50		50		50		25
	Sulfameter		5		5		5		5		2.5
	Sulfanilamide		50		50		50		50		20
	Dapsone	Forbidden	0.2	Forbidden	0.2	Forbidden	0.2	Forbidden	0.2	5	0.1
Macrolides	Tilmicosin	50	50	50	50	75	50	50	50	50	12.5
	Tylosin A	100	20	100	20	100	20	100	20	50	10
	Tylvalosin	50	125	50	125	50	125	50	125	200	20
	Tildipirosin	1200	1200	400	1200	400	1200	400	1200	*	500
	Spiramycin	250	250	200	200	200	200	200	200	250	200
Lincosa	Lincomycin	100	25	100	25	100	25	100	25	150	20
	Clindamycin	-	150		500	-	300	-	500	-	100
	Colistin	150	150	150	150	150	150	150	150	50	25

		Porcine muscle		Bovine muscle		Poultry muscle		Fish		Milk	
Family	Antibiotic	MRL	CCβ	MRL	CCβ	MRL	CCβ	MRL	CCβ	MRL	CCβ
Tetracyclines	Chlortetracycline	100	25	100	100	100	100	100	100	100	25
	Doxycycline	100	25	100	25	100	25	100	25	*	12.5
	Oxytetracycline	100	50	100	100	100	100	100	100	100	50
	Tetracycline	100	50	100	50	100	50	100	50	100	50
	Demeclocycline	-	50	-	100	-	100	-	100	-	50
	Methacycline	-	25	-	50	-	50	-	50	-	25
(Fluoro)Quinolones	Marbofloxacin	150	20	150	20	150	20	150	20	75	2
	Flumequine	200	800	200	2000	400	2000	600	2000	50	50
	Enrofloxacin	100	5	100	5	100	5	100	5	100	1
	Ciprofloxacin		10		20		20		20		2
	Danofloxacin	100	250	200	200	200	200	100	200	30	30
	Oxolinic acid	100	800	100	1000	100	1000	100	1000	*	62.5
	Difloxacin	400	50	400	50	300	50	300	50	*	25
	Norfloxacin	-	2.5	-	10	-	10	-	10	-	2
	Sarafloxacin	30	125	30	125	30	125	30	125	10	5
	Pefloxacin	-	10	-	20	-	20	-	20	-	2
	Enoxacin	-	50	-	50	-	50	-	50	-	5
	Lomefloxacin	-	15	-	15	-	15	-	15	-	3
	Ofloxacin	-	15	-	15	-	15	-	15	-	1
	Cinoxacin	-	800	-	800	-	800	-	800 (lean) 900 (fat)	-	50
	Nalidixic acid	-	2000	-	2000	-	2000	-	2000	-	50
Phenicols	Chloramphenicol	0.3	0.45	0.3	0.45	0.3	0.45	0.3	0.45	0.3	0.3
	Florfenicol	300	2100	200	2100	100	2100	1000	2100	*	1600
	Thiamphenicol	50	100	50	100	50	100	50	100	50	100
Pleuro	Valnemulin	50	50	50	50	50	50	50	50	50	10
	Tiamulin	100	25	100	25	100	25	100	100	1000	12.5

*Not for use in animals from which milk are produced for human consumption

Validation of Beadyplex - Summary

Screening of 74 antibiotics (test report) from 10 antibiotic groups:

TETRACYCLINES, ß-LACTAMS, SULFONAMIDES (incl. Dapsone), QUINOLONES, AMINOGLYCOSIDES, POLYMYXINE (Colistin), MACROLIDES, LINCOSAMIDES, PLEUROMUTILINS, AMPHENICOLES

Sulfaclozin, Cefalexin, Florfenicol were excluded, because CCß >> MRL

Tylvalosin, Tildipirosin (cattle, goat), Thiamphenicol, Chloramphenicol: CCß > MRL/RPA

70 antibiotics (incl. Tildipirosin for pig): CCß ≤ MRL/RPA

4-epi-Oxytetracycline is sensitive: CCß = 25 µg/kg, 4-epi-Chlortetracycline and 4-epi-Tetracycline are insensitive, but Chlortetracycline and Tetracycline CCß = 25 µg/kg; more than 50 % 4-epimer is unlikely => screening below MRL = 100 µg/kg should be no problem (see analyses of reference materials)

Le micotossine

Le vacche da latte sembrano essere protette dall'esposizione alle micotossine dalla loro flora del rumine.

Tuttavia, diverse micotossine superano questa barriera o vengono convertite in metaboliti che conservano l'attività biologica.

Tra gli effetti indesiderati delle micotossine esercitati nei ruminanti è presente un'attività antimicrobica, il cui esito è una compromissione della funzione della flora del rumine che causa:

- **uno scarso utilizzo dei mangimi**
- **un ridotto aumento di peso**
- **una riduzione della produttività**

Sebbene le aflatossine (specialmente l'aflatossina M1) siano micotossine di maggiore incidenza nel latte e nei prodotti caseari, in questi prodotti si possono trovare altre micotossine, come fumonisina, ocratossina A, tricoteceni, zearalenone, tossina T-2 e deossinivalenolo

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Table 2–Occurrence of AFM_1 contamination in bovine milk.

Country	Sample	Frequency (%)	Min–Max (μg/L)	Reference
Serbia	Pasteurized milk	(35/36) 97.2	0.06–1.2	Kos and others (2014)
	UHT milk	(69/70) 98.5	0.02–0.41	
	Organic milk	(6/6) 100	0.01–0.08	
	Raw milk	(40/40) 100	0.08–1.2	
	Raw milk	(382/678) 56,3	0.282–0.358	Tomasevic and others (2015)
	Heat treated milk	(143/438) 32,6	0.09–0.145	
Lebanon	Raw milk	(28/38) 73.6	0.0026– 0.126	Assem and others (2011)
	Pasteurized milk	(17/25) 68.0	0.0033–0.084	
	Powder milk	(5/14) 35.7	0.0092–0.016	
Spain	UHT milk	(68/72) 94.4	0.002–0.014	Cano-Sancho and others (2010)
Brazil	Pasteurized milk	(26/30) 87	0.009–0.437	
	UHT milk	(13/17) 76	0.008–0.215	
	Milk with additives	(13/17) 76	0.009–0.061	Iha and others (2013)
	Powder milk	(12/12) 100	0.02–0.76	
	Infant formula	(0/7) 0	–	
	Fluid milk	(26/65) 40	0.009–0.069	Jager and others (2013)
	Powder milk	(2/4) 50	0.5–0.81	
	UHT milk	(23/75) 30.7	1.0–4.1	Oliveira and others (2013)
	UHT milk	(133/152) 87.5	0.002–0.121	Silva and others (2015)
South corea	Raw milk	(48/100) 48	0.002–0.08	Lee and others (2009)
Portugal	UHT and pasteurized milk	(11/40) 27.5	0.007–0.07	Duarte and others (2013)
Italy	Infant formula	(2/185) 1,1	0.012–0.015	Meucci and others (2010)
Thailand	Raw milk	(240/240) 100	0.05–0.101	Ruangwises and Ruangwises (2010)
Turkey	Fluid milk	(43/50) 86	0.001–0.030	Ertas and others (2011)
	UHT milk	(75/129) 58.1	0–0.544	Unusan (2006)
	UHT milk	(67/100) 67	0.01–0.63	Tekinsen and Eken (2008)
Morocco	Pasteurized milk	(54/61) 88.8	0.001–0.117	Zinedine and others (2007a)
Índia	Infant formula	(17/18) 94	0.143–0.77	Rastogi and others (2004)
Taiwan	Pasteurized milk	(44/48) 90.9	0.002–0.083	Rastogi and others (2004)
Iran	Raw milk	(85/111) 76.6	0.015–0.28	Kamkar (2006)
	Pasteurized milk	(83/116) 71.5	0.006–0.528	Fallah (2010)
	UHT milk	(68/109) 62.3	0.006–0.516	
China	Raw milk	(12/12) 100	0.16–0.5	Pei and others (2009)
	Raw milk	(45/100) 32.5	0.005–0.06	Han and others (2013)
	UHT milk	(84/153) 54.9	0.006–0.16	Zheng and others (2013)
	Pasteurized milk	(25/26) 96.2	0.023–0.154	
Sudan	Raw milk	(42/44) 95.5	0.22–6.9	Elzupir and Elhussein (2010)
Pakistan	Raw milk	(76/107) 71	0.004–0.845	Iqbal and Asi (2013)
Croatia	Raw milk	(72/1543) 2	0.006–0.027	Bilandzic and others (2015)

Table 3–Occurrence of AFM_1 contamination in dairy products.

Country	Sample	Frequency (%)	Min–Max (μg/kg)	Reference
Kuwait	White cheese	(32/40) 80	0.024–0.452	Dashti and others (2009)
Turkey	Cheese	(68/72) 94.4	0.012–0.378	
	Yoghurt	(28/50) 56	0.0025–0.078	Ertas and others (2011)
	Dairy dessert	(26/50) 52	0.0015–0.08	
	Butter	(92/92) 100	0.01–7.0	Tekinsen and Uçar (2008)
	Cream cheese	(99/100) 99	0–4.1	
	Yoghurt	(70/80) 87.5	0.01–0.475	Atasever and others (2011)
Iran	White cheese	(93/116) 80.1	0.052–0.745	Fallah and others (2009)
	Cream cheese	(68/94) 72.3	0.058–0.785	
	Lighvan cheese	(49/75) 65.3	0.03–0.313	Fallah and others (2011)
	Cheese	(47/88) 53.4	0.082–1.254	Rahimi and others (2009)
	White cheese	(30/50) 60	0.041–0.374	Tavakoli and others (2012)
	Feta cheese	(66/80) 82.5	0.15–2.41	Kamkar (2006)
Libya	Cheese	(15/20) 75	0.11–0.52	Elgerbi and others (2004)
Brazil	Cheese	(3/10) 30	0.091–0.3	Jager and others (2013)
	Yoghurt	–	–	
Greece	Feta cheese	(0/54) 0	–	Kaniou-Grigoriadou and others (2005)
Pakistan	White cheese	(93/119) 78	0.004–0.595	
	Cream cheese	(89/150) 59	0.004–0.456	Iqbal and Asi (2013)
	Butter	(33/74) 45	0.004–0.413	
	Yoghurt	(59/96) 61	0.004–0.616	
Serbia	Milk products	(122/322) 37,8	0.268–0.952	Tomasevic and others (2015)

L'ampia presenza di diversi tipi di micotossine non solo nel latte ma anche in altri prodotti lattiero-caseari, nonché la preoccupazione per la loro tossicità animale e umana, rendono necessaria l'adozione di misure adeguate per ridurre al minimo la contaminazione degli alimenti da tali micotossine.

Czeh et al. hanno introdotto un immunodosaggio competitivo fluorescente con microsfere basato sulla citometria a flusso (CFIA) per la determinazione simultanea di sei micotossine dallo stesso campione dopo una singola fase di estrazione. La CFIA potrebbe diventare una tecnica di routine robusta con l'obiettivo principale di consentire alle autorità di procedere a interventi rapidi in circostanze critiche

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Research paper

A flow cytometry based competitive fluorescent microsphere immunoassay (CFIA) system for detecting up to six mycotoxins

Arpad Czeh [a,b,*], Frank Mandy [a], Szilvia Feher-Toth [a,b], Livia Torok [a], Zoltan Mike [a], Balazs Koszegi [a], Gyorgy Lustyik [a,b]

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ORIGINAL ARTICLE

Cytometry PART A Journal of the International Society for Advancement of Cytometry

Cytometry Part A - 83A: 1073-1084, 2013

Comparison and Evaluation of Seven Different Bench-Top Flow Cytometers with a Modified Six-Plexed Mycotoxin Kit

Arpad Czeh,[1,2*] Abe Schwartz,[3] Frank Mandy,[2] Zsuzsanna Szoke,[2] Balazs Koszegi,[2] Szilvia Feher-Toth,[1,2] Gyorgy Nagyeri,[2] Pal Jakso,[4] Robert L. Katona,[5] Agnes Kemeny,[6] Gabor Woth,[7] Gyorgy Lustyik[1,2]

Table 1
Summary of major fungi species, and corresponding mycotoxins including impact on humans and domestic animals.

Mycotoxins	Fungal species	Deleterious effects
Aflatoxins (B_1,B_2,G_1,G_2 and M_1)	*A. flavus*	Acut toxicity: LD_{50} values, 1.0–17.9 mg/kg BW (laboratory animals),
	A. parasiticus	0.5 mg/kg BW (ducklings); hepatic lesions; teratogenic.
	A. nomius	Reduced feed efficiency, immune function and reproductive performance in ruminants.
		Carcinogenic in humans. Hepatotoxic and carcinogenic
Ochratoxin-A	*P. ochraceus*	Teratogenic, carcinogenic, decreased foetal weight, immunosuppressive,
	A. alliaceus	strong inhibition of protein synthesis, nephrotoxicity, hepatotoxicity, strong acute toxicity
	P. verrucosum	
	P. nordicum	
Fumonisins	*F. moniliforme*	Hepatic lesions in pigs and cattle, Equine leukoencephalomalacia.
	F. proliferatum	Porcine pulmonary oedema. Implicated in oesophageal cancer in humans,
	F. subglutinans	hepato- and nephrotoxicity
Zearalenone	*F. culmorum*	Infertility, reduced milk production and hyperoestrogenism in cows.
	F. graminearum	
	F. sporotrichioides	
Deoxynivalenol (Vomitoxin)	*F. culmorum*	Feed refusal, decreased weight gain and vomiting, teratogenic
	F. gmminearum	
	F. sporotrichioides	
	F. poae	
	F. acuminatum	
T-2 toxin	*F. sporotrichioides*	Feed refusal, nervous system disturbances, diarrhoea,
	F. poae	decreased milk production, acute toxicity, inhibition
		of protein synthesis, immunosuppressive

Text:
Table 1 identifies the six major fungi species and mycotoxins they produce. Some of the major deleterious health effects for both humans and animals are included (Barna-Vetro, 2002; Varga et al., 1996).

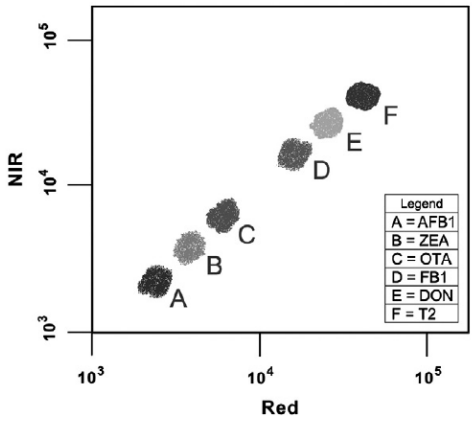


Fig. 1. Two dimensional dot plot display on the FACSArray™ for six mycotoxins. Text: Based on dual fluorescent scatter ploting, the 6 distinct microsphere populations are easily discriminated from each other.

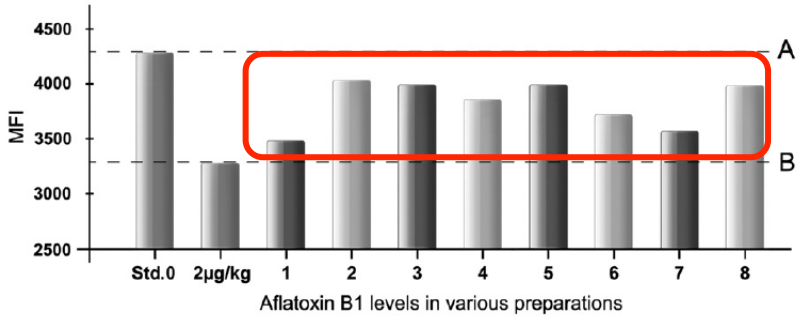
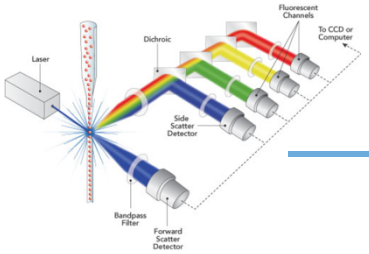


Fig. 2. Histograms for aflatoxin B1 levels with 8 different toxin free grain types. Legends. 1 = sunflower 5 = white lupine. 2 = wheat 6 = spring barley. 3 = pea 7 = chickpea. 4 = mung bean 8 = rye and pea feed mixture. Text: In this histogram the first column illustrates the toxin free standard, next the maximum permitted toxin level for EU, following with 8 varieties of grain illustrating the naturally existing levels that are all well below zero standard.

Riassumendo…..


Controllo micotossine
nel foraggio e nel latte

citofluorimetro



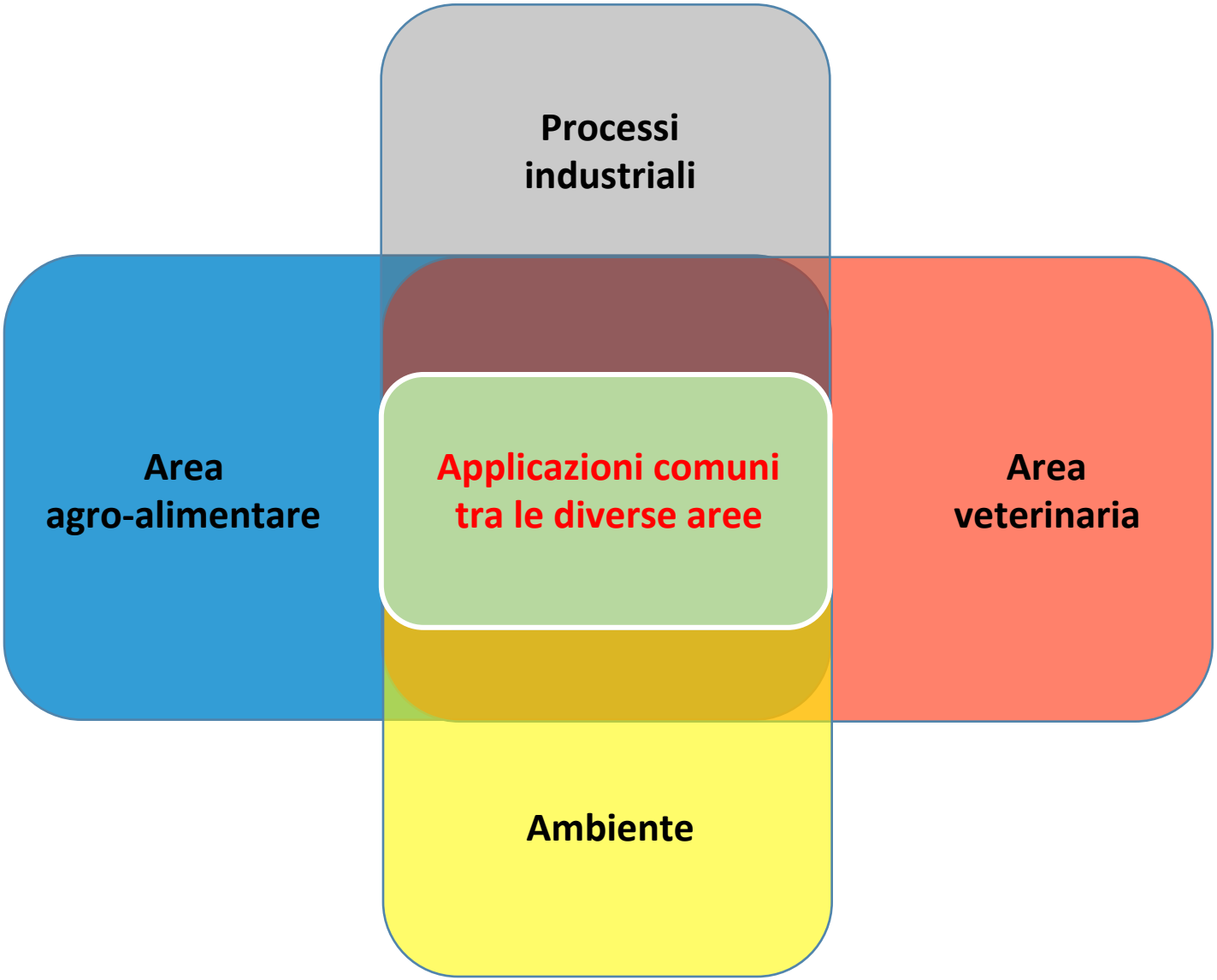
Controllo batteri e cellule
somatiche nel latte
Sviluppo nuovi marker predittivi

Controllo antibiotici
negli animali e nel latte


Fogli di calcolo da
diverse acquisizioni

Sviluppo di software
per l'analisi differenziata

L’impiego della citofluorimetria in campi non strettamente biomedici
Possibili interessi comuni e risparmio economico



Possibili procedure comuni per analisi citofluorimetrica in settori diversi:

Analisi carica batterica:	**Agroalimentare**	**controllo probiotici**
	Ambiente	**controllo batteri patogeni e cianobatteri**
	Processi industriali	**controllo starter**
	Veterinaria	**controllo batteri nel latte**
Analisi residui antibiotici:	**Agroalimentare**	**controllo in diverse matrici alimentari**
	Ambiente	**controllo nei bacini di allevamenti ittici**
	Veterinaria	**controllo in animali destinati al consumo e nei prodotti da loro derivati**
Micotossine:	**Agroalimentare**	**controllo in cereali destinati al consumo**
	Veterinaria	**controllo nel foraggio per animali destinati al consumo e nei prodotti alimentari da loro derivati.**

Suggerimenti

Riconoscimento

- **Contribuire a sviluppare nuovi sistemi di analisi basati sulla citofluorimetria e proporre relativi percorsi di convalida**

Semplificazione

- **La realizzazione di sistemi basati sull'uso di un unico strumento, in grado di rispondere rapidamente e con affidabilità a istanze analitiche di differenti problemi biologici relativi a prodotti destinati al consumo**

Integrazione

- **Questo impianto analitico potrebbe inoltre essere ulteriormente migliorato con l'introduzione di un sistema di tracciabilità dei prodotti**

Tali obiettivi dovrebbero essere realizzati in accordo e in stretta collaborazione fra settore imprenditoriale e ricerca, sfruttando i benefici della ricerca e dell'innovazione.

E dovrebbero:

- **portare a una maggiore garanzia per la tutela ambientale, la salute animale e sanità pubblica, rispettando inoltre le esigenze dei consumatori;**
- **e sostenere i consumi promuovendo l'immagine e la qualità dei prodotti alimentari.**