XLIX CONGRESSO NAZIONALE AMCLI

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Zoonoses and flow cytometric applications

Sessione 2 AMCLI-ISCCA
Applicazioni Citometriche in ambito Microbiologico

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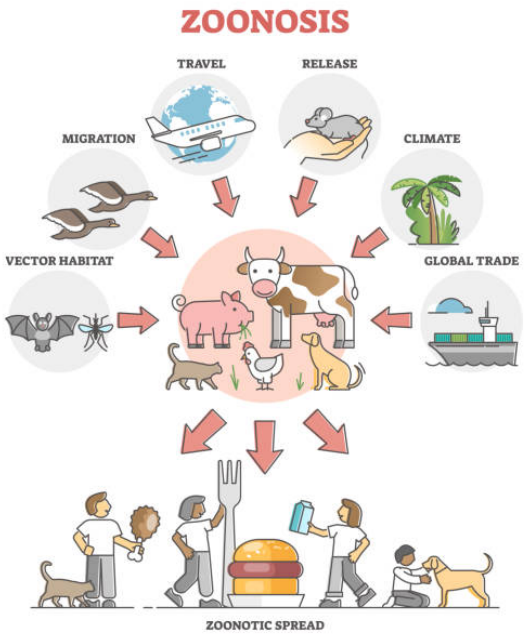
Zoonoses

Zoonoses are diseases that can be transmitted from animals to humans.

About 75% of the emerging human diseases recognized in recent decades have a zoonotic origin.

The agents responsible for zoonoses can be bacteria, viruses, parasites or prions.



Zoonoses can be transmitted through:

- consumption of contaminated foods (salmonellosis, listeriosis);
- vectors (Lyme disease, West Nile fever);
- direct or indirect contact (rabies, avian flu).

One Health 2020 - Zoonoses Directive

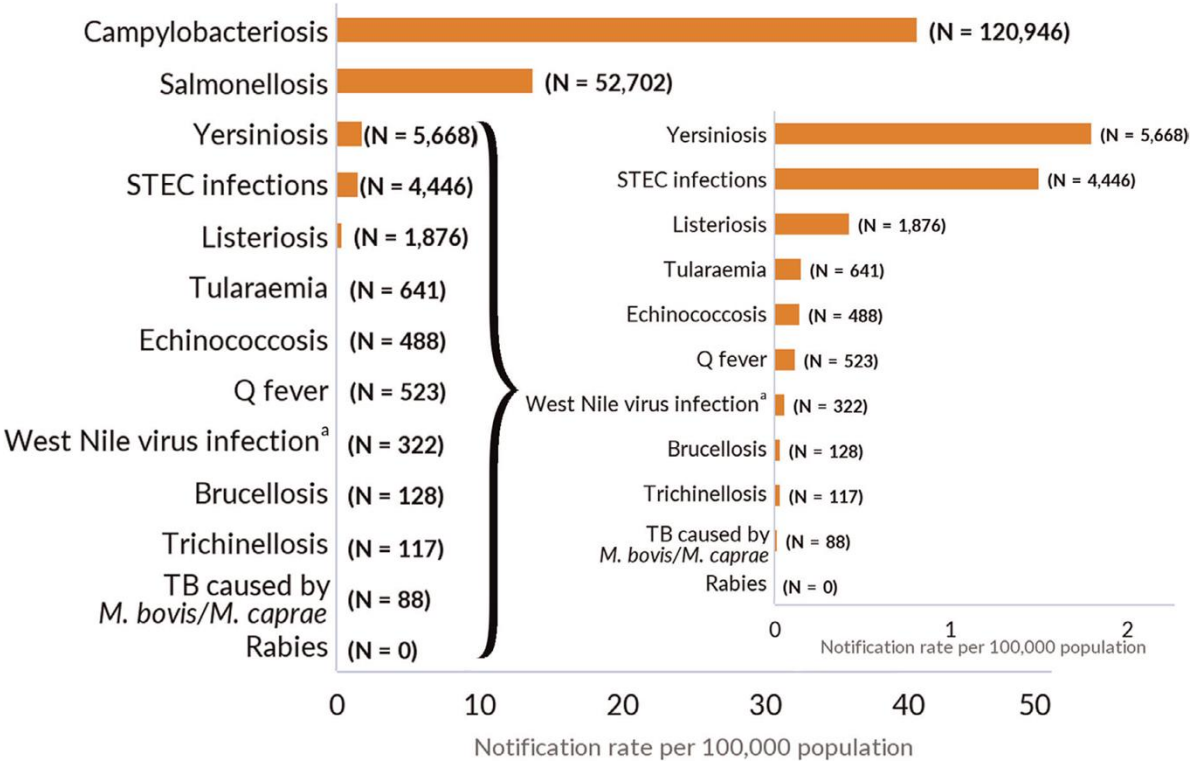
- The European Union (EU) system for the monitoring and collection of information on zoonoses is based on Zoonoses Directive 2003/99/EC1, which obliges EU Member States (MS) to collect relevant and, when applicable, comparable data on zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks.

- Since 2008, data on human cases have been received via The European Surveillance System (TESSy), maintained by ECDC.

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

ej EFSA

Reported numbers of cases of confirmed human zoonoses in the EU 2020



EFSA - EU One Health Zoonoses Report 2020

Surveillance and Monitoring of Zoonoses

- Effective surveillance is essential to understand pathogen epidemiology and consequently essential to the development of disease control and prevention programmes.
- Towards integrated surveillance-response systems for the prevention of future outbreaks.
- The usual difficulties encountered in diagnosing zoonotic and/or emerging diseases in humans and animals constitute a major limitation in their surveillance.

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

EFSA

Methods for rapid diagnosis of zoonosis

- The clinical manifestations caused by these pathogens are often poorly specific.

Direct methods:

- isolation in culture from clinical samples;

- PCR-based methods.

Indirect methods:

- Immunoserological (e.g., microagglutination, complement fixation tests; titration of IgM- and IgG-type antibodies, such as the indirect immunofluorescence assay and enzyme immunoassays).

Flow Cytometry:

new gold standard method?

Possible applications of flow cytometry technology in microbiological areas

Pathogen characterization: Detection, enumeration, size, shape, viability, and functional state; identification; resistance and susceptibility; isolation for further study

Application areas:

Clinical microbiology: Laboratory analysis of clinical specimens for the presence of pathogens;

Environmental quality: Detection of pathogen contamination in air, water, soil, and buildings

Food biosafety: Detection and identification of pathogens in food and beverages; farms; livestock;

Therapeutics development: Antibiotics, antivirals; large-scale library screening for drug candidates; susceptibility testing

Possible applications of flow cytometry technology in microbiological areas

Host-cell characterization:

Toxic and immune response to infection; pathogen load; cytotoxicity; T-cell epitopes; innate immune response;

adaptive immune response

Application areas

Disease diagnostics: Infection, bacteremia, viral load in clinical specimens

Epidemiology: Testing the population for markers of infection or disease to determine the spread

Vaccine development: Screening vaccine candidates for T-cell responses in experimental animals or humans

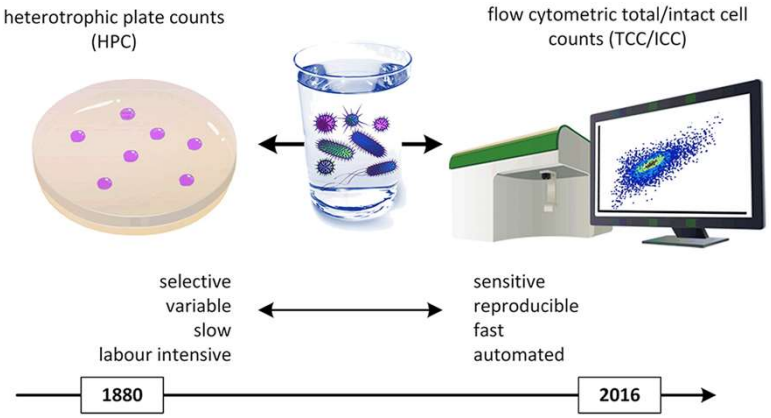
Drug testing and trials: Large-scale screening of candidate therapeutics for cell permeability and efficacy; cytotoxicity testing

Basic R&D: Understand mechanisms of pathogenicity and host response to pathogens in experimental cell or animal model systems

Environmental Surveillance of Pathogens Using Flow Cytometry

Flow cytometry is an extremely useful tool for analysis of microbial populations in nature whether from collected aerosol samples or from water samples.

It characterizes heterogeneous populations of environmental microbes, particularly in aqueous environments such as drinking water systems.

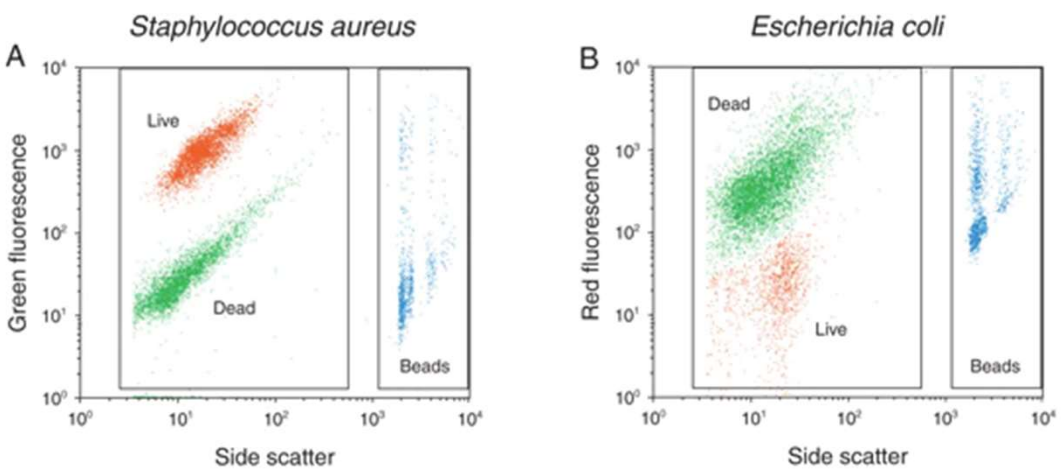


https://doi.org/10.1016/j.watres.2017.01.065.

Flow cytometry assays: viability of bacteria

Traditionally, bacterial viability is measured using plate growth techniques.

These are not always ideal, especially for slow-growing or hard to culture bacteria

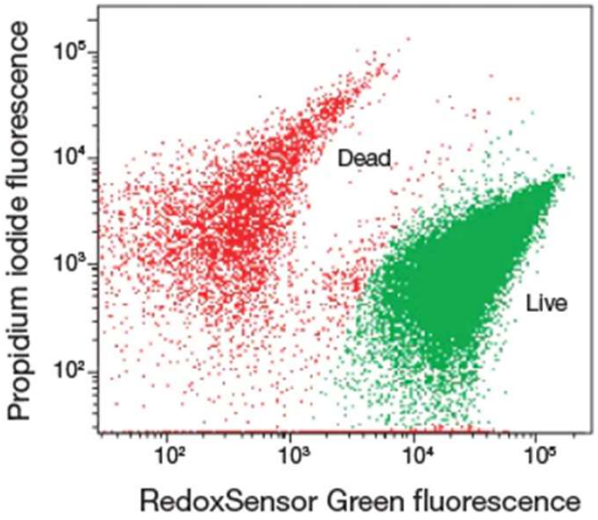


kit Live / Dead BacLight ThermoFisher
(green-fluorescent SYTO 9 dye and red-fluorescent propidium iodide)

Flow cytometry assays: viability and metabolic activity

This assay allows to evaluate the health and viability of bacterial cells based on the detection activity of bacterial oxidase and reductase.

The enhanced fluorescent signal is observed in bacterial cells with active metabolic processes



BacLight RedoxSensor Green Bacterial Vitality Kit

Flow cytometry gave birth to flow virometry

- Recent advances in the field of flow cytometry have allowed the detection of microparticles that range between 100 nm and 1000 nm in size (e.g exosomes, or intruders of cells, such as small bacteria or viruses).

- Flow virometry has the potential to be used as a tool for several applications:

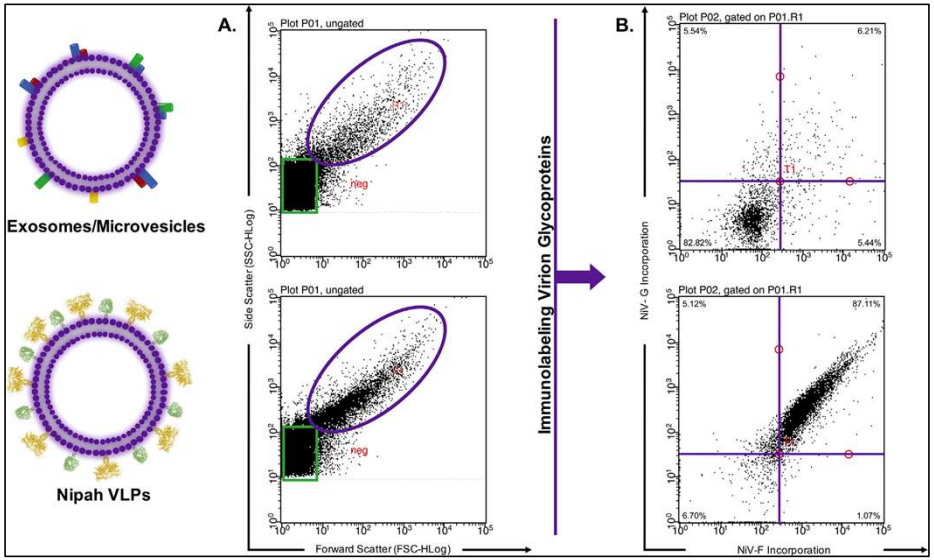
✓ enumeration of viruses,

✓ discrimination of viral populations from environments

✓ determination of virus glycoprotein and lipid topography of viral particles.

Flow cytometry gave birth to flow virometry

- Immunolabeling to distinguish virions from other microparticles.



J.L.R. Zamora, H.C. Aguilar / Methods 134–135 (2018) 87–97

Virions and exosomes/microparticles are similar in size.

(A) Detection utilizing light scattering parameters show similar sized microparticles.

(B) Immunolabeling clearly distinguishes virions from other microparticles.

Flow cytometry is the most commonly used technology in immunology research

It has been widely used for several decades to:

- monitor immune responses to vaccination and infection in bulk cell populations;
- track the phenotypic and functional characteristics of antigen-specific cells;
- largely applied in routine clinical settings for the diagnosis, prognosis and monitoring of disease

Flow cytometry is the most commonly used technology in immunology research

For example:

- for the detection of minimal residual disease;
- to distinguish different stages of tuberculosis infection;
- to monitor changes in cell populations after some surgery for the prediction of infections in risk patients;
- in HIV for the determination of CD4+ T cell counts;
- Urine flow cytometry (UFC)
- to analyze the contribution of innate immunity to vaccine efficacy and disease pathogenesis

Diagnosis of Tularemia by Flow Cytometry

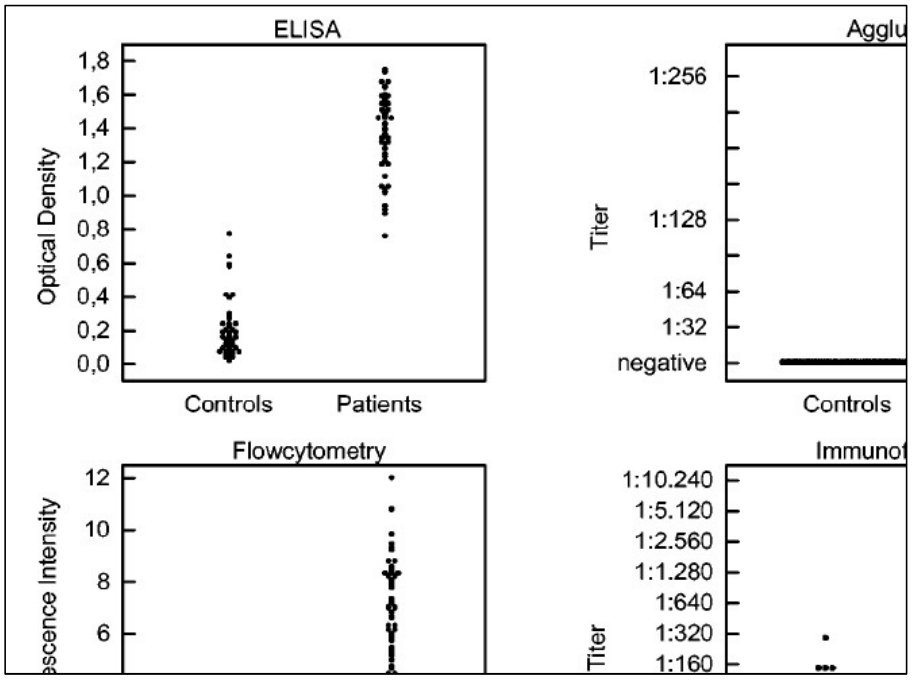
* Tularemia is a zoonotic disease caused by *Francisella tularensis*.
* In Europe, >500 cases are reported annually
* The infection is acquired by direct contact with *F. tularensis* from animal reservoirs, infected vectors, or contaminated water.

- No clinical or laboratory manifestations are pathognomonic for tularemia;
- preliminary diagnosis is based on exposure risk and compatible clinical presentation

Diagnosis of Tularemia by Flow Cytometry

Flow cytometry has been tested for the first time as a new platform for serodiagnosis of tularemia, with superior efficiency compared to ELISA and comparable to Western blotting and microagglutination.

The distributions of F. tularensis LPS-specific immunoglobulin levels obtained by the different diagnostic approaches



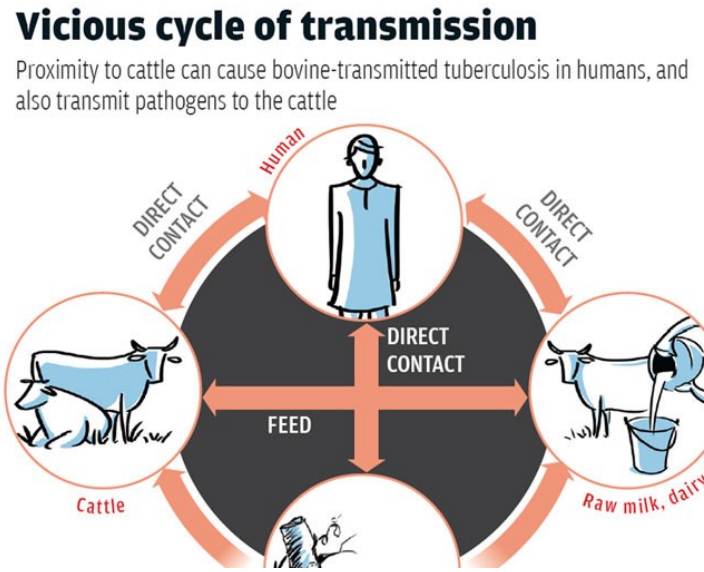
ASM Journals, Clinical and Vaccine Immunology, doi:10.1128/CDLI.11.6.1008–1015.2004

Notes:
Levels in sera of patients (n 50) and healthy controls (n 50) were determined by the methods.
Sera for ELISA were diluted 1/640.

Flow Cytometric assay for Mycobacterium bovis detection

- Zoonotic tuberculosis (bTB) is predominantly caused by *M. bovis*
- 143,000 new cases of zoonotic TB and 12,300 deaths disease-related in humans in 2018 (WHO, 2019).

- The detection of bTB is complicated, and no diagnostic gold standard is currently available.
- Conventional diagnostic tools (detection of antibodies or antigens) can only be used in late stages of the disease.
- The most widely used bTB diagnostics are based on cell-mediated immune response (Tuberculin skin test).



Flow Cytometric assay for Mycobacterium bovis detection

frontie
in Veter

Clinical and Vaccine Immunology, Dec. 2006, p. 1343–1348
1556-6811/06/$08.00+0 doi:10.1128/CVI.00291-06
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Flow Cytometric Detection of Gamma Interferon Can E
Discriminate *Mycobacterium bovis* BCG-Vaccinated

Veterinary Immunology and Immunopathology 228 (2020) 110112

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Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

ELSEVIER

Short communication

Development of a flow cytometry assay for bovine interleukin-2 and its preliminary application in bovine tuberculosis detection

Aihong Xia [a], Zhengzhong Xu [b], Ting Hu [a], Xin Li [b], Zhaocheng Zhu [a], Xiang Chen [a,*], Xinan Jiao [b,*]

Flow Cytometric assay for Mycobacterium bovis detection

- Evidence from different studies:

- higher secretion of IL-2 (post-lymphocytes stimulation by TB-specific antigens) in cattles/humans with bTB than in healthy individuals

IL-2 (from CD4+) could be an ideal target for the diagnosis of Mycobacterium infection

Flow Cytometric assay for Mycobacterium bovis detection

- Evidence from different studies:

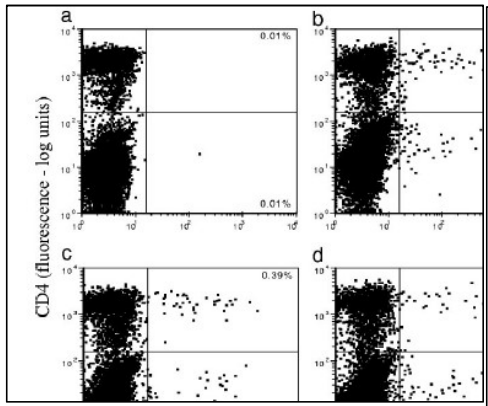
✓ accurate diagnosis of M. bovis-infected animals and the potential to distinguish these animals from vaccinates.

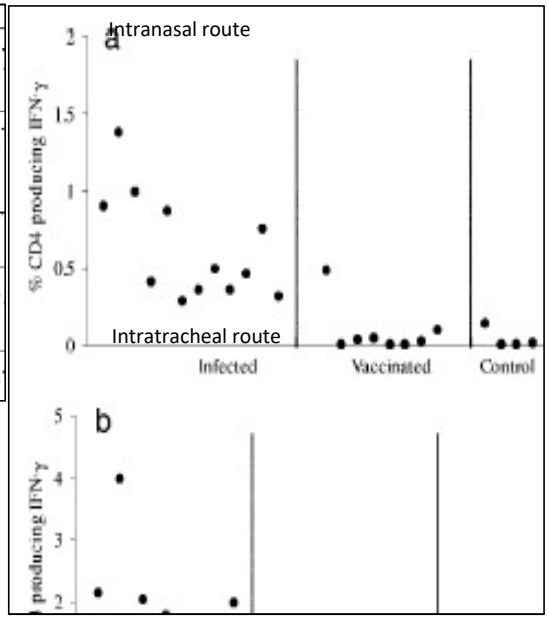
✓ the current used diagnostic tests, the skin test and gamma interferon (IFN-) blood test, have a sensitivity of up to 95%.

Flow Cytometric assay for Mycobacterium bovis detection

Cytokine flow cytometry (CFC):

- extremely sensitive methodology for assaying numbers of antigen-specific cytokine-expressing cells in PBMC and whole blood
- useful indicators of immune status





Detection of IFN-gamma by CFC allows differentiation of cattle infected with M. bovis from BCG vaccinates (cells from infected cattle higher numbers of IFN-gamma+-CD4+ T cells than BCG-vaccinated cattle)

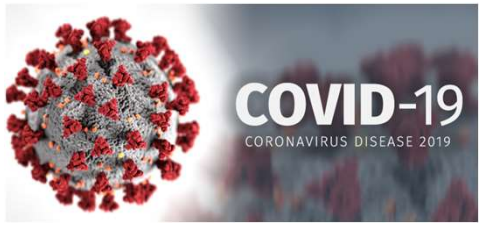
2006, American Society for Microbiology; doi:10.1128/CVI.00291-06

Flow Cytometric assay for Mycobacterium bovis detection

- Polychromatic flow cytometry can provide more detailed information for one sample, which increases the method capacity for discriminating between infection and healthy states.
- This assay can be used repeatedly, increasing the frequency of surveillance, whereas a tuberculin skin test requires 6 weeks before repeat testing.
- The flow cytometry assay can be performed simultaneously with the skin test.

Flow cytometry-based assay for serological detection of antibodies in COVID-19 patients

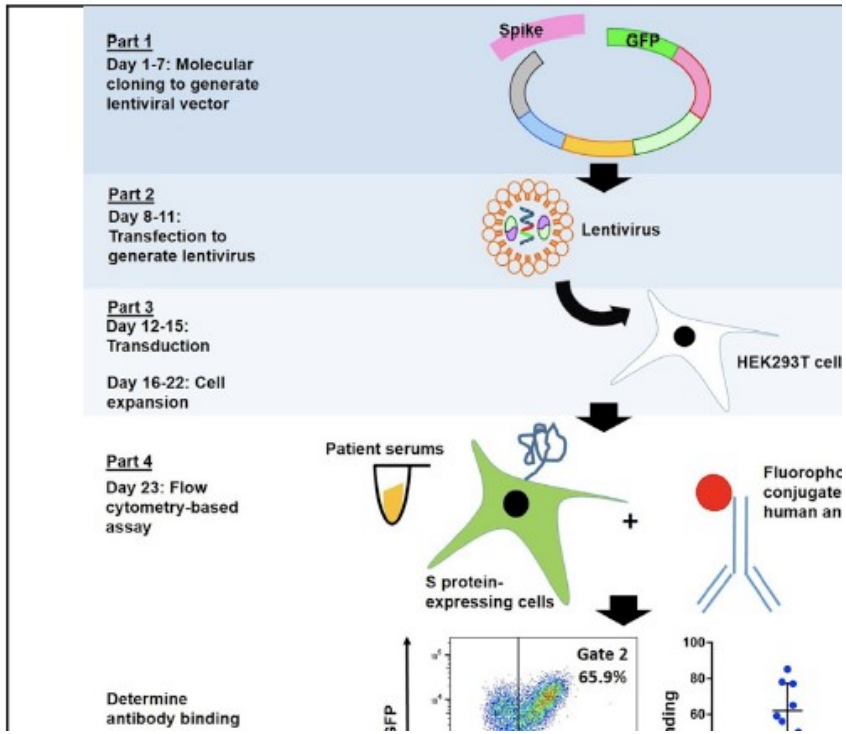
- Ongoing need of developing sensitive and specific methods for the determination of SARS-CoV-2 seroconversion
- Serological assays are important to detect previously infected individuals and perform epidemiological seroconversion studies
- ELISA limitation (individual plates/wells for each antigen or antibody class to be tested; hide epitopes or increase the background noise; low antibody titres are not detected; values close to the cut-off).



FC assay offers an alternative to perform serology:

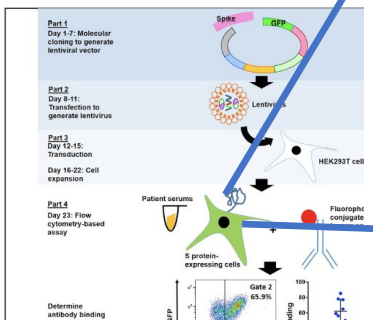
- ✓ rapid identification of multiple analytes simultaneously on a multiplexed manner;
- ✓ less amounts of sample than traditional immunoassays.

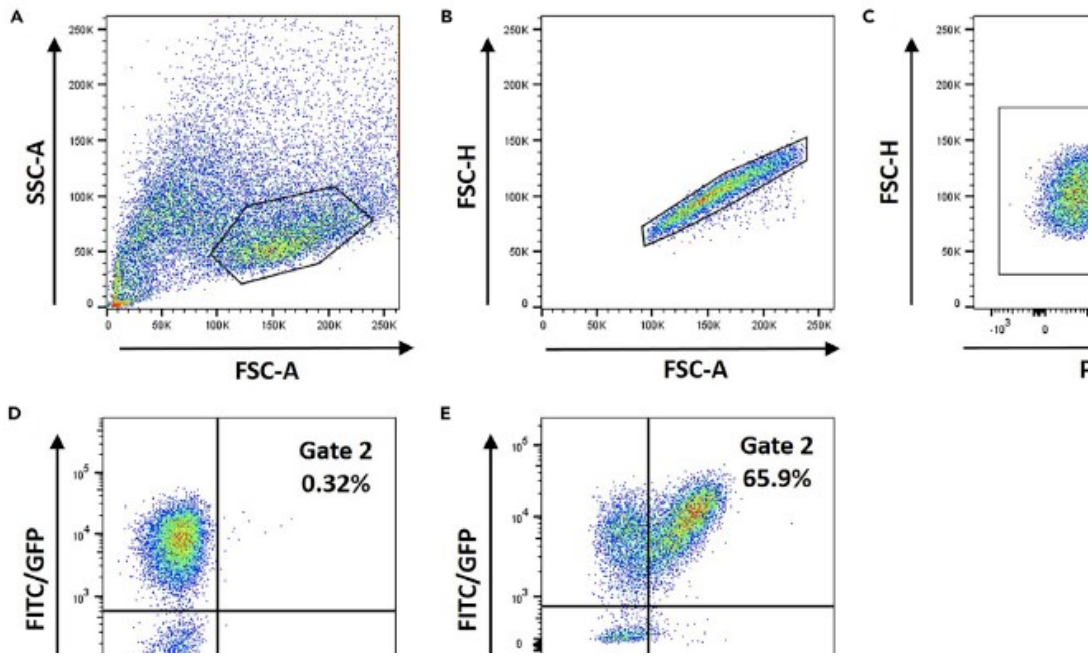
Flow cytometry-based assay for serological detection of anti-spike antibodies in COVID-19 patients



CellPress STAR Protocols, 100671; 2021

Flow cytometry-based assay for serological detection of anti-spike antibodies in COVID-19 patients





CellPress STAR Protocols, 100671; 2021

Flow cytometry-based assay for serological detection of anti-spike antibodies in COVID-19 patients

communications biology

European Journal of Clinical Microbiology & Infectious Diseases
https://doi.org/10.1007/s10096-020-04072-7

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TRANSPARENT PROCESS

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Flow cytometry multiplexed method for the detection of neutralizing human antibodies

Salmonellosis

* **Pathogen**:

- nontyphoidal salmonellosis (Salmonella enterica)

- typically acquired by the oral ingestion of contaminated food or water

* **Symptoms**:

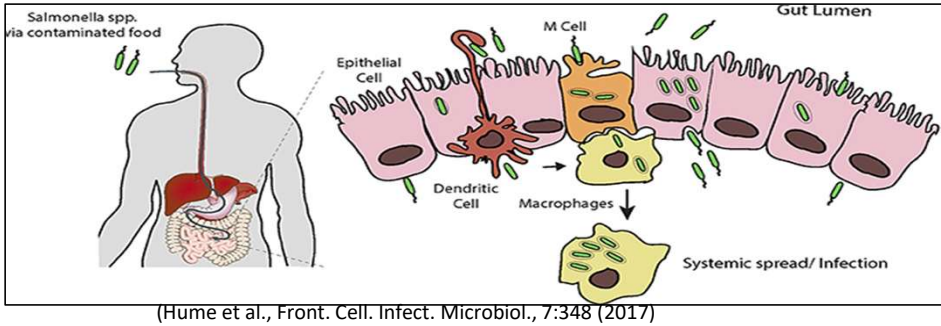
- Nausea, abdominal pain, diarrhea 12-36 hours after eating

- Possible fever

- Septic shock can occur in the infants and elderly

* **Treatment**:

- Oral rehydration

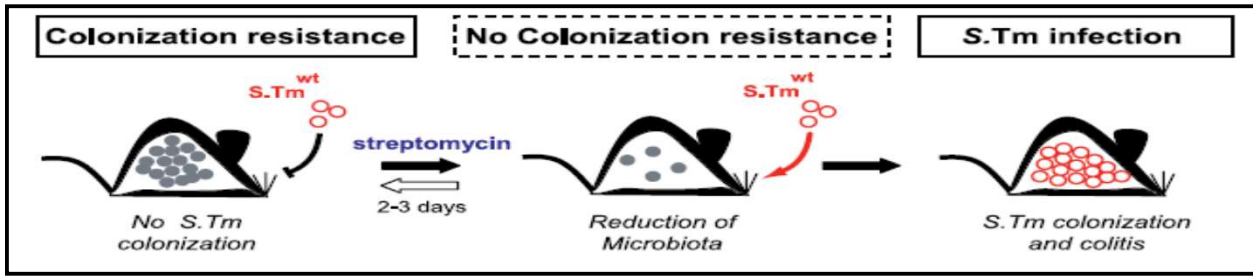


(Hume et al., Front. Cell. Infect. Microbiol., 7:348 (2017)

Animal models for studying Salmonella Typhimurium infection

* Many pathogens use inflammation and the accompanying dysbiosis for their advantage in order to overcome colonization resistance
* STM exploits inflammation to promote its own colonization, out-competing the resident microbiota

Streptomycin mouse model

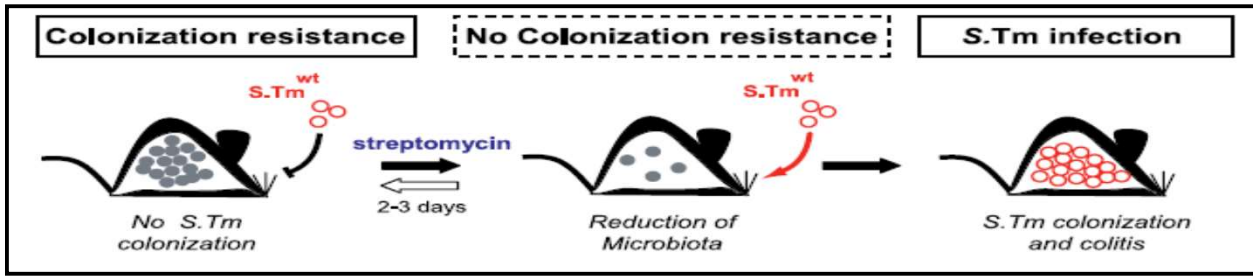


(Stecher et al., PLoS Biol 2007)

Animal models for studying Salmonella Typhimurium infection

However this model is based on the lack of an intact microbiota, limiting a comprehensive evaluation of the complex interactions of STM within the gastrointestinal environment.

Streptomycin mouse model

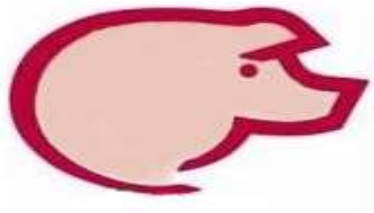


(Stecher et al., PLoS Biol 2007)

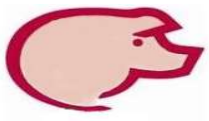
Piglets as model of choice for studying salmonellosis

* Pig is a natural host of Salmonella;

* STM induces a disease closely resembling that occurring in humans



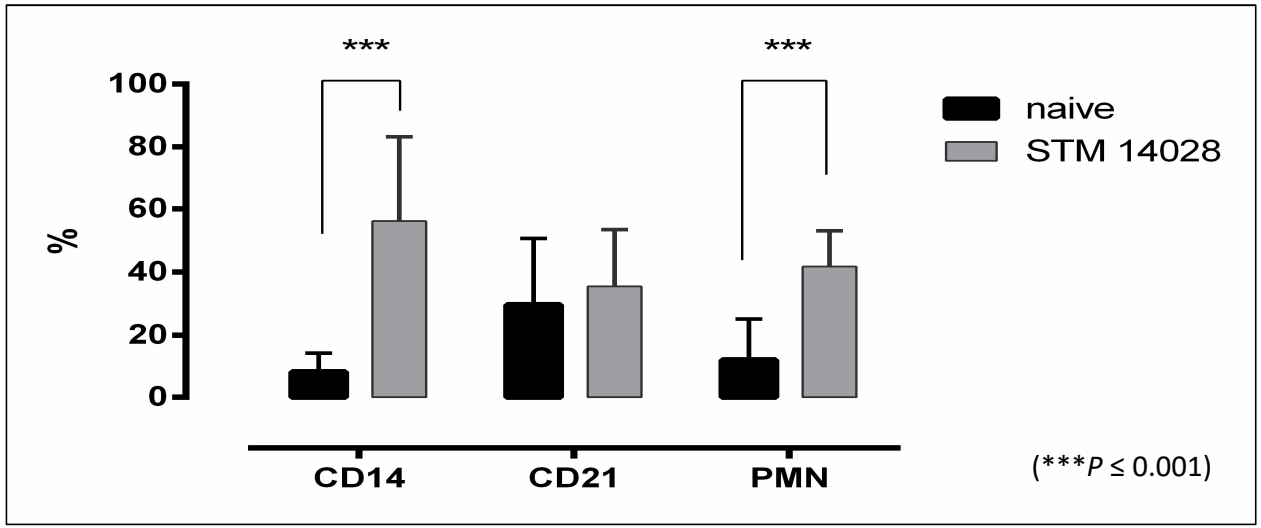
Salmonella wt (STM 14028) infection induces an innate immune response



10^9 CFU
STM 14028 – OS

frontiers in Microbiology

Salmonella Typhimurium exp
inflammation to its own adva
piglets



(Chirullo et al., Frontiers 2015)

Bacterial infections and Vitamin D

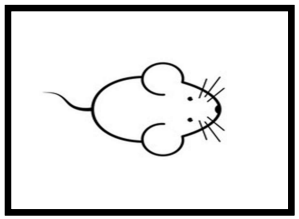
Vitamin D induces many biological effects:

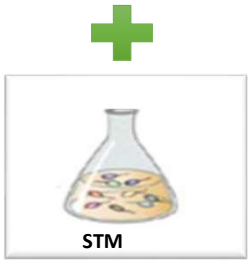
- ✓ calcium homeostasis and important functions for bone health;
- ✓ beneficial effect in the innate and acquired immune system.
- ✓ The increase in vitamin D leads to an increase in calcium which is effective in preventing bacterial infections and promoting bone marrow stem cell osteogenesis.

But the role of this vitamin in infections is unclear

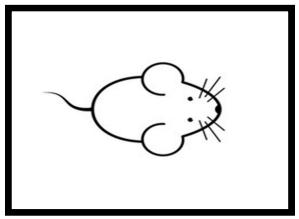
S.Typhimurium and Vitamin D

A1

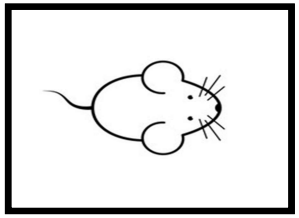




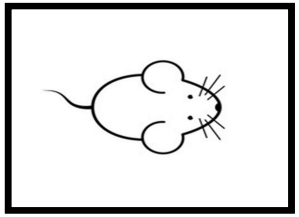
B1



B3



A3



A1 = vit D + STM
B1 = STM
B3 = (no Vit D no STM) CTRL doppio neg
A3 = vit D (no STM) CTRL neg

(dati preliminari, studi in corso)

STM and Vitamin D: blood count

Blood count panel

Vit D
CD45+
CD3+ CD4+
CD3+
CD3+ CD4-
CD8+ (CD3+ CD4- CD56-)
Nk+ (CD3-)
B220+
Cd11b+ Ly6g+ (neu)
Cd11b+ (mono)
F4/80+

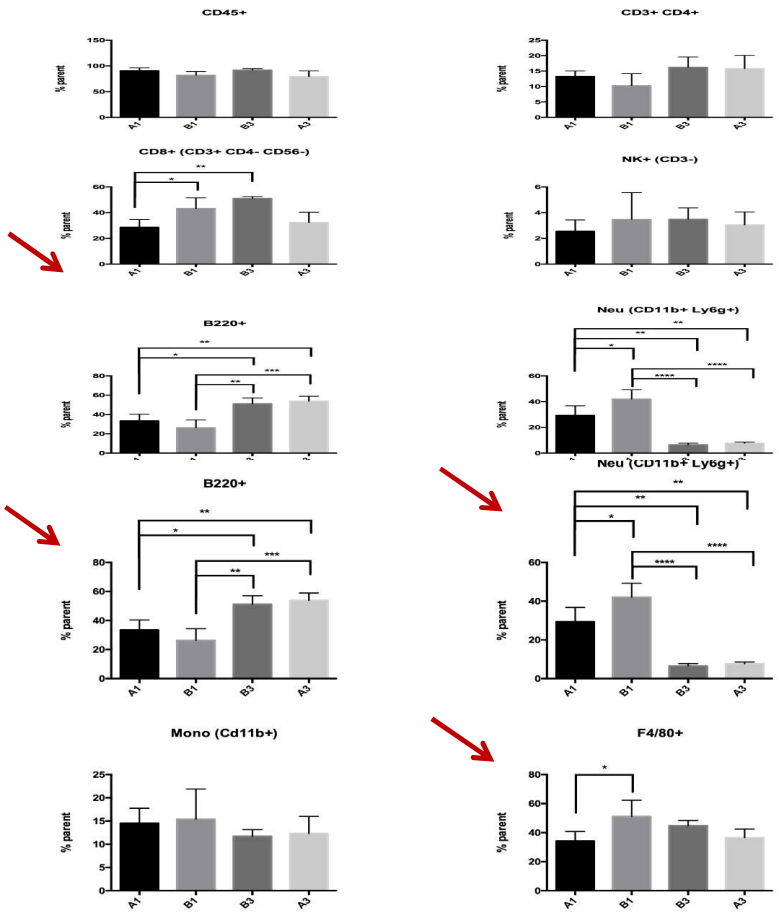
(prelimilary data, ongoing studies)

STM and Vitamin D

- ✓ The STM + vit D (A1) group has fewer neutrophils, macrophages and CD8 T lymphocytes compared to the STM group (B1).

Less inflammation.

- ✓ B lymphocytes are slightly higher in group A1 rather than B1



A1 = vit D + STM
B1 = STM
B3 = (no Vit D no STM) CTRL neg neg
A3 = vit D (no STM) CTRL neg

(prelimilary data, ongoing studies)

STM and Vitamin D

- Vitamin D has a positive effect on immune cells.
- Vitamin D has an inhibitory effect on the inflammatory response, shifting the balance from the pro-inflammatory to the anti-inflammatory process.

Acknowledgement

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