Developing two in vitro assays to measure antibody mediated protection against intracellular bacteria

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CHLAMYDIA TRACHOMATIS

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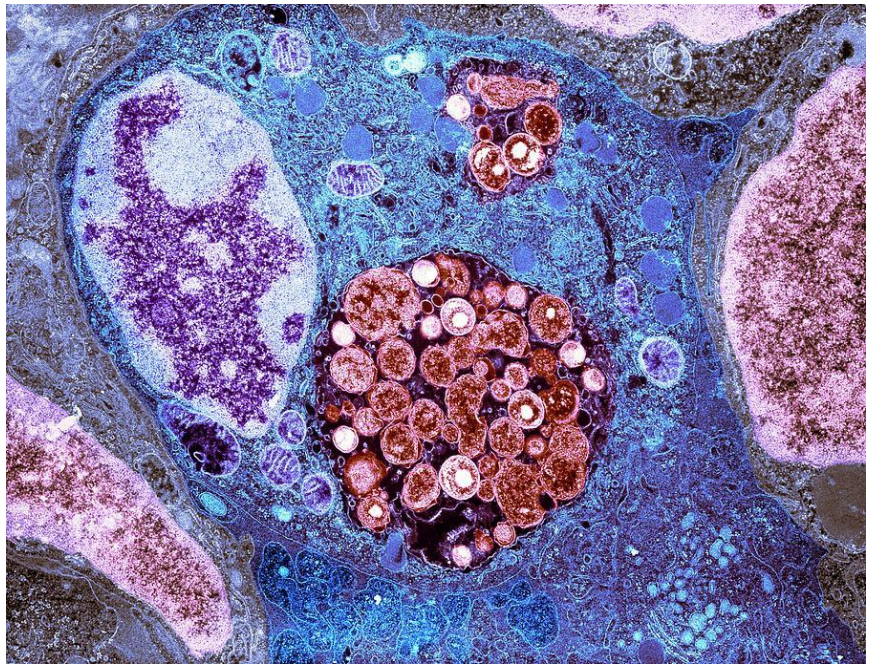
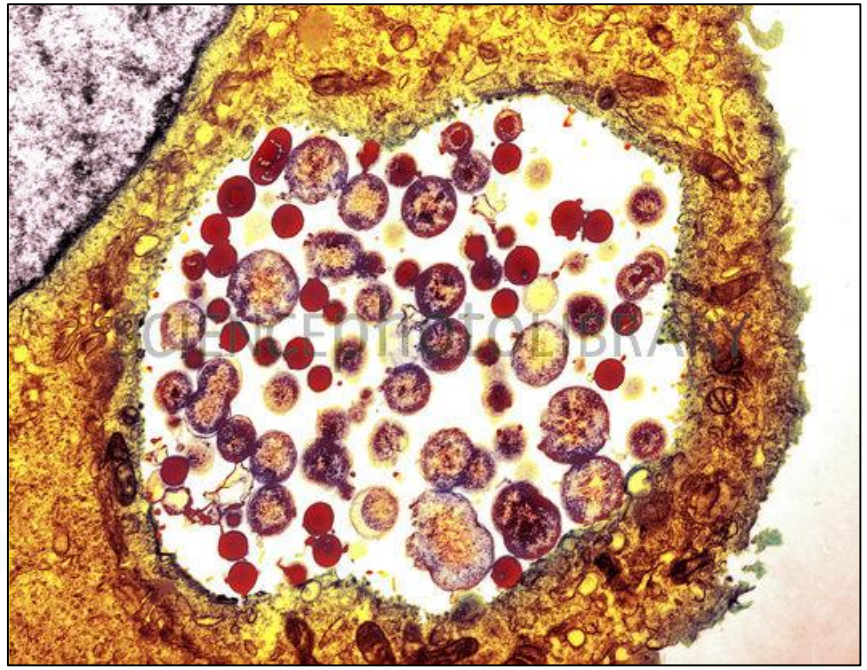
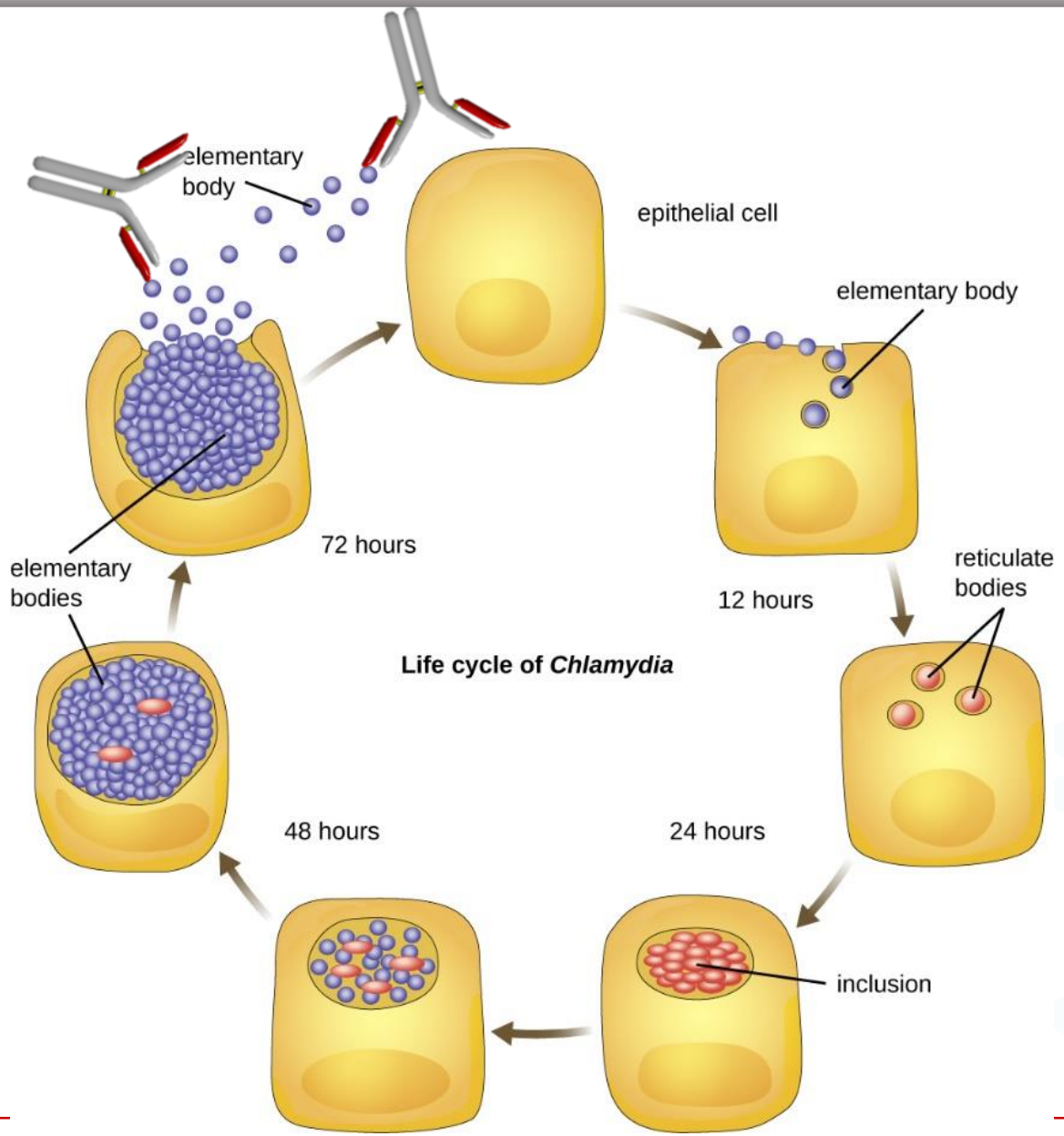
Chlamydia, caused by infection with *Chlamydia trachomatis*,
More than 100 million chlamydial infections are estimated annually
Cause serious damage in the upper genital tract which can lead to infertility.

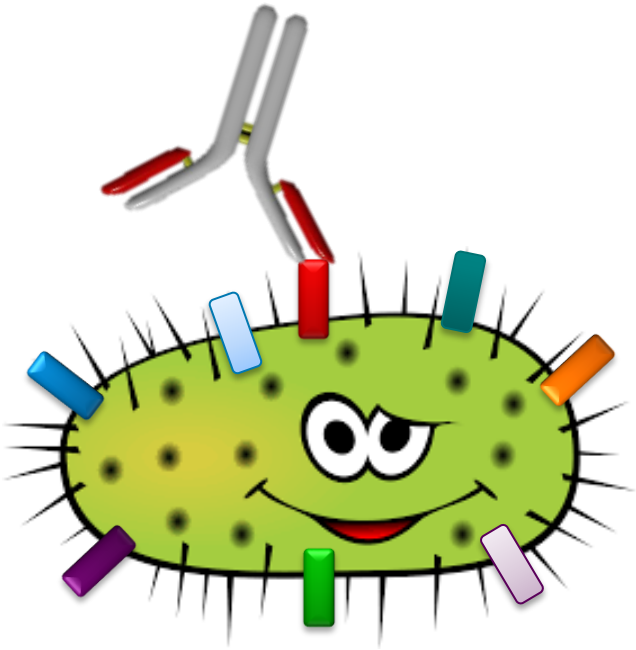




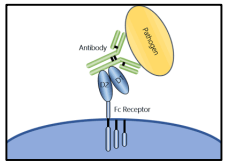
CHLAMYDIA LIFE CYCLE



Which antigen?

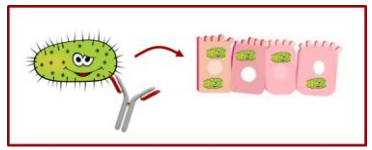


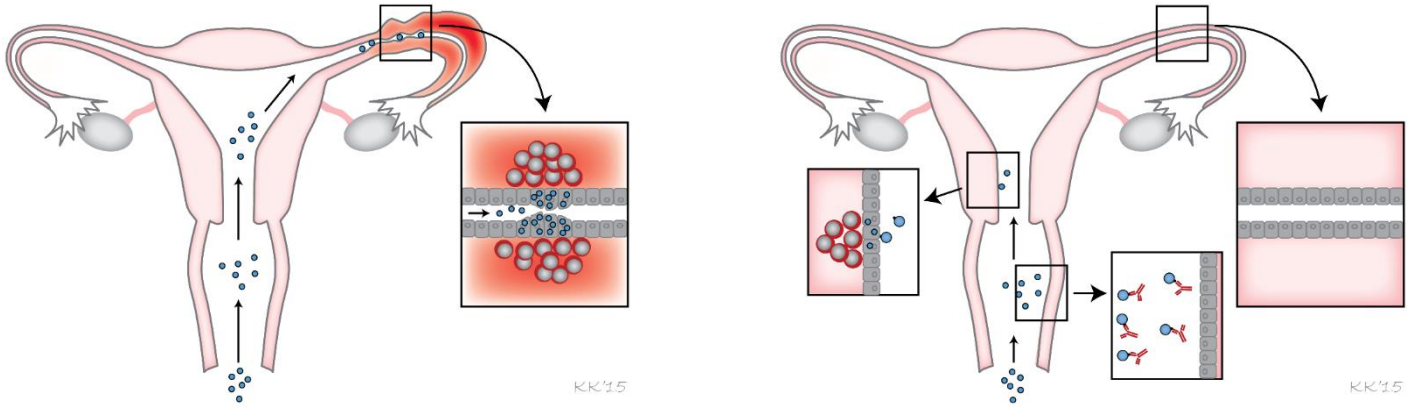
ANTIBODIES



Induction of phagocytosis

Neutralization

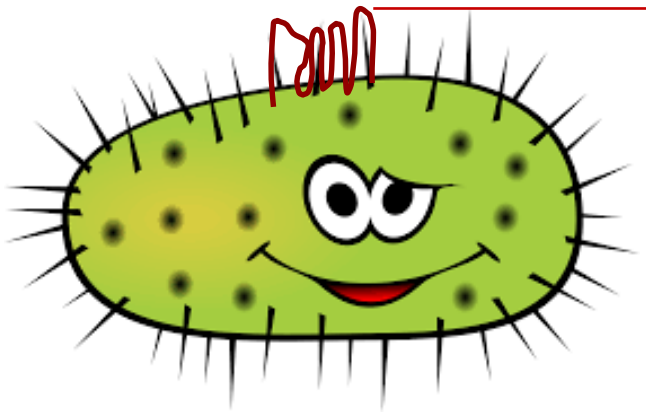


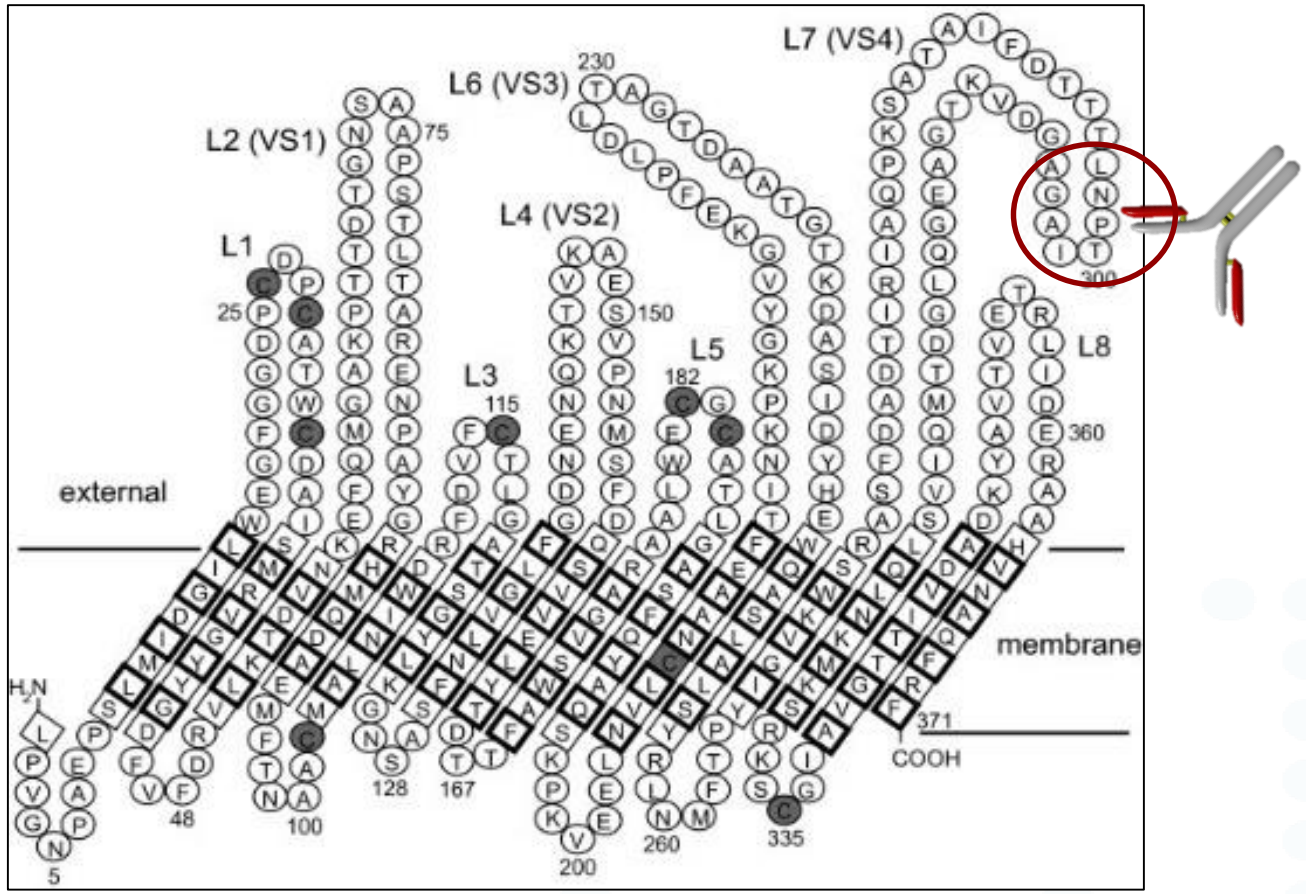


Prevent pathology in upper genital tract

FINDING THE RIGHT ANTIBODY

MOMP





MOMP protein

SCREENING FOR PROTECTIVE ANTIGENS

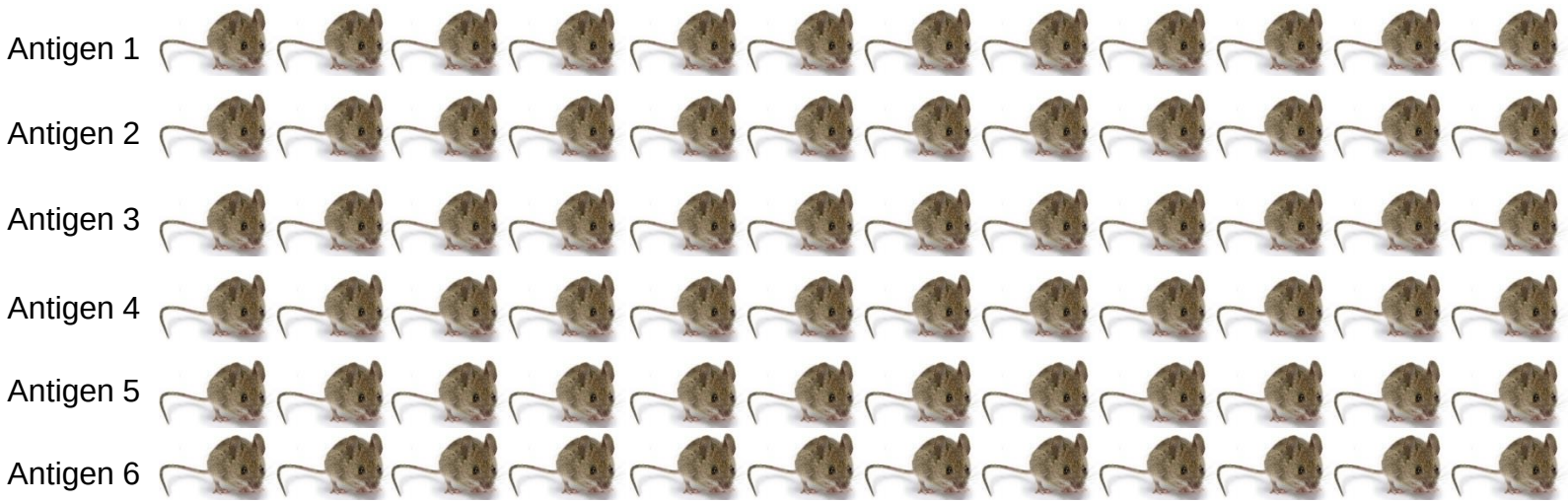
Produce the antigens

⇩

Vaccinate animals with the antigens and test for Protection against infection

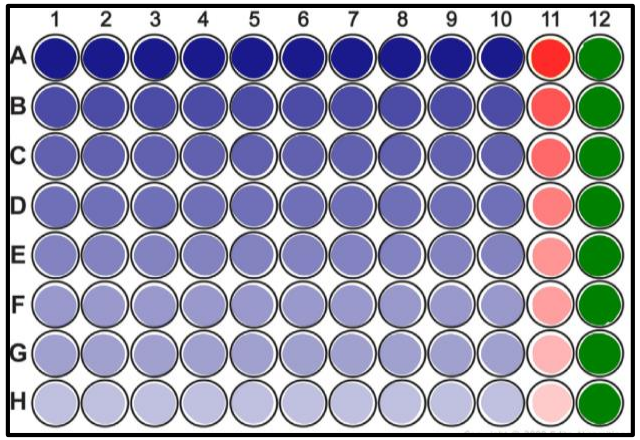
⇩

Select protective antigen



Exchange with in vitro assays ⇩

In vitro experiments using bacteria and cell lines



SCREENING FOR PROTECTIVE ANTIGENS

Produce the antigens

⇩

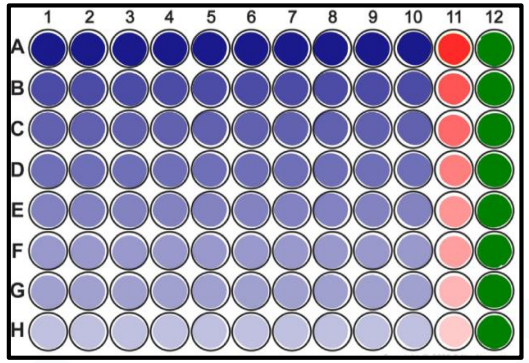
Vaccinate 1 mouse to produce the Abs

⇩

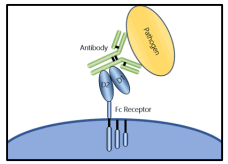
In vitro screening for protection ←

⇩

Select protective antigen



DEVELOP TWO ASSAYS



Induction of phagocytosis

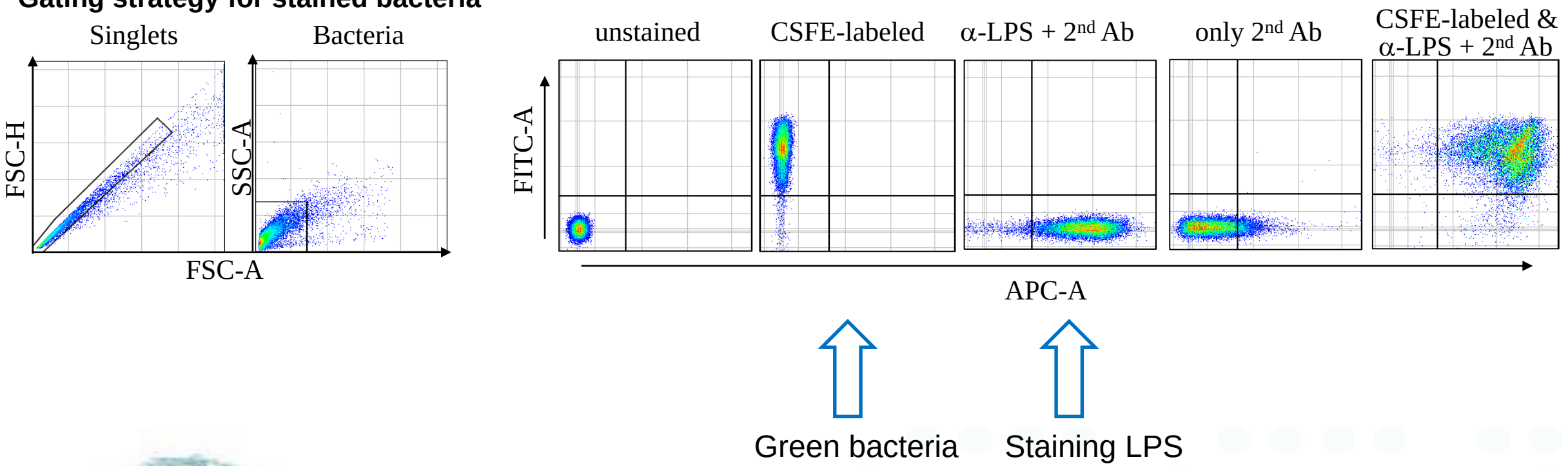
Neutralization

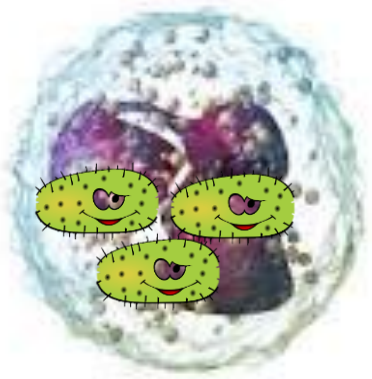
Produce a green Chlamydia bacteria (visible in a flow cytometer)

Show that an antibody binding to it
can mediate uptake into neutrophil or macrophage

STAINING THE BACTERIA

Gating strategy for stained bacteria

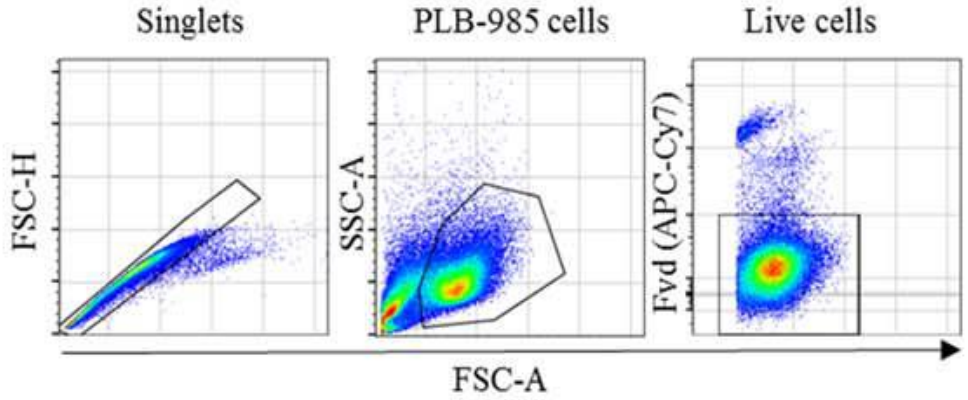




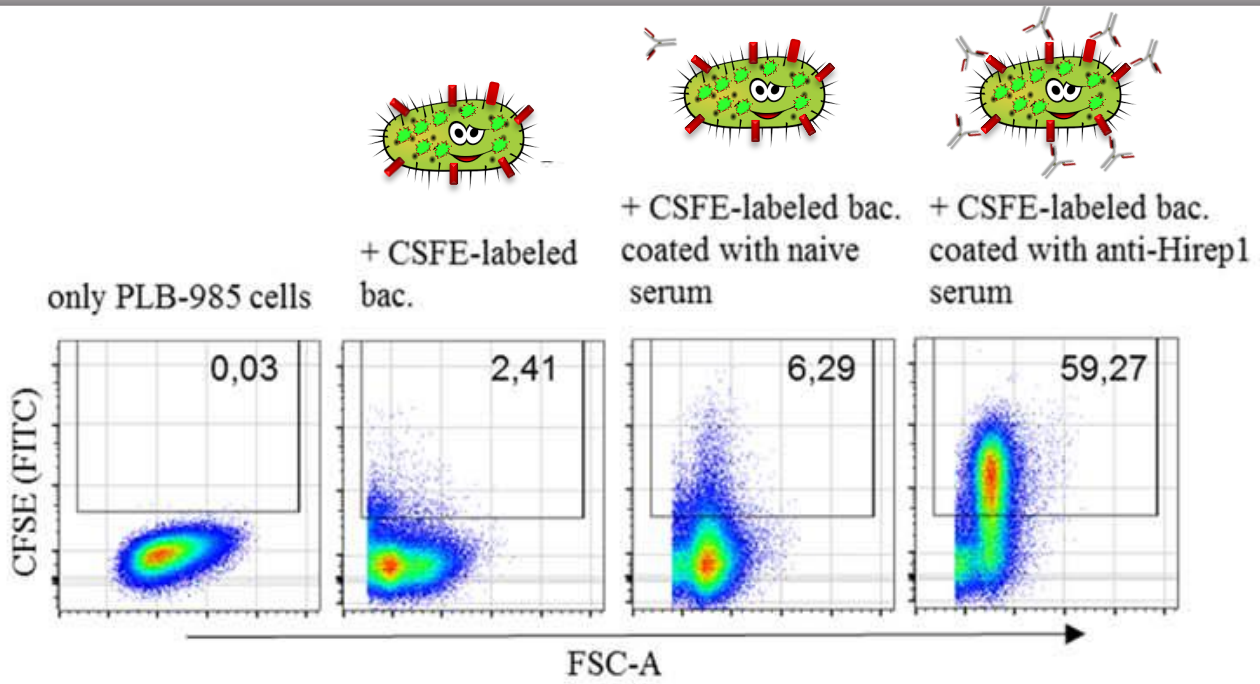
Two ways to measure uptake :

1. Permeabilize the cells, stain the bacteria, and FACS
2. FACS (because the bacteria are green)

UPTAKE INTO PLB-985 CELLS

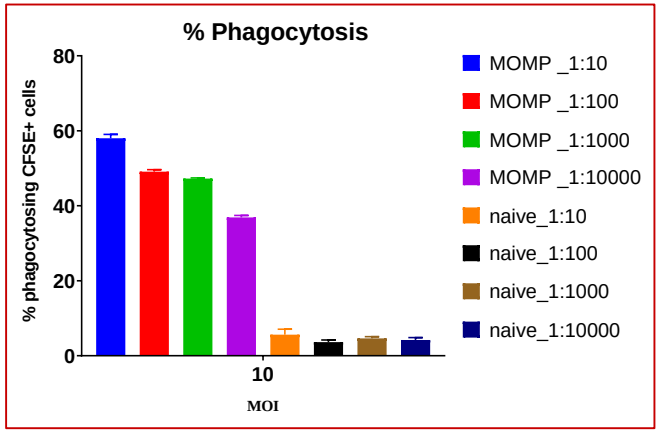


Gating strategy for phagocytosis assay



PLB-985 cells with/without phagocytosed *C. trachomatis* bacteria

CFSE-labeled SvD bacteria were preincubated with no serum, serum from naıve rabbits, or serum from rabbits vaccinated with Hirep1 for 40 min at 37C and incubated for 4 h with DMF-stimulated PLB-985 cells



TESTING TWO SURFACE ANTIGENS – MOMP AND CT043

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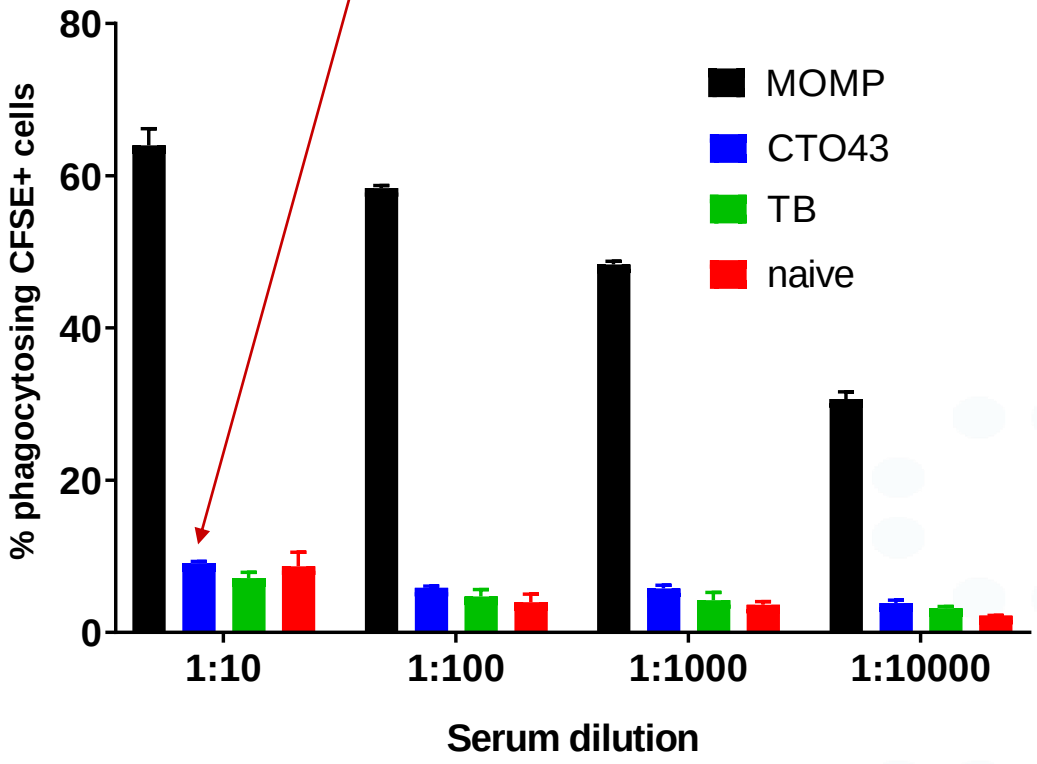
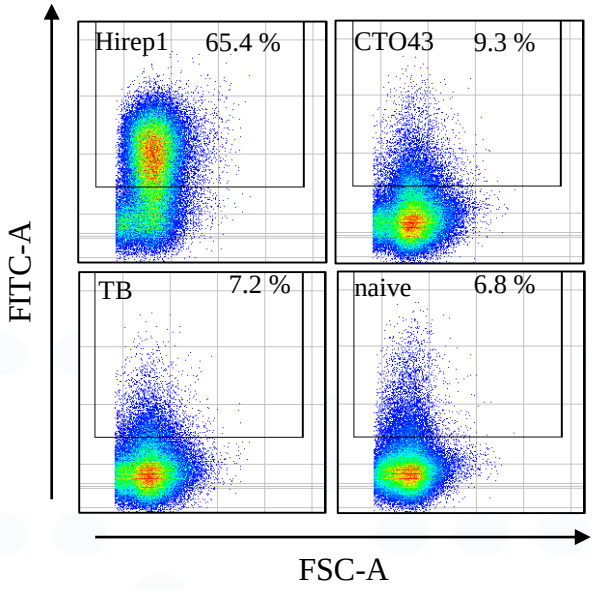
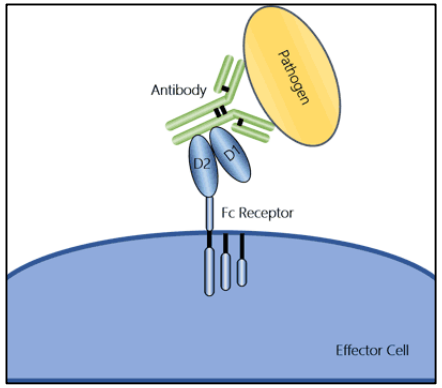
Being surface exposed does not automatically lead to phagocytosis.

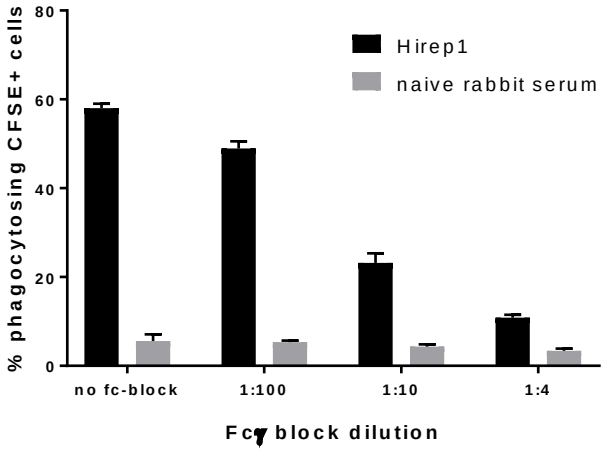


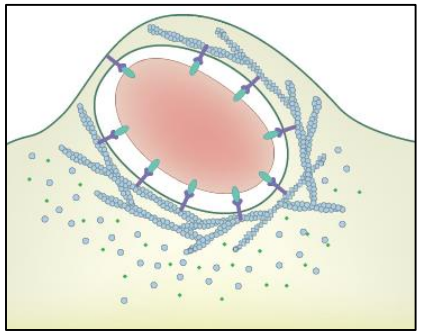


INHIBITING FC RECEPTORS OR ACTIN POLYMERIZATION

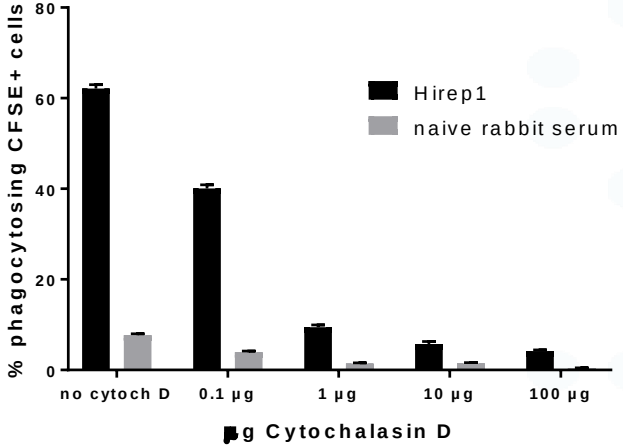


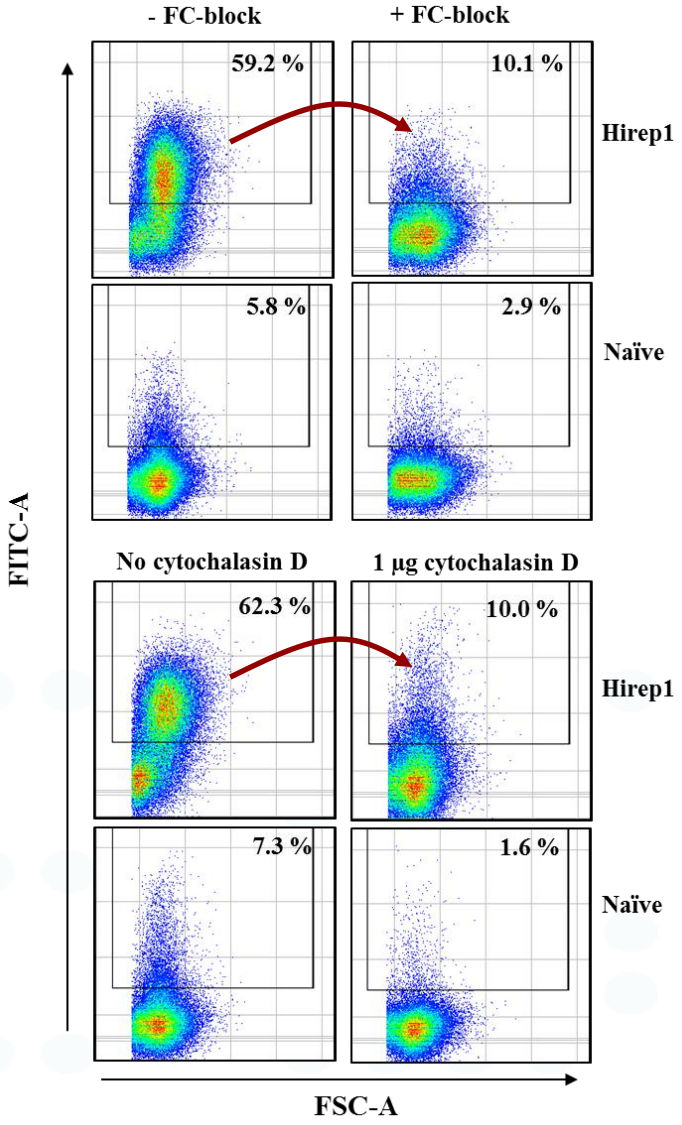
Fcγ receptor blocking





Actin-polymerization inhibition

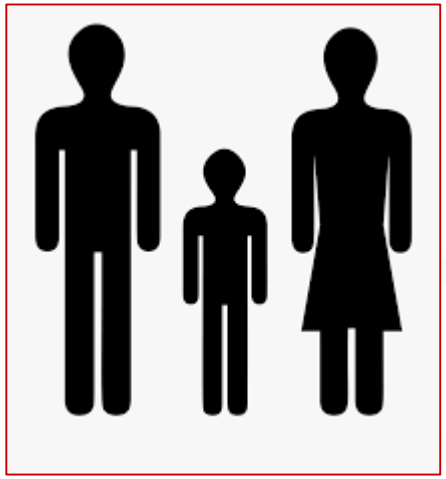


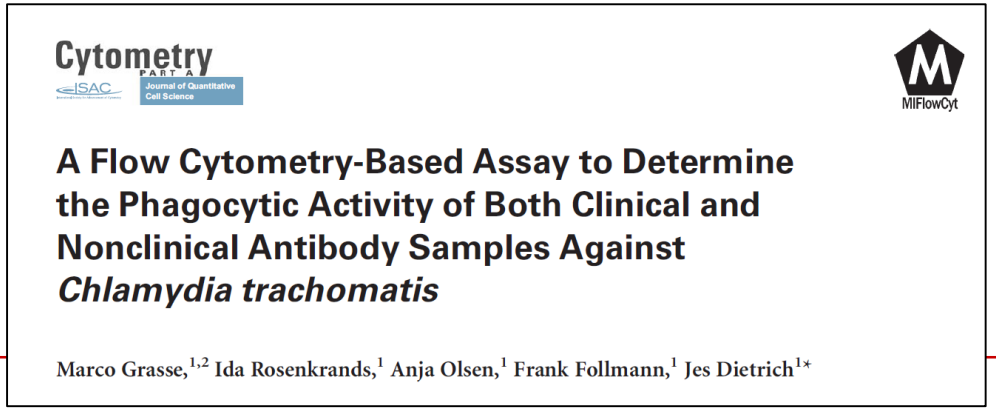


OTHER USED FOR THE ASSAY

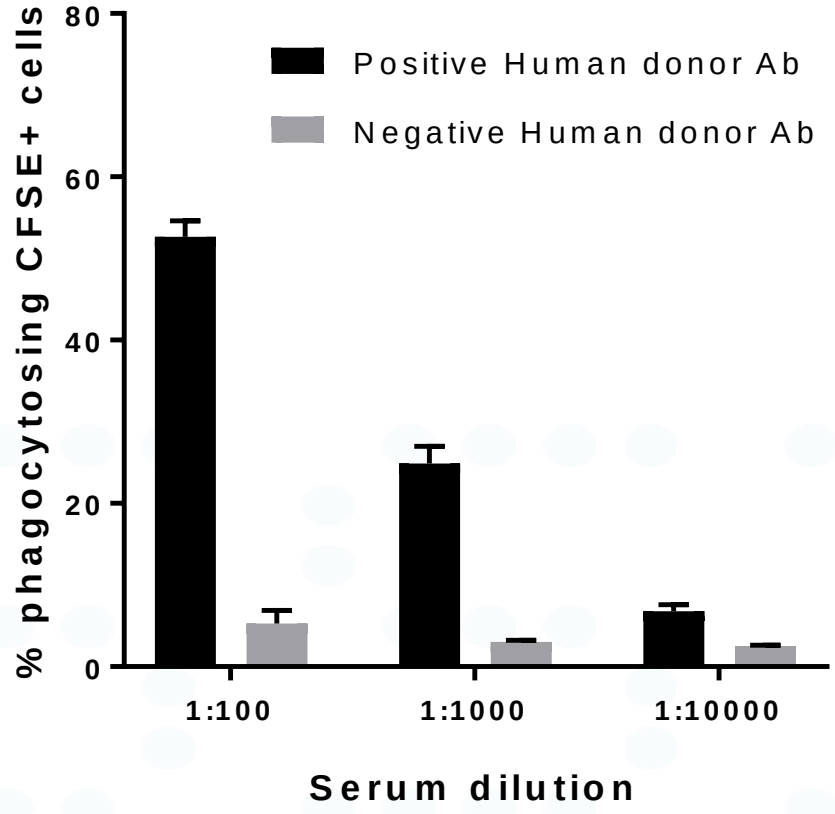
Another obvious use for a FACS based phagocytosis assay is **the high-throughput testing of serum samples from human donors**.

It was, therefore, important to show that the assay is also suitable for human and murine serum

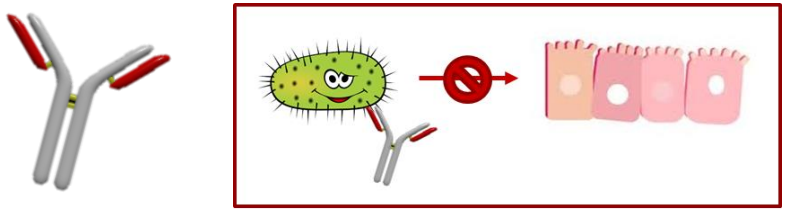




Phagocytosis induced by human serum

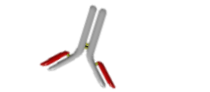


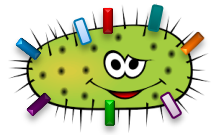
NEUTRALIZATION ASSAY – PREVENTION OF UPTAKE

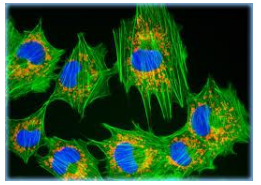


Induction of phagocytosis Neutralization

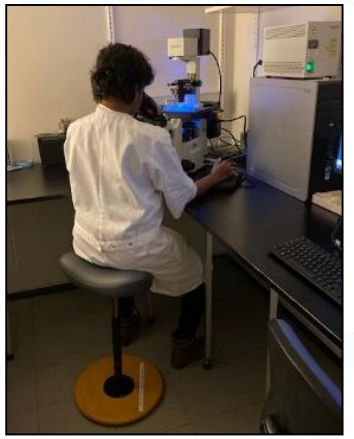
The assay





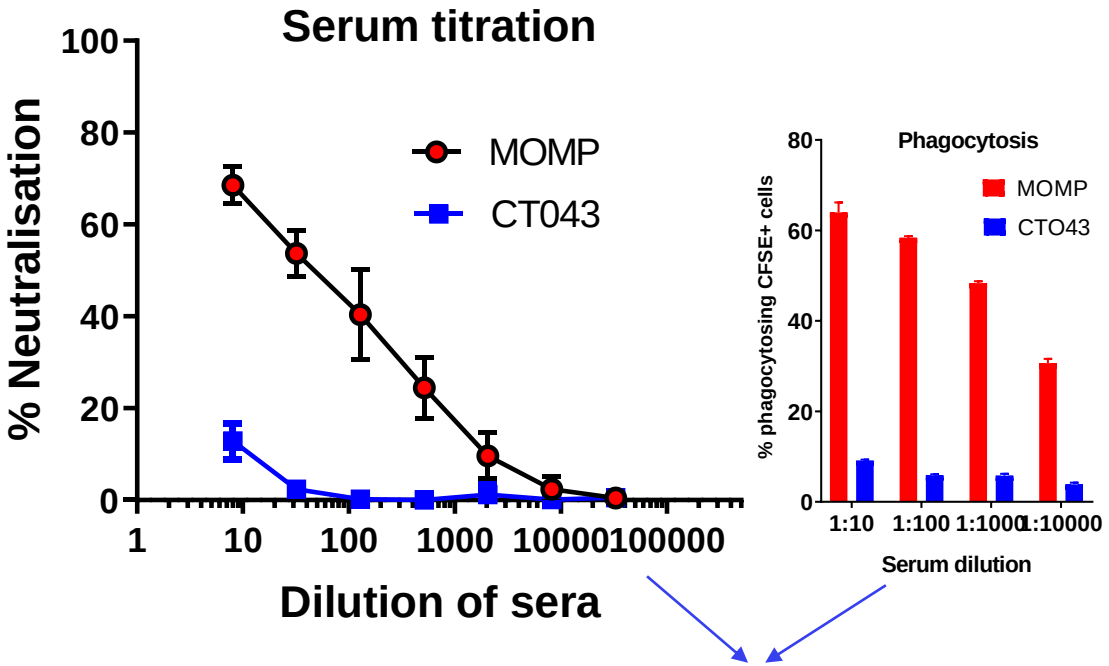


- The standard assay includes incubation of *C. trachomatis* with serum followed by infection of a HaK cell line (20h) and microscopy counting of inclusions
- Labour intensive



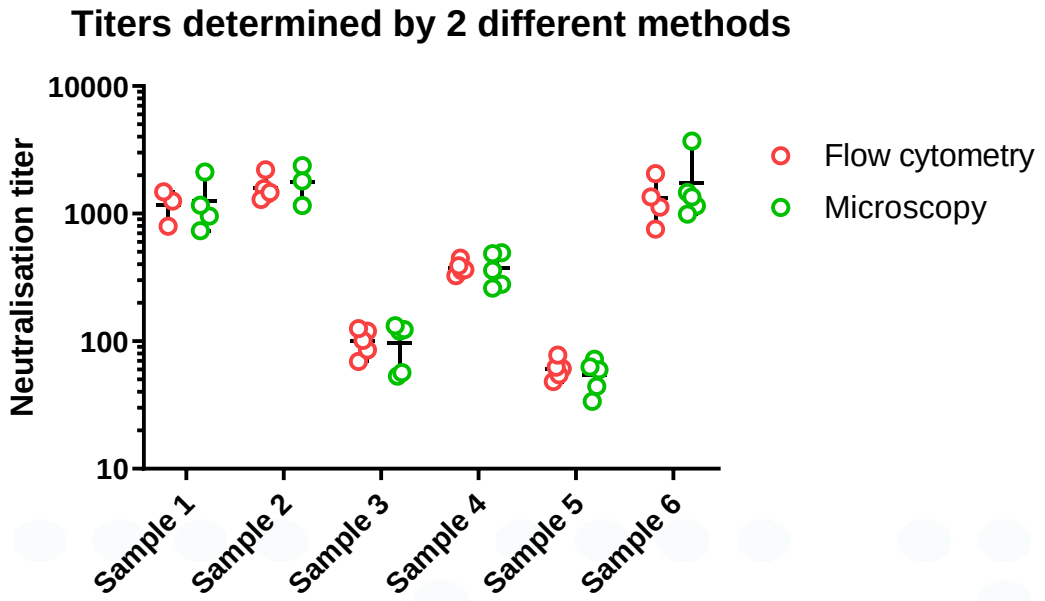
COMPARISON OF NEUTRALISATION METHODS

Mouse serum



Antibodies against surface exp. proteins does not automatically lead to phagocytosis or neutralization

Human serum



Conclusion: Flow cytometry is an alternative to the standard microscopy method

CONCLUSIONS

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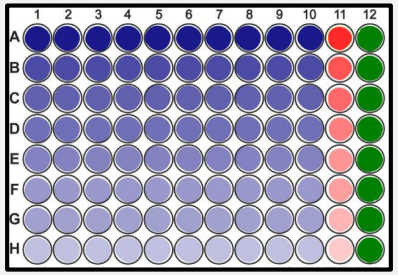
Developing two in vitro assays to measure antibody mediate protection against intracellular bacteria

Danmarks 3R-Center

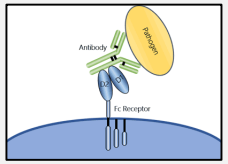
RRR

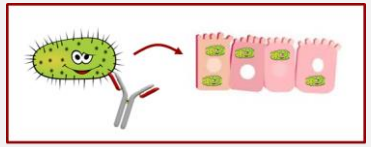
We can use these assays in the screening part of the antigen discovery process

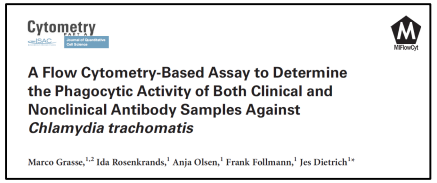


Phagocytosis assay

Neutralization assay







Cytometry

A Flow Cytometry-Based Assay to Determine the Phagocytic Activity of Both Clinical and Nonclinical Antibody Samples Against *Chlamydia trachomatis*

Marco Grasse,[1,2] Ida Rosenkrands,[1] Anja Olsen,[1] Frank Follmann,[1] Jes Dietrich[1]*