# Antibody-based flow cytometry method for the detection of *A. muciniphila* in complex cell suspensions

**Filardi R** [1], Tytgat H [2], Reunanen J [3], De Vos W M [2,4], Mora D [1], Arioli S [1]

(1) University of Milan, DeFENS; (2) Wageningen University, Laboratory of Microbiology; (3) University of Oulu, Oulu Biocentre & Cancer and Translational Medicine Research Unit; (4) University of Helsinki, Human Microbiome Research Program Unit

## Introduction

***Akkermansia muciniphila* is a** mucin-degrading bacterium abundantly present in the human gut. Since its discovery, *A. muciniphila* has been recognized as a beneficial intestinal symbiont and is considered a **next-generation probiotic** (Zhang *et al* 2019). However, the isolation of new strains of this species is known to a be a challenge. In this study, **polyclonal antibodies** against *A. muciniphila* DSM22959 (type strain) were used to develop an immuno-flow cytometry (FCM) method **for the specific detection of *A. muciniphila* in complex cell suspensions**. Preliminary results shown that our method could be applied for the detection and quantification of *A. muciniphila*. This protocol will be further combined with fluorescence-activated cell sorting **(FACS) for improving the isolation** of new strains of this species.

## Methods

*A.muciniphila* cells (6 to 8 Log cells/ml) were labeled using indirect immunofluorescence in pure culture or in mixed cell suspensions

### Primary antibodies production

1. Polyclonal antibodies against *A. muciniphila* DSM22959 were raised in rabbit
2. Primary antibodies were purified from serum proteins using NAb Protein A Spin Columns (Thermo Fisher)

### Indirect immunolabeling protocol

1. Total cells number were determined using SYBR™ green I staining
2. As blocking step, cells were incubated in PBS, 5% goat serum (30' at RT)
3. 1 µg/ml of the primary antibodies was added (incubation 1 h at RT)
4. Cells were washed and resuspended in PBS, 3% (v/v) bovine serum albumin (BSA)
5. 1 µg/ml of the secondary antibodies (Alexa-488 goat anti-rabbit IgG) was added (1 h at RT in the dark)
6. FCM analysis was carried out by C6 plus BD Accuri® (excitation: 488 nm; emission: 530 nm).

## Results

A. Polyclonal antibodies resulted to be specific at species level being able to:

1. Detect *A. muciniphila* DSM22959 and new isolated strains of *A.muciniphila* **Fig.1A**
2. Not bind other intestinal species **Fig.1B**

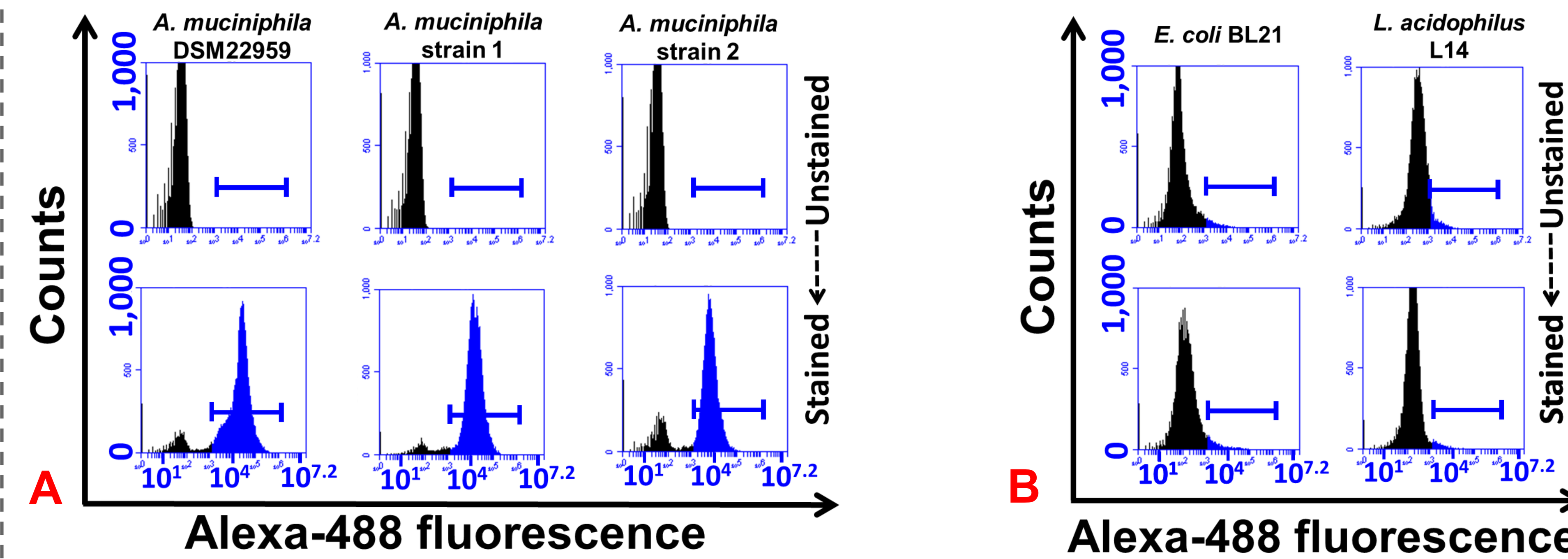


**Figure 1.** Examples of target cells (A) and not target cells (B) are reported as histograms before (unstained) and after (stained) the antibody labeling. Antibody-stained cells were identified in the blue gate.

B. The immune-FCM method was applied to detect and quantify *A. muciniphila* in multi-species samples, containing *A. muciniphila* DSM22959 and *Escherichia coli* BL21 or *Lactobacillus acidophilus* L14 at known concentrations. Pure culture of each species was quantified separately by SYBR™ green I staining, before being used for assembling the mixed cultures. Results show:

1. *A. muciniphila* was specifically detected in mixed cell suspensions **Fig. 2**
2. There is a good correlation between the quantification made by antibodies and SYBR™ green I staining in mixed cell suspensions **Fig. 3**
3. The quantification accuracy of *A. muciniphila* (in pure colture) made by antibodies compared to SYBR Green I staining, varies according to cell density **Fig. 4**

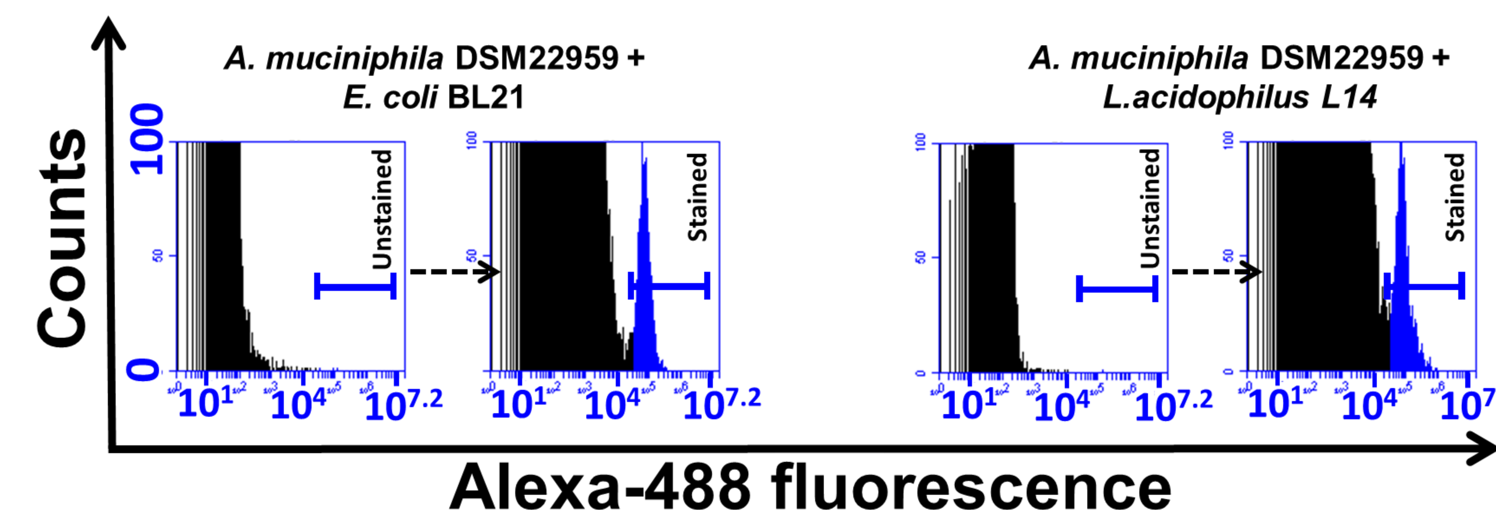


**Figure 2.** Flow cytometry histograms show the detection of *A.muciniphila* in mixed cell suspension containing *E.coli* (left) or *L.acidophilus* cells (right) after antibody staining.

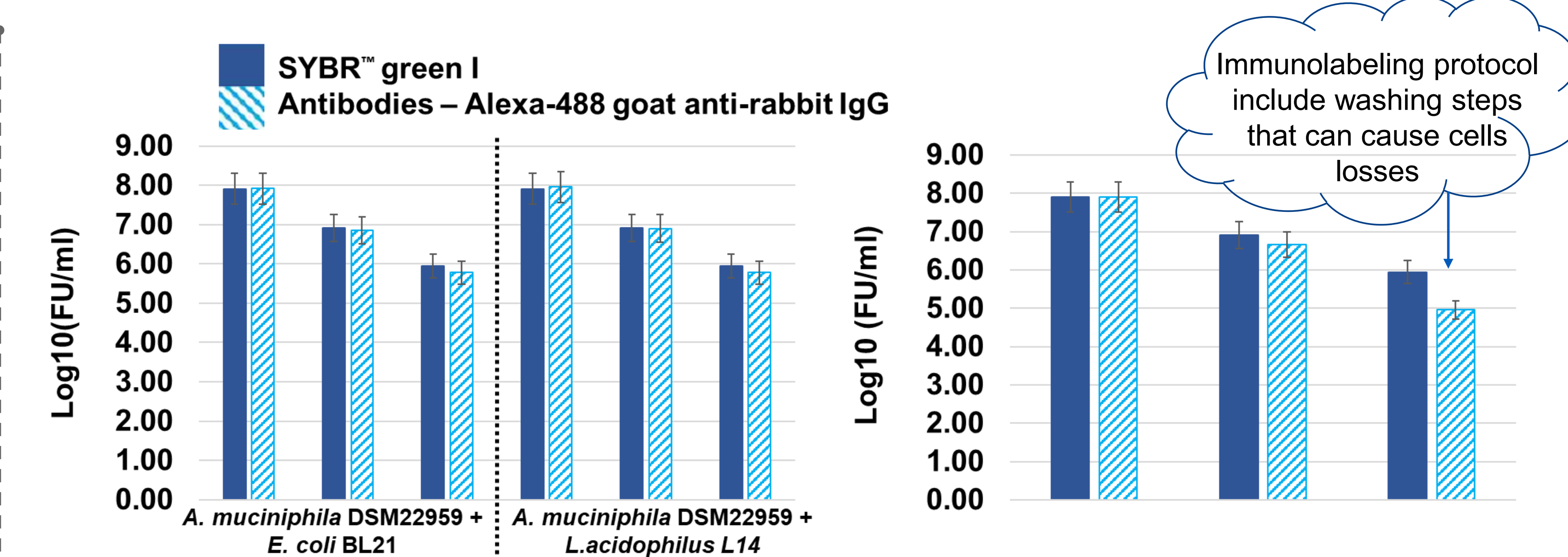


**Figure 3.** Quantification of different concentrations of *A. muciniphila* DSM22959 using SYBR ™ green I staining, before being mixed with *E. coli BL21* or *L.acidophilus L14 cells.* Specific quantification of *A. muciniphila* cells, in the mixed cell suspensions, was performed by antibodies labeling and compared with SYBR ™ green I data. Analytical imprecision is not higher than 5%. FU, fluorescent units.

**Figure 4.** Quantification of different concentrations of *A. muciniphila DSM22959* measured by FCM using SYBR™ green I or antibodies staining. Analytical imprecision is not higher than 5%. FU, fluorescent units.

C. The developed method was also able to detect *A. muciniphila* in enrichment cultures obtained inoculating fecal samples in minimal medium supplemented with mucin (Derrien *et al.* 2004) **Fig. 5**

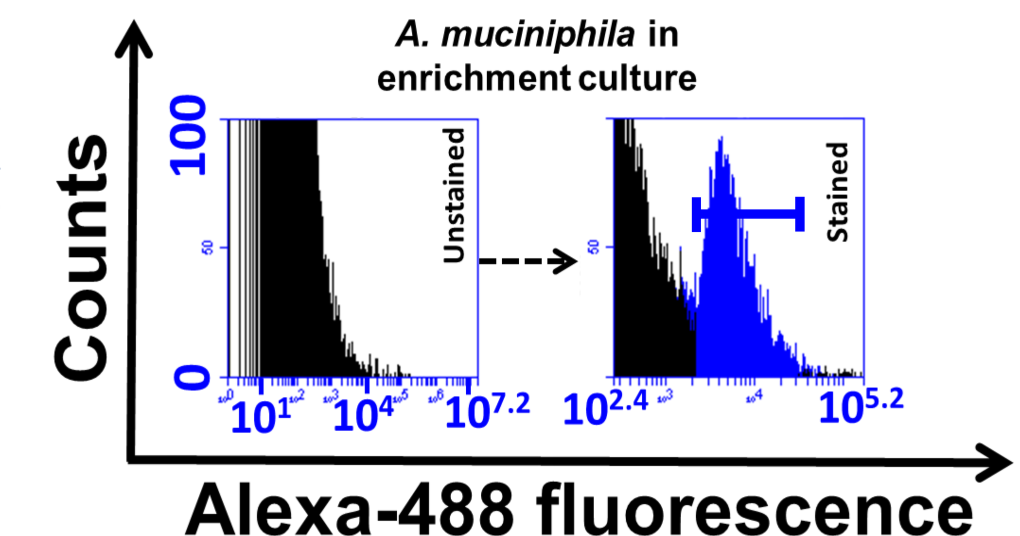


**Figure 5.** Flow cytometry histograms show the detection of *A.muciniphila* in enrichment cultures after antibody staining. Antibody-stained cells were identified in the blue gate.

## Conclusion

Our method enabled us to selectively detect and quantify *A. muciniphila* in mixed samples and in enrichment cultures. Considering that antibody-labelled cells shown to retain their viability, our protocol will be further combined with FACS analysis as a new strategy to directly isolate new *A. muciniphila* strains. Improving the isolation process could greatly enhance our knowledge of this bacterial species.

## References

1. Zhang T *et al*. Microbial Biotechnology (2019) 12(6), 1109–1125
2. Derrien M *et al. International Journal of Systematic and Evolutionary Microbiology (2004), 54, 1469–1476*

**rossella.filardi@unimi.it**