Detection of *Lactobacillus* and *Gardnerella* species in vaginal samples by PNA-FISH.

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Introduction

Bacterial vaginosis (BV) is a common vaginal infection of women in reproductive age. This infection is initially asymptomatic and late diagnosis can increase the health costs and hamper treatment. Therefore, an easy and quick method to detect the transition between normal microflora to the predecessors of infection is of upmost importance.

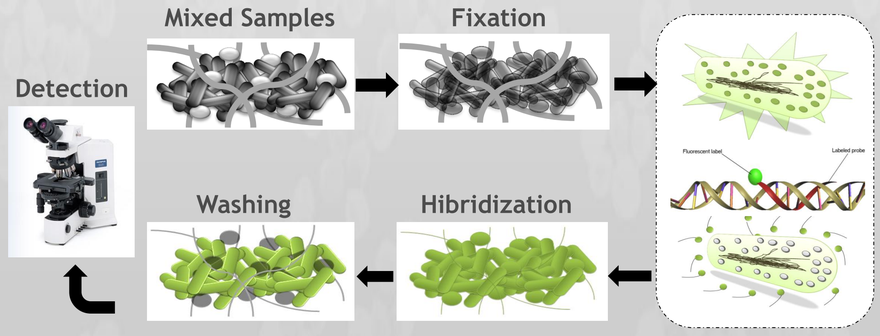
Although BV etiology remains unknown, it is commonly accepted that normal to BV microflora transition is characterized by a decrease in *Lactobacillus* species and an increase in *Gardnerella vaginalis* (*GV*) number. *GV* is found in normal vaginal epithelium but its biofilm could be responsible for the establishment of BV.

In this work, our goal was to develop two novel Peptid Nucleic Acid (PNA) probes for Fluorescence *in situ* Hybridization (FISH) multiplex analysis, aimed to detect *Lactobacillus* spp. and *G. vaginalis* in vaginal samples.

Methods

Fluorescence *In Situ* Hybridization - FISH

1. Probe design and evaluation (using RDPII database and Primrose);
2. Specificity and sensitivity test (using 85 representative culture collection strains);
3. Application to vaginal samples.



Results

PNA-FISH Methodology Evaluation

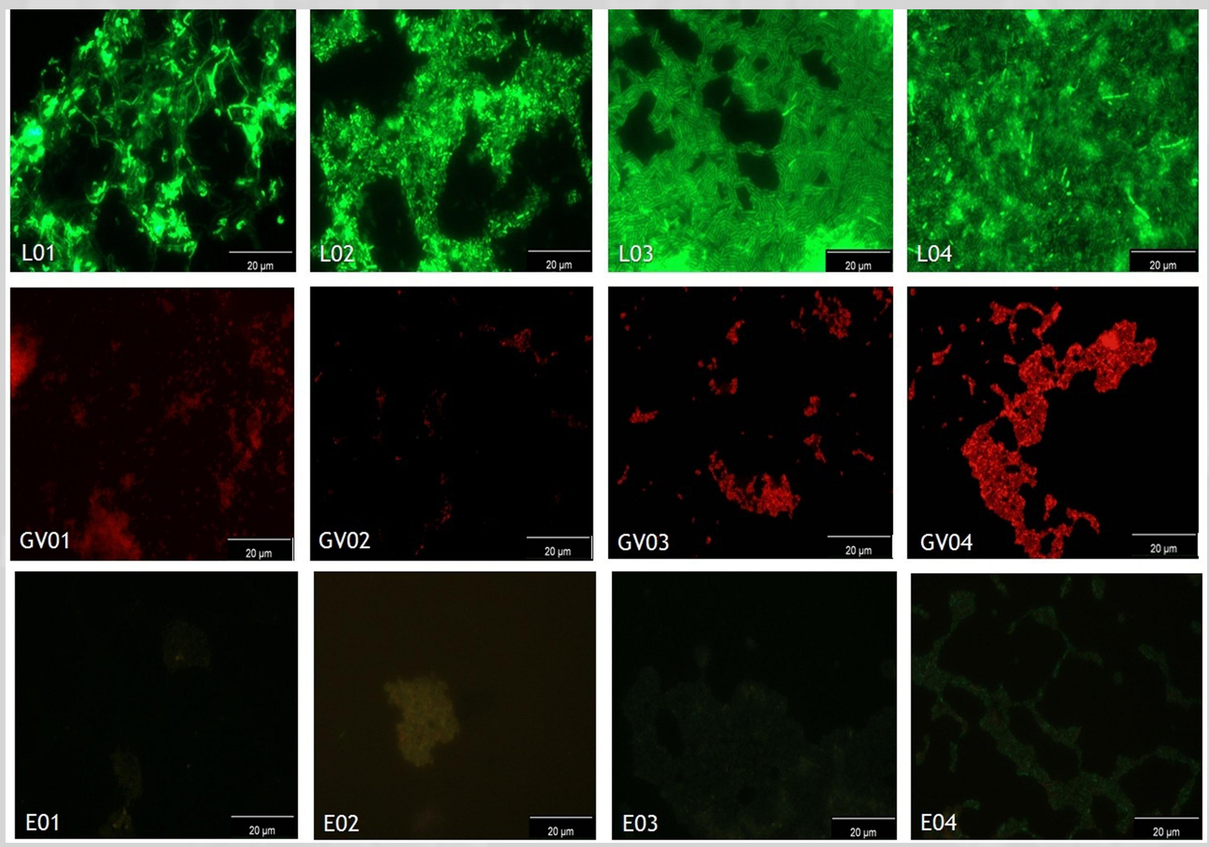


Figure 1 – Fluorescence microscopy pictures of *Lactobacillus spp.* species (L01-04), *Gardnerella vaginalis* (GV01-04) and others related bacteria (E01-04) by specific PNA probe (Lac663 and Gard162) associated with Alexa Fluor 488 and 594 fluorochromes, respectively.

Legend – L01, *L. paracasei* CECT227; L02, *L. delbrueckii* ATCC9649; L03, *L. murinus* ATCC35020; L04, *L. salivarius* 438; GV01, *G. vaginalis* 5-1; GV02, *G. vaginalis* ATCC; GV03, Belgian *G. vaginalis* isolate 17; GV04, Belgian *G. vaginalis* isolate 18; E01, *Streptococcus thermophilus* A; E02, *Leuconostoc mesenteroides*; E03, *Enterococcus faecium*; E04, *Enterococcus faecalis*.

Table 1 – Results of the Multiplex PNA-FISH assays for *Lactobacillus* spp. and *Gardnerella* spp. detection in vaginal samples.

Legend – BV diagnostic in the vaginal samples were classified with the following evaluation: (+) presence confirmed; (-) absence confirmed. While PNA probes (Lac663 and Gard162) efficiencies were tested in each sample, with the following a hybridization PNA-FISH qualitative evaluation: (-) Absent hybridization; (+) Poor hybridization; (++) Moderate hybridization; (+++) Good hybridization; Optimal hybridization. The table shows the media value from the three experiments for each strain.

Vaginal samples	BV diagnostic	Multiplex PNA-FISH assay	
		Lac663 Probe efficiency	Gard162 Probe efficiency
S01	-	+++	+++
S02	-	++++	-
S03	-	+++	+++
S04	-	++	+++
S05	-	++++	-
S06	-	++	++
S07	-	++	++
S08	-	+++	+++
S09	-	++	++
S10	-	++	-
S11	+	-	++
S12	+	-/+	-
S13	+	-	++
S14	+	-	++++
S15	+	-	+++
S16	+	-	+++
S17	+	-	-/+

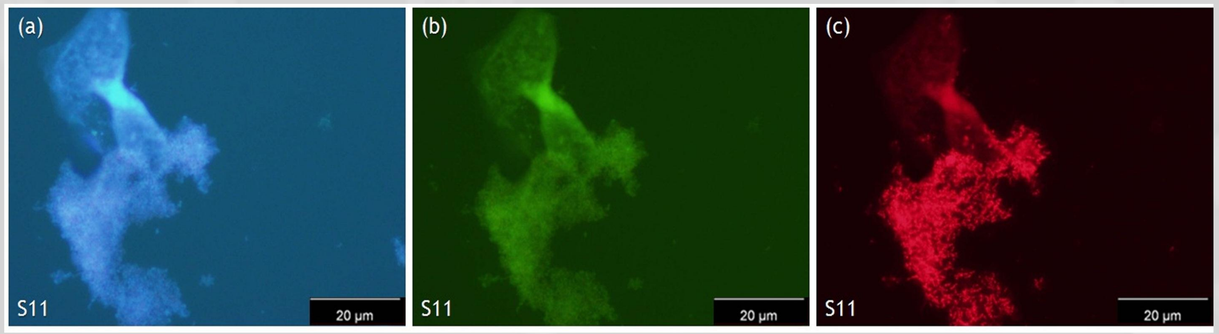


Figure 2 – Fluorescence microscopy pictures of *Lactobacillus* spp. and *G. vaginalis* species from a BV vaginal sample (S11) by specific PNA probe (Lac663 and Gard162) associated with Alexa Fluor 488 and 594 fluorochromes, respectively.

Legend – (a) blue filter; (b) green filter; (c) red filter.

Conclusions

We designed, evaluated and validated both probes by using 36 representative *Lactobacillus* strains, 22 representative *Gardnerella vaginalis* strains and 27 others taxonomically related or pathogenic bacteria strains usually found in vaginal samples. The probes were also tested in 17 vaginal samples, collected from women with normal vaginal microflora or women with BV. These probes proved to be a powerful tool for the simultaneous detection and visualization of *Lactobacillus* spp. and *G. vaginalis* in bacterial strain collections and vaginal samples.

In conclusion, the PNA-FISH methodology represents a reliable tool for *Lactobacillus spp.* and *G. vaginalis* detection in clinical samples, allowing specific and direct detection. Furthermore, this methodology also provides spatial organization information in relation to the presence or absent of clue cells as well as *Lactobacillus spp.* or/and *G. vaginalis* adherence against vaginal epithelial cells.

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