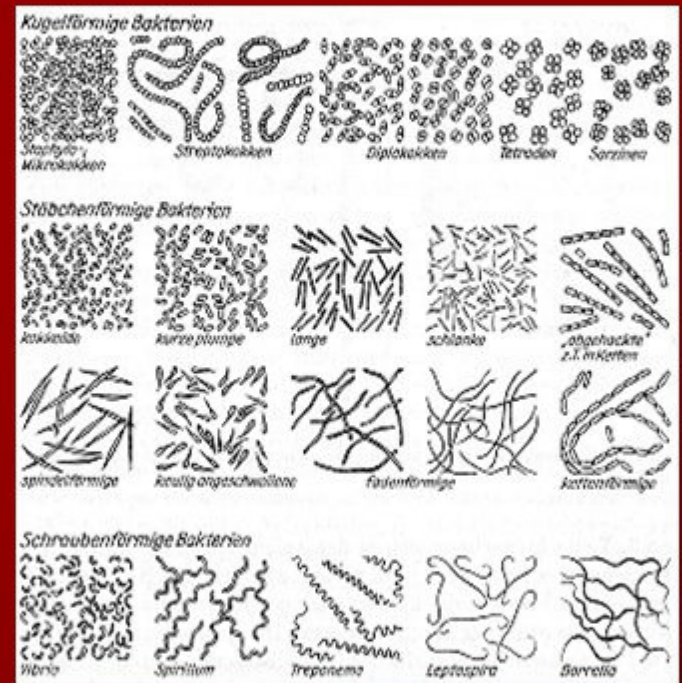
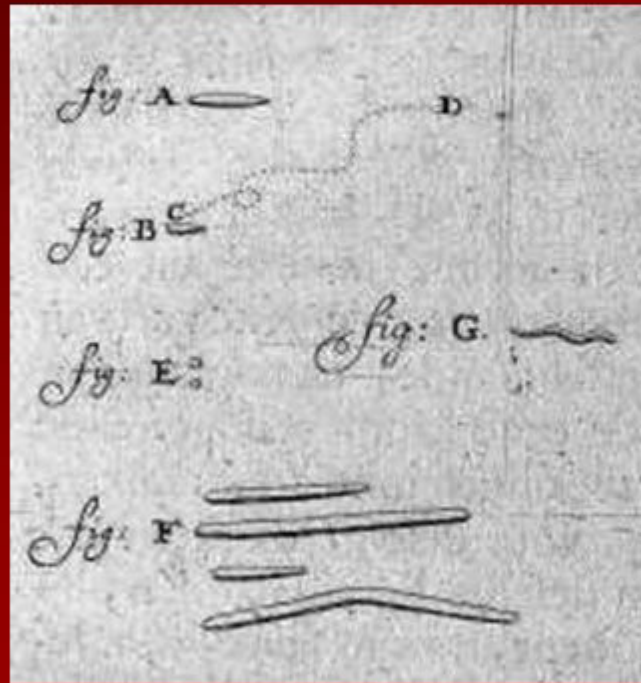


# Anno 1683

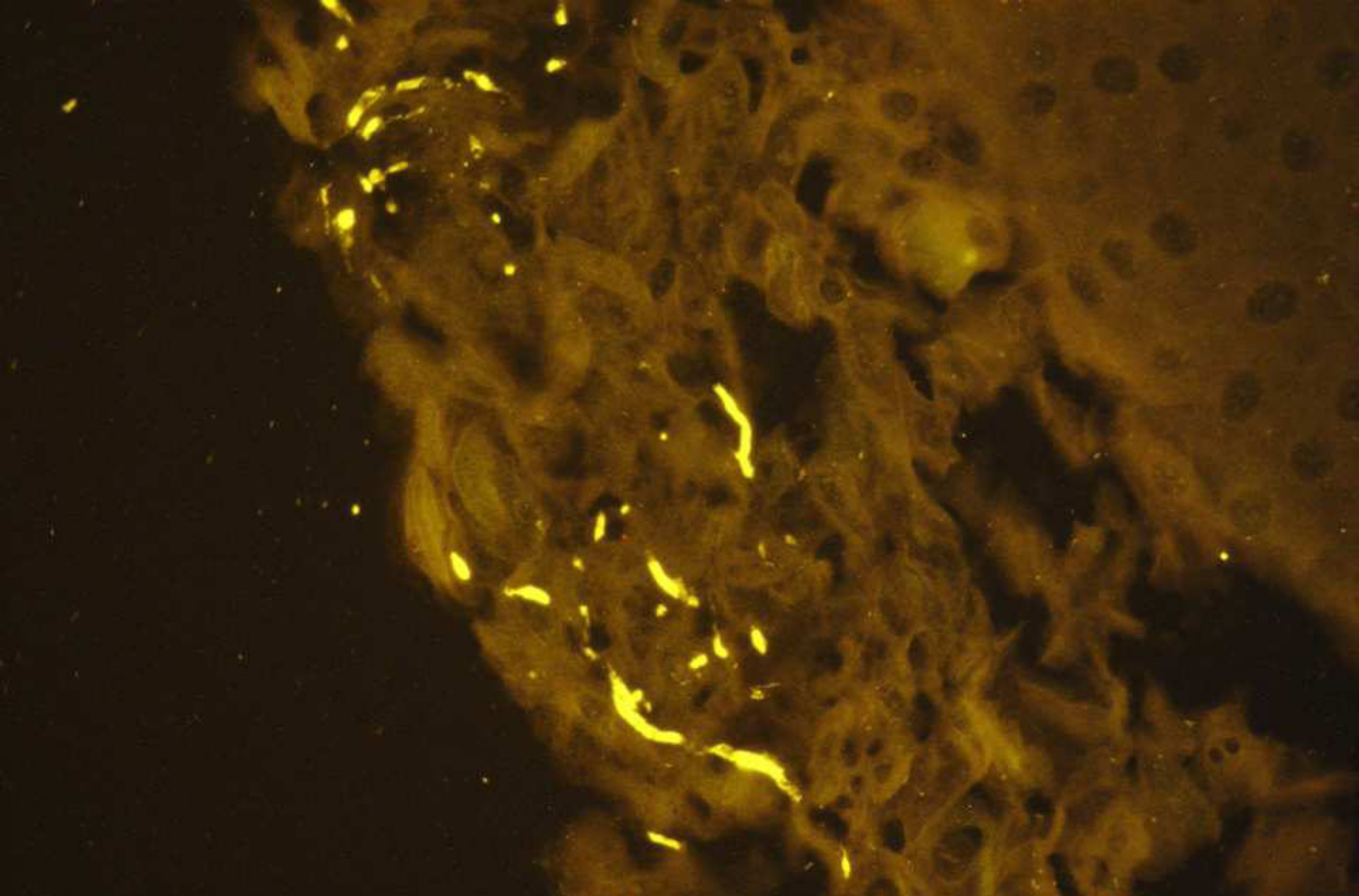


# Archeogenesis Hypothesis

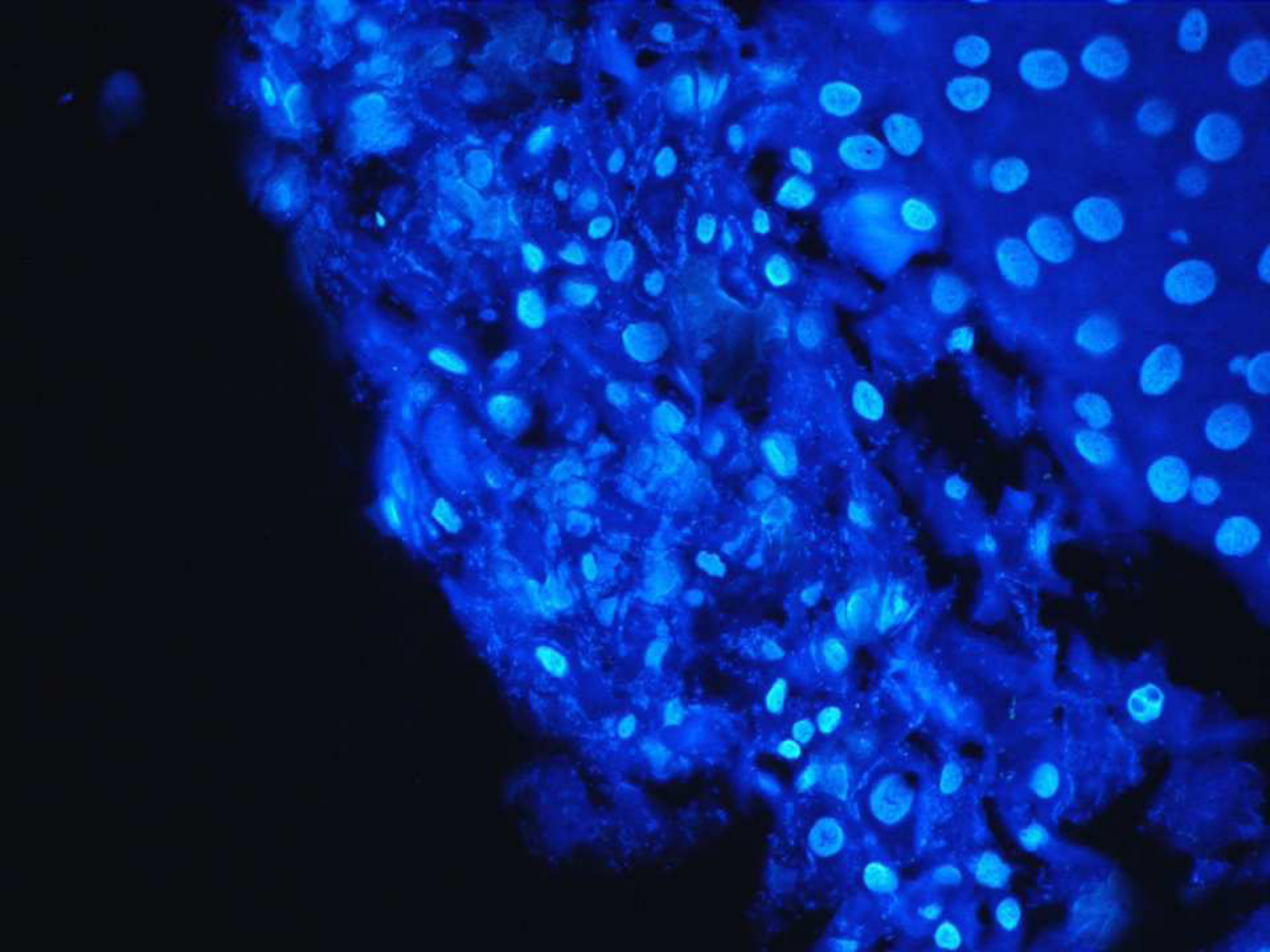
A high-magnification light micrograph showing a cross-section of tissue. The upper portion shows a layer of stratified epithelium. Below it, the subepithelial connective tissue is exposed, showing a dense network of collagen fibers and scattered cells. Numerous small, dark, rod-shaped structures, identified as bacteria in the caption, are visible within the subepithelial tissue, particularly in the lower right quadrant. The overall appearance is that of a biopsy site where the epithelial barrier has been disrupted, allowing for the mechanical introduction of bacteria into the underlying tissue.

Subepithelial tissue exposed due to biopsy tear.  
Bacteria are introduced mechanically here.

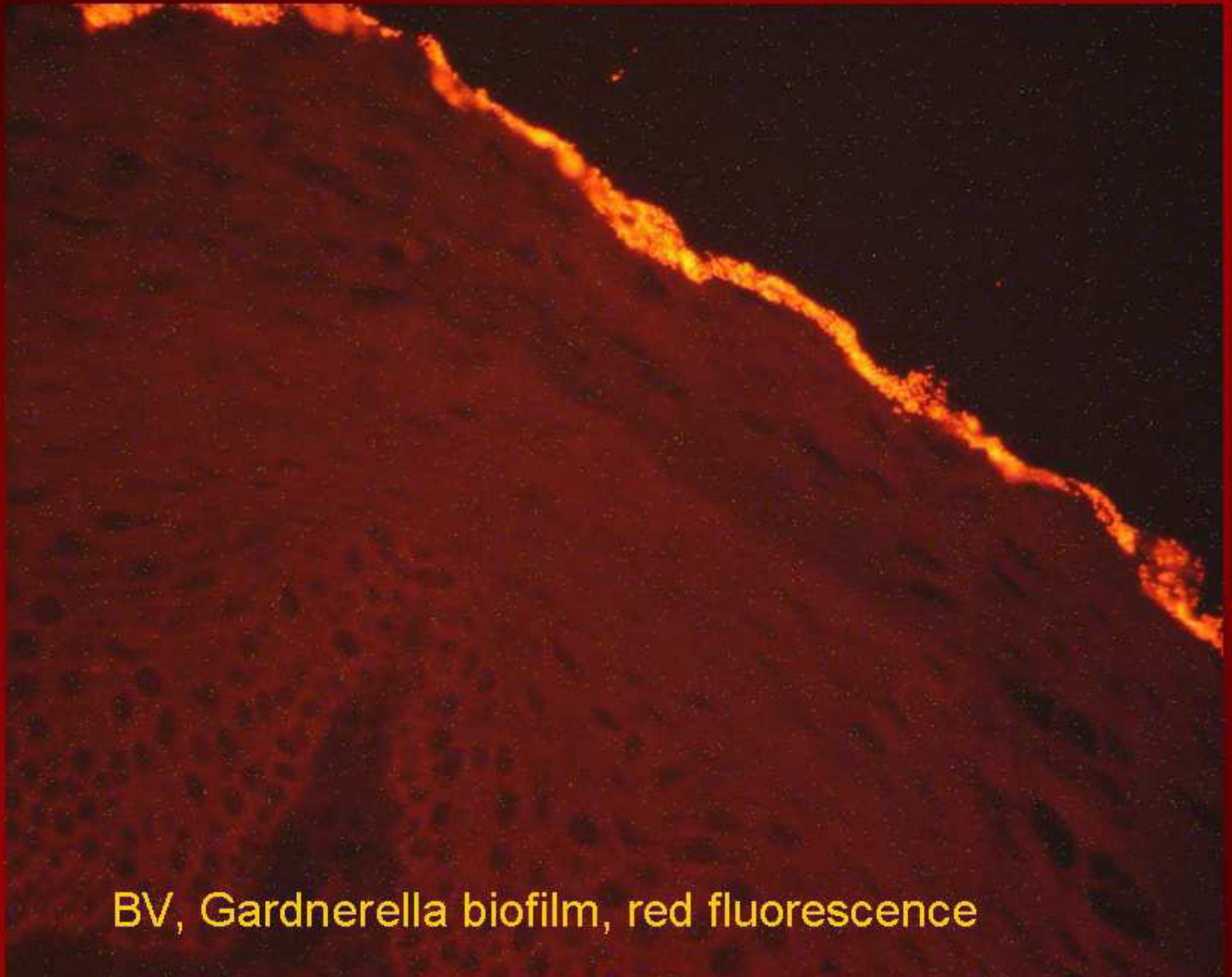




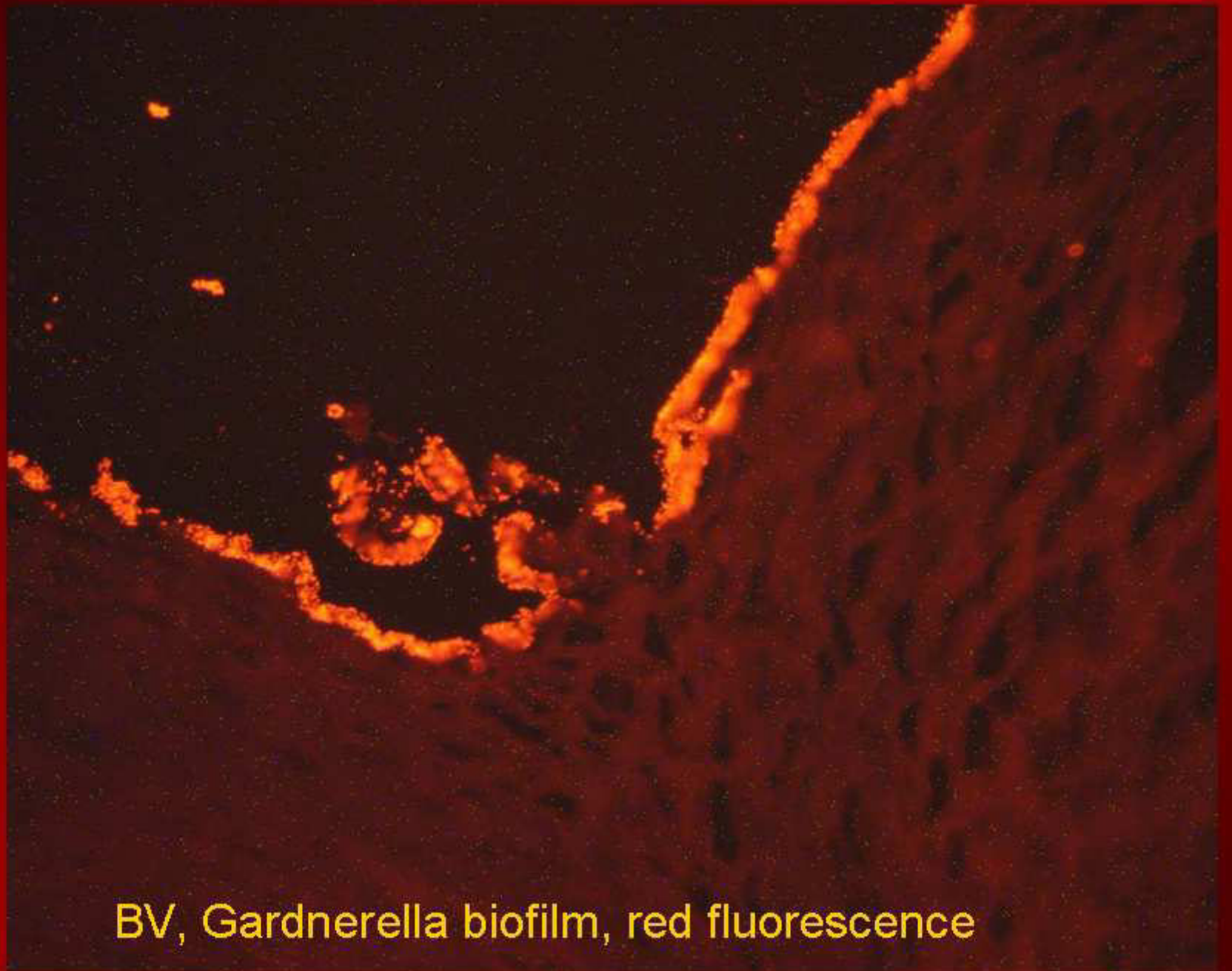
Fungal infection of the vaginal epithelium







BV, Gardnerella biofilm, red fluorescence



BV, Gardnerella biofilm, red fluorescence

- What is the role of
  - Gardnerella
  - Lactobacilli
  - Other bacterial groups?

## Occurrence of bacteria in vaginal biopsies as detected by FISH (N=141)

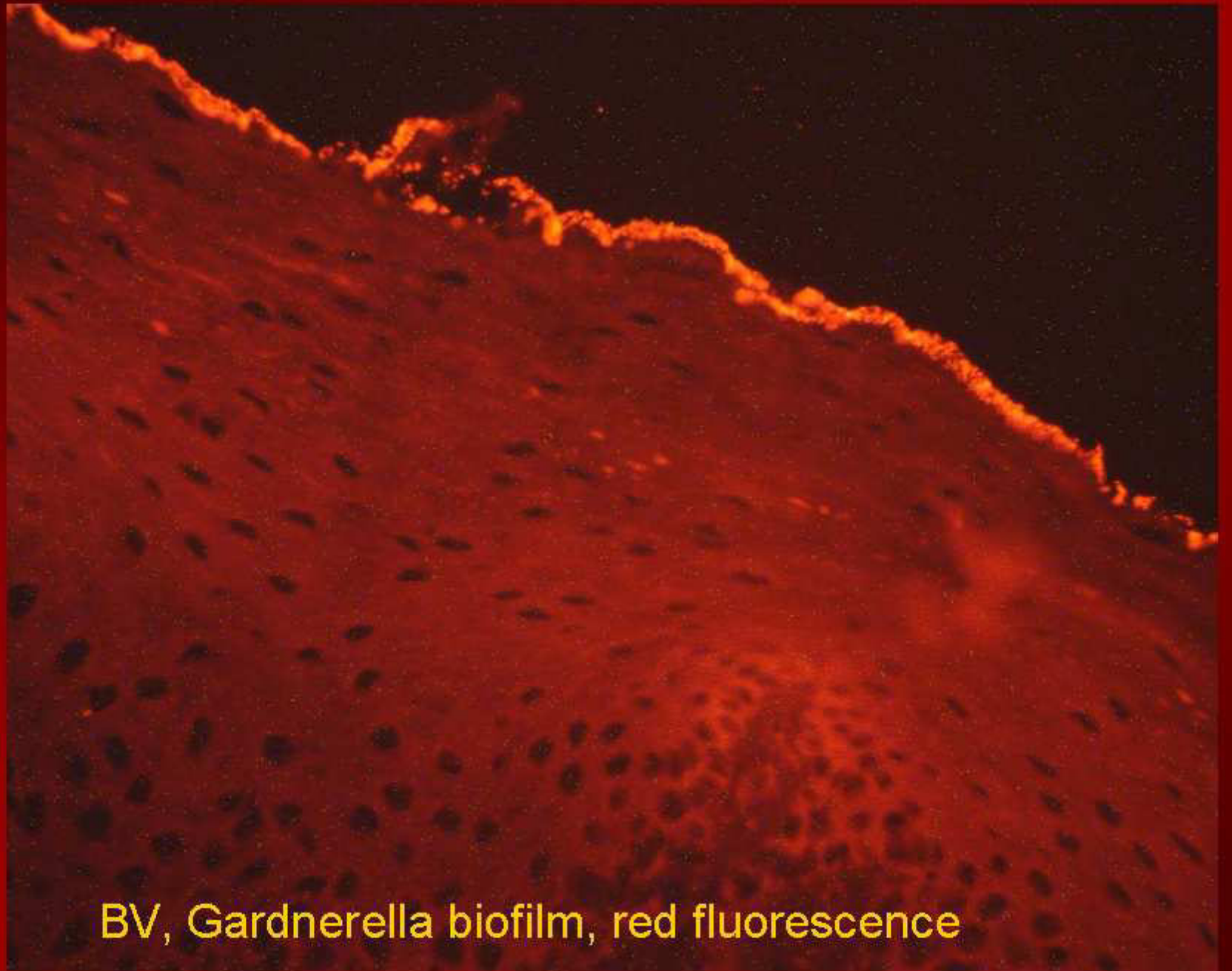
	Structured polymicrobial <i>Gardnerella</i> biofilms	Mixed islands of bacterial accumulations attached to the vaginal epithelium	Diffuse nonadherent bacteria in secretions	Singular or no bacteria
Healthy controls N=20	<b>1 (1)</b>	7	<b>4 (2)</b>	<b>14</b>
Healthy controls postmenopausal N=31	<b>1 (1)</b>	<b>4 (1)</b>	<b>13 (2)</b>	<b>12</b>
Anaerobe vaginitis N=12	<b>0</b>		<b>1</b>	<b>11</b>
Vaginal candidiasis N=10	<b>7 (7)</b>	<b>2 (1)</b>	<b>1 (1)</b>	<b>0</b>
<b>Bacterial vaginosis</b> N=68	<b>66 (66)</b>		<b>2 (2)</b>	<b>0</b>

Numbers of patients with positive *Gardnerella* detection are given in parenthesis



Slime over vaginal surface, *Gardnerella* is red





BV, Gardnerella biofilm, red fluorescence



Abundant Lactobacilli, yellow fluorescence  
within Gardnerella biofilm

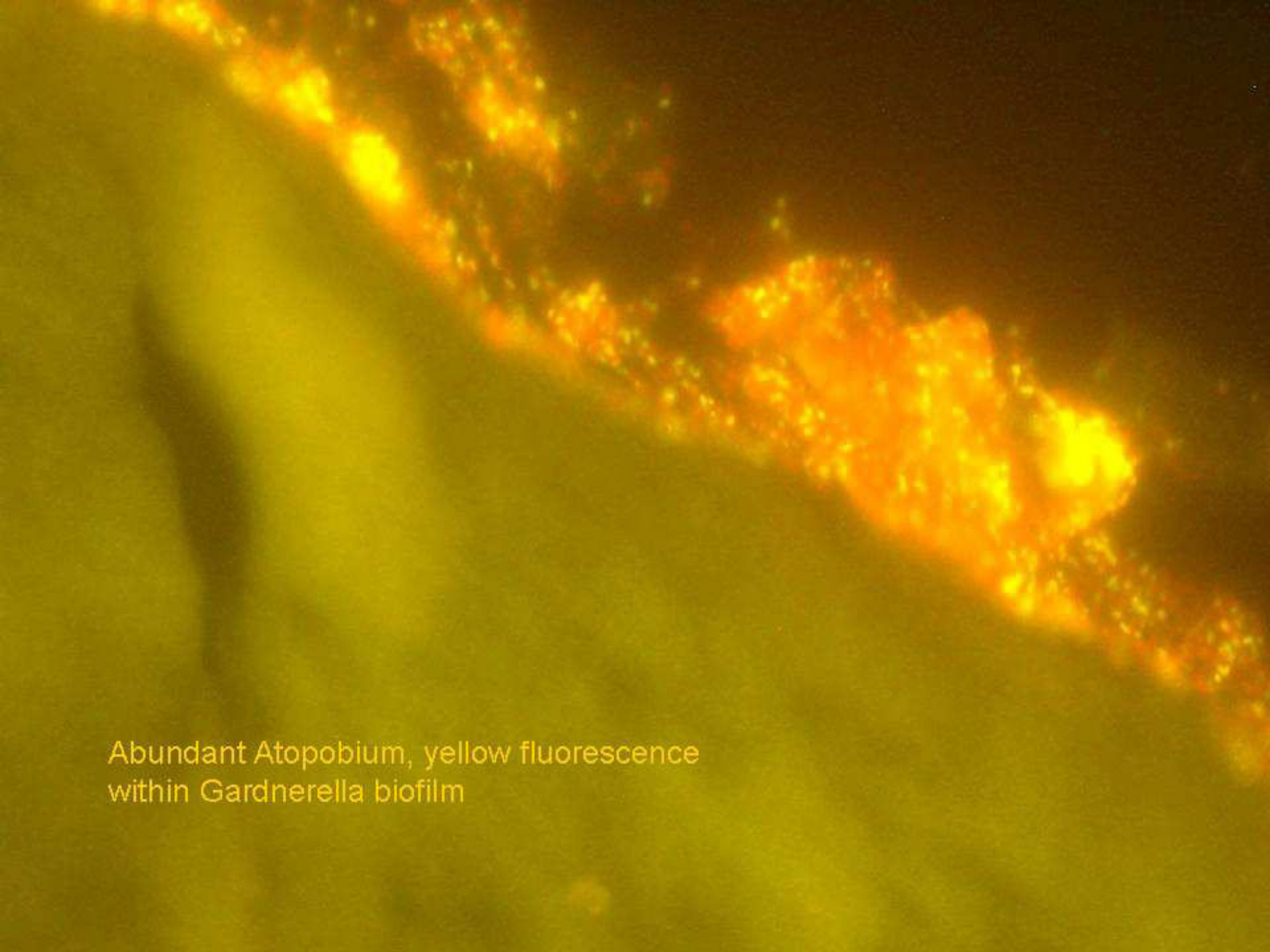


- **A surgeon was not required to wash his hands before seeing a patient because such practices were not considered necessary to avoid infection. In 1871 a guest wrote that Lister regularly “wore an old blue frock-coat for operation, which he had previously worn in the dissecting room,” and which was “stiff and glazed with blood.” Dirty coats were seen as a sign of a surgeon’s knowledge and experience, and the smell was referred to as “good old surgical stink.”**

A fluorescence microscopy image showing a Gardnerella biofilm. The biofilm is a dense, yellowish structure on the left side of the frame. A prominent, bright yellow fluorescent line runs diagonally from the top right towards the bottom right, representing abundant Lactobacilli. The background is dark, and there are some faint, blurry spots of light.

Abundant Lactobacilli, yellow fluorescence  
within Gardnerella biofilm



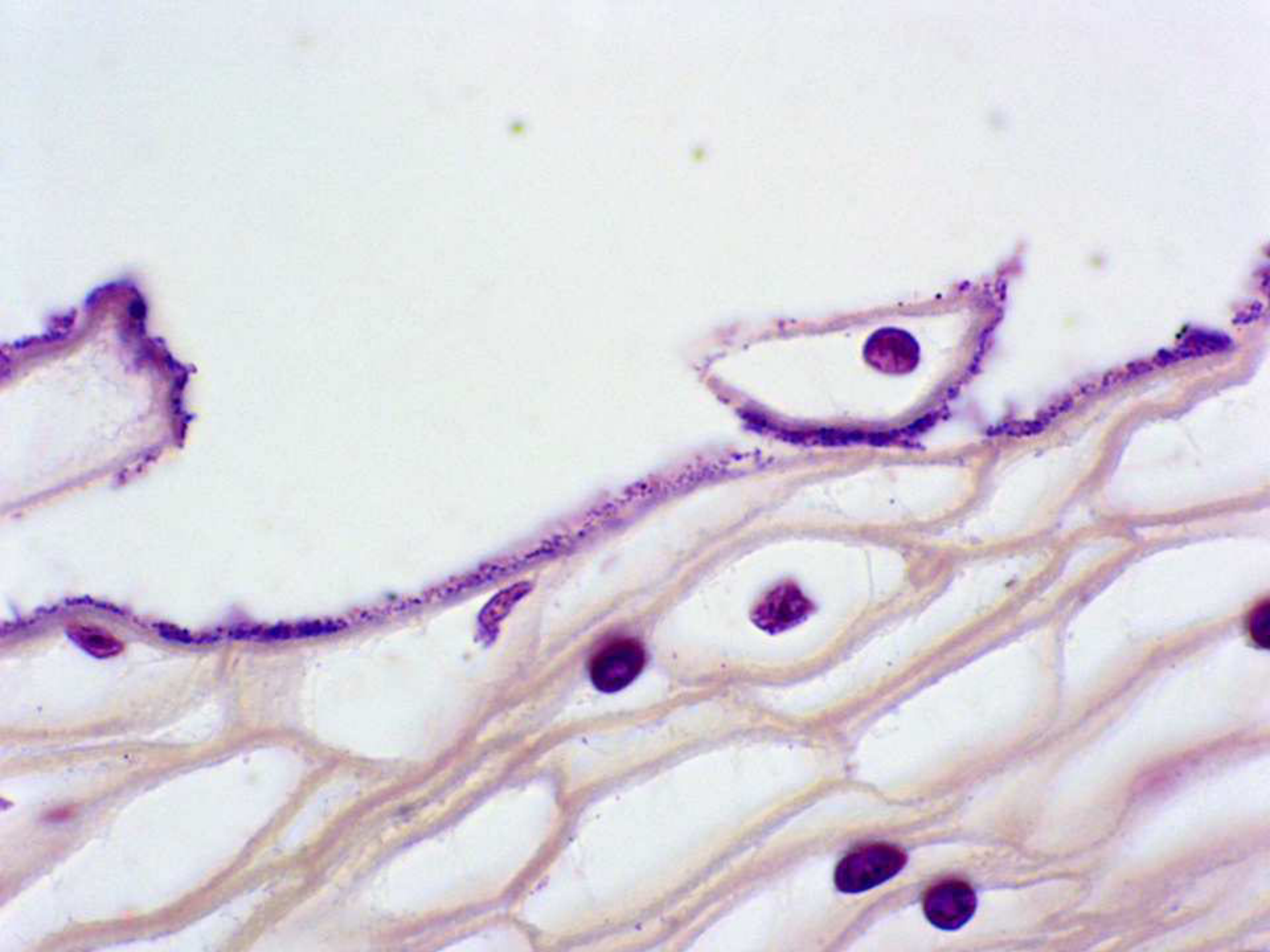


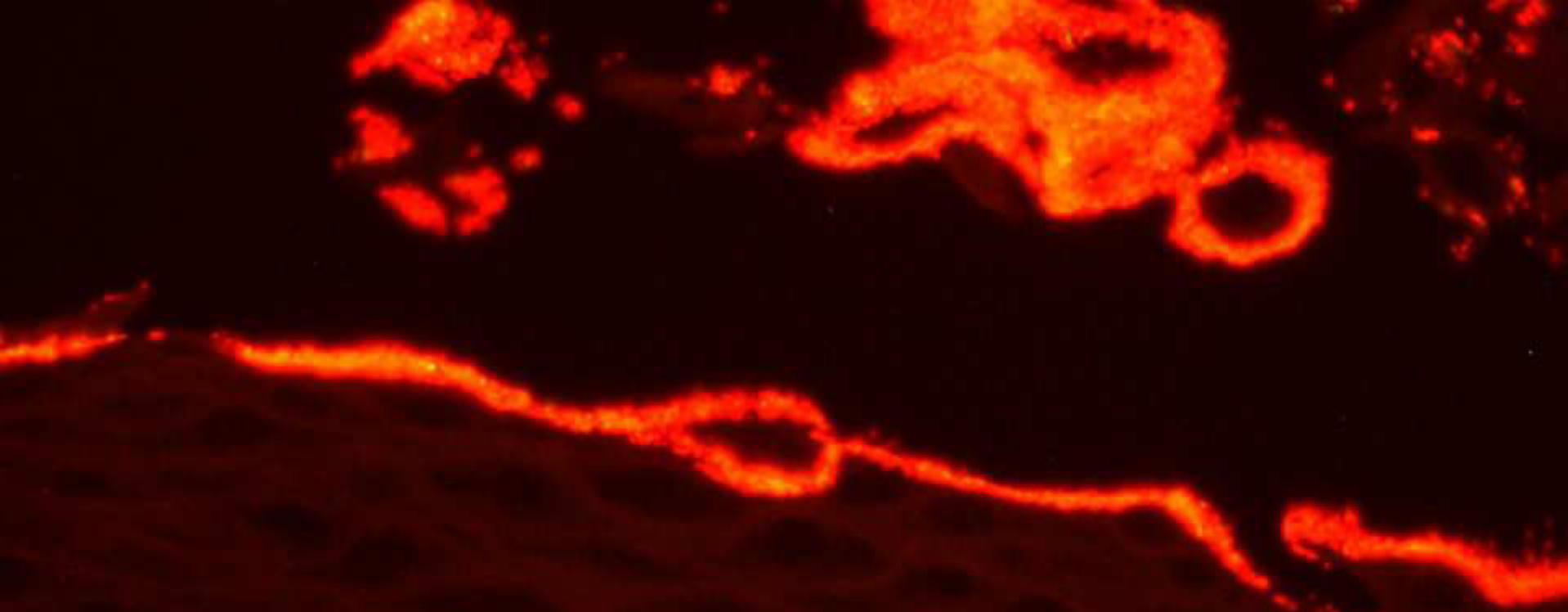
Abundant *Atopobium*, yellow fluorescence  
within *Gardnerella* biofilm



## Occurrence and mean concentrations of bacteria in the vaginal biopsies as detected by FISH

	Healthy N=51 (49)**		BV N=68	
	Mean±SD*/Max. concentration x 10 <sup>9</sup> /ml	Occurrence	Mean±SD*/Max. concentration x 10 <sup>9</sup> /ml	Occurrence
<b>Gardnerella (Gard 5)</b>	0.03±0.04/0.1x10 <sup>8</sup>	14% (7)	<b>31.8 ± 21.8/ 120 x 10<sup>8</sup></b>	<b>97% (66)</b>
Atopobium (Ato)	0.17±0.26/0.5x10 <sup>8</sup>	8% (4)	<b>5±4.1 /30 x 10<sup>8</sup></b>	<b>60% (41)</b>
Lactobacillus (Lab)	0.7 ±1/4x10 <sup>8</sup>	39% (20)	<b>2.8±3.8/20 x 10<sup>8</sup></b>	<b>81% (55)</b>
Enterobacteriaceae (Ebac)	0.01 - 3 x 10 <sup>8</sup>	4% (2)	0.5±0 /3 x 10 <sup>8</sup>	12% (8)
Coriobacterium (Cor)	0.01-0.1 x10 <sup>8</sup>	6% (3)	1.9±1.7/4 x 10 <sup>8</sup>	15% (10)
Cytophaga-Flavobacteria (CF)	0	0	1±1/3 x 10 <sup>8</sup>	13% (9)
Veillonella (Veil)	0	0	0.18 ± 0.35 /1 x10 <sup>8</sup>	12% (8)
Bacteroides (Bac)	0	0	0.17±0.19/0.5x10 <sup>8</sup>	10% (7)
Clostridien (Clit, Chis, Erec)	0	0	0.12±0.19/0.5x10 <sup>8</sup>	9% (6)
Fusobacterien (Fus)	0	0	0.2±0.05/0.1x10 <sup>8</sup>	6% (4)



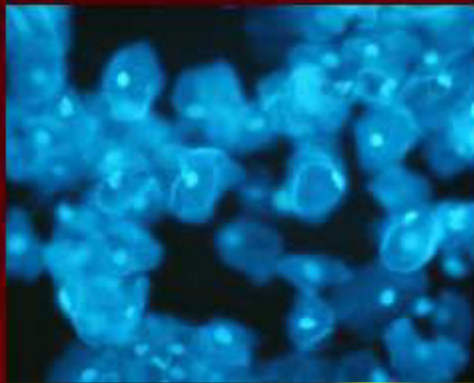


Clue cells

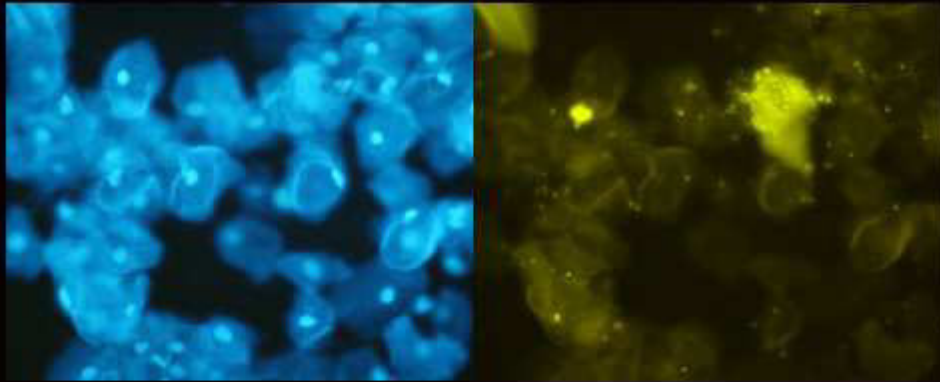


A microscopic image showing a dense population of vaginal epithelial cells. The cells are stained with DAPI, which highlights their nuclei in a bright blue color. The cells themselves are larger and have a more granular, pinkish appearance. The overall field is filled with these cells, with some showing distinct nuclei and others appearing more confluent.

**Urine sediment, vaginal epithelial cells,  
DAPI stain,  
healthy women**



**All bacteria (universal bacterial probe – yellow fluorescence), healthy women**

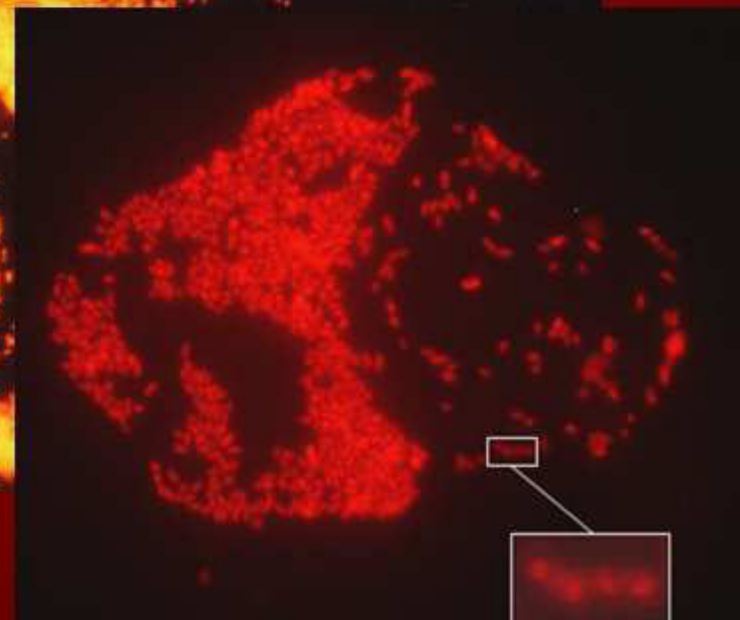
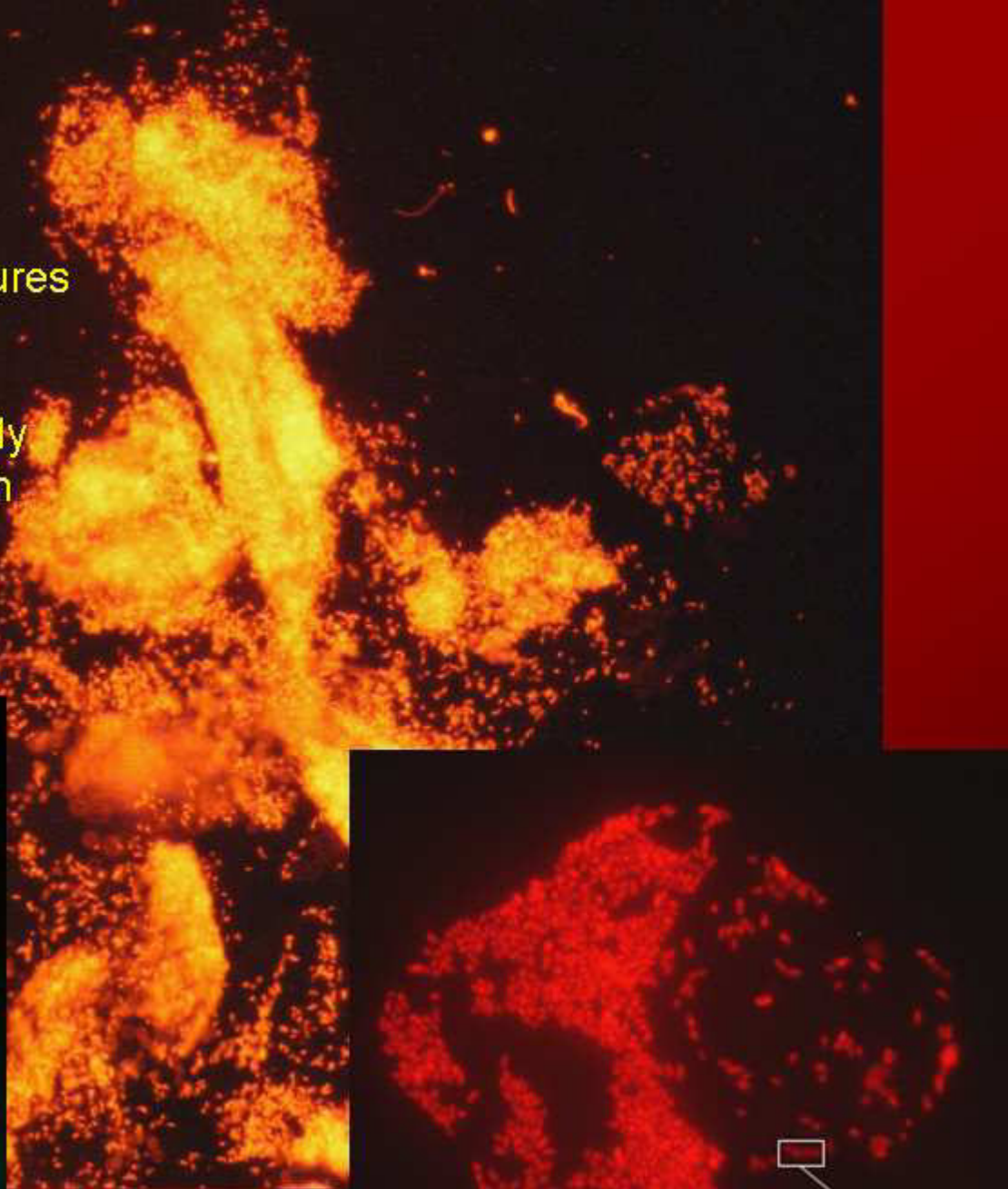
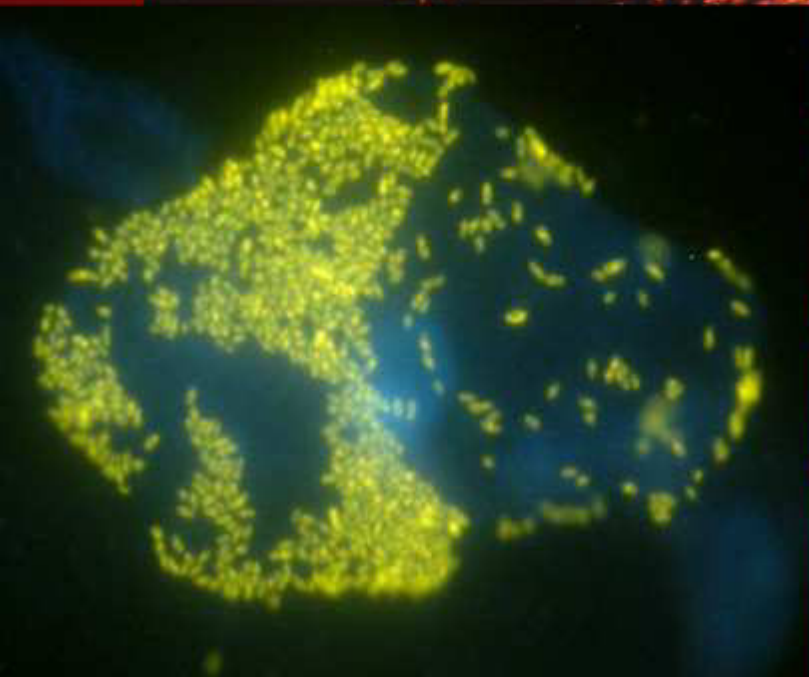


**Dispersed Gardnerella (reds fluorescence) in relation to epithelial cells (DAPI, left insertion) and other bacteria (Eub 338, right insertion)**



*Gardnerella* arrangement to structures resembling masonry of brickwork.

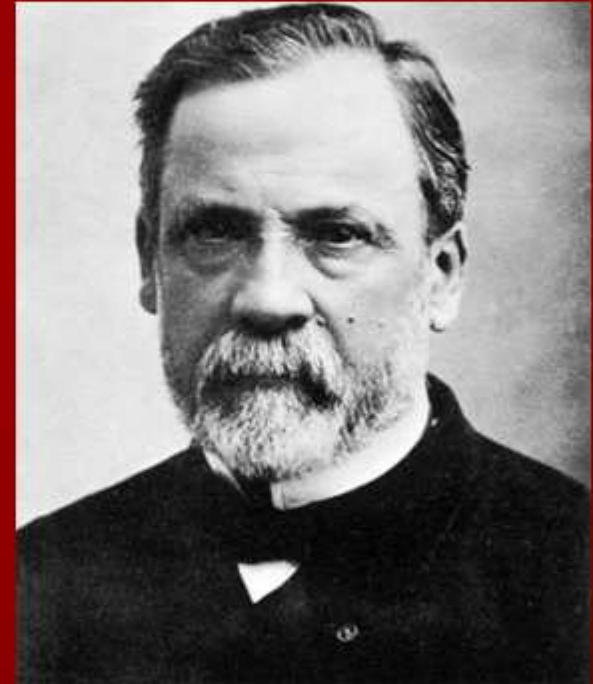
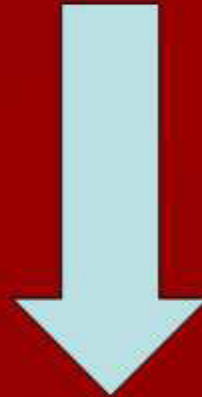
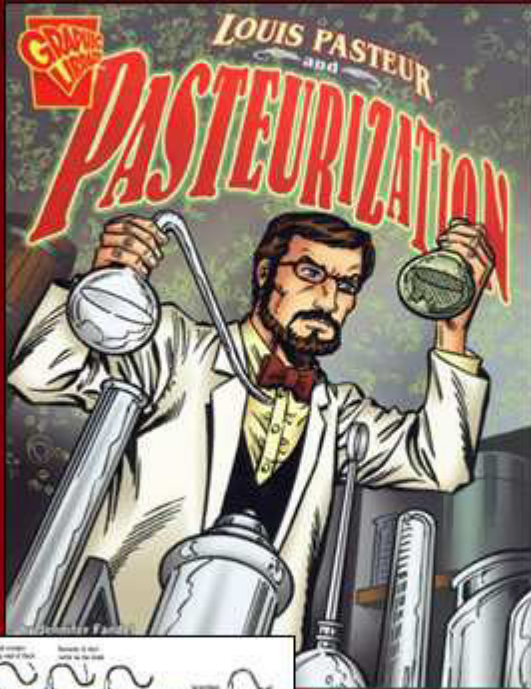
*Gardnerella* is a short rod with a dark spot in the center of the body. Because of this spot, the bacterium can be mistaken as a short chain of cocci (insertion)



Occurrence (patients/samples) and concentrations of selected bacterial groups associated with desquamated vaginal epithelial cells in urine sediments of bacterial vaginosis and healthy women collected over duration of 8 weeks

N=patients/ samples	StPM- <i>Gardnerell</i> <i>a</i>	Disperced <i>Gardnerella</i>	Lab	Ato
Healthy N=10/150	0/150 (0)	18/150 (2) 12%	93/150 (10) 62%	10/150 (3) 7%
BV N=4/60	4/60 (56) 94/100%*		49/60 (4) 82%	42/60 (3) 70%

# Anno 1864



## Definition of Pathogens

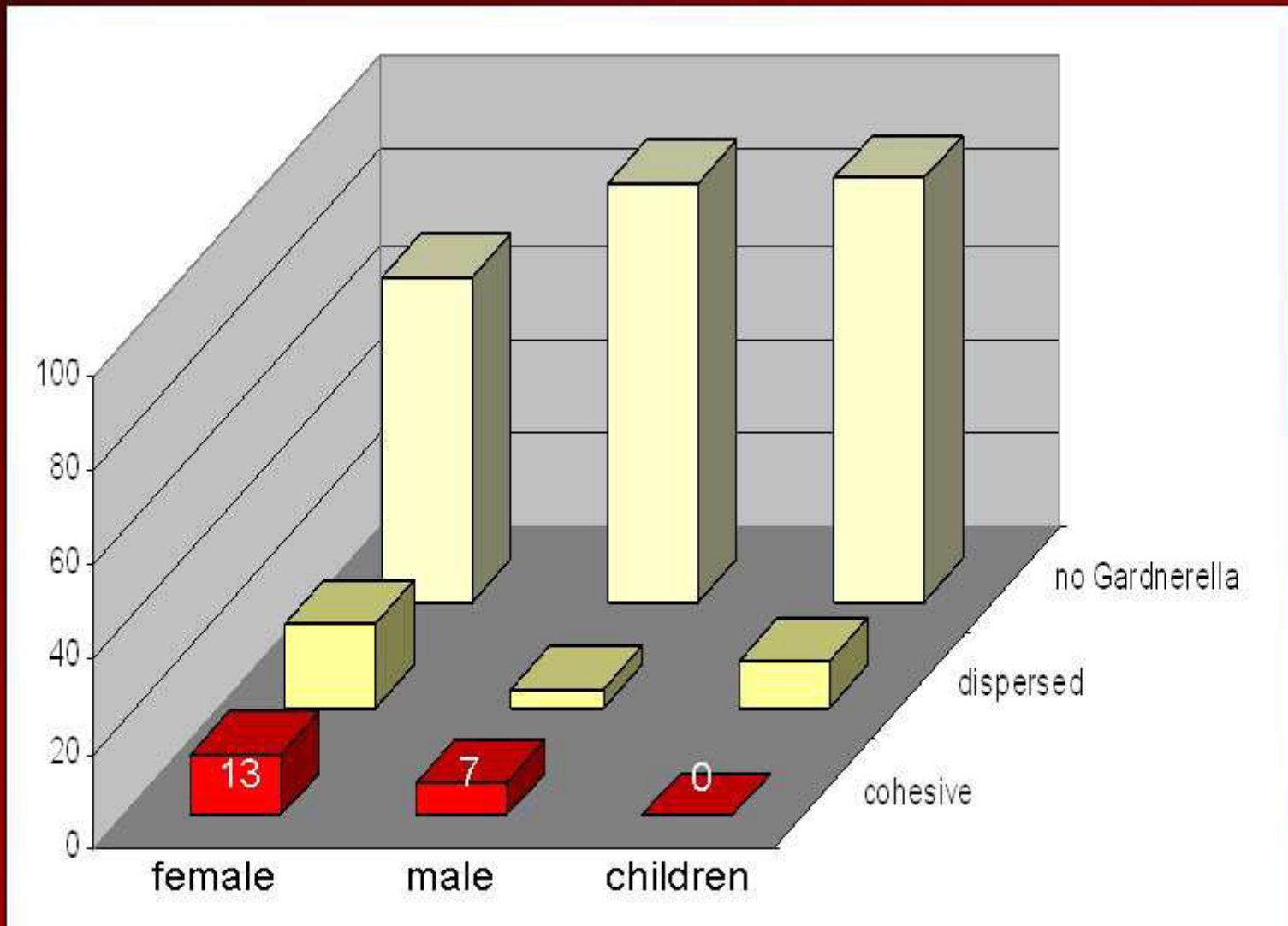


The image is a fluorescence micrograph showing a complex, multi-colored biofilm. The background is a dense network of red and orange filaments and clusters. There are several bright, yellowish-green spots scattered throughout, particularly in the upper and central regions. The overall appearance is that of a highly structured and diverse microbial community.

Multicolor FISH. prolific *Gardnerella* biofilm (red fluorescence)  
is associated with high concentrations of *Lactobacilli* (orange fluorescence),  
symptomatic BV

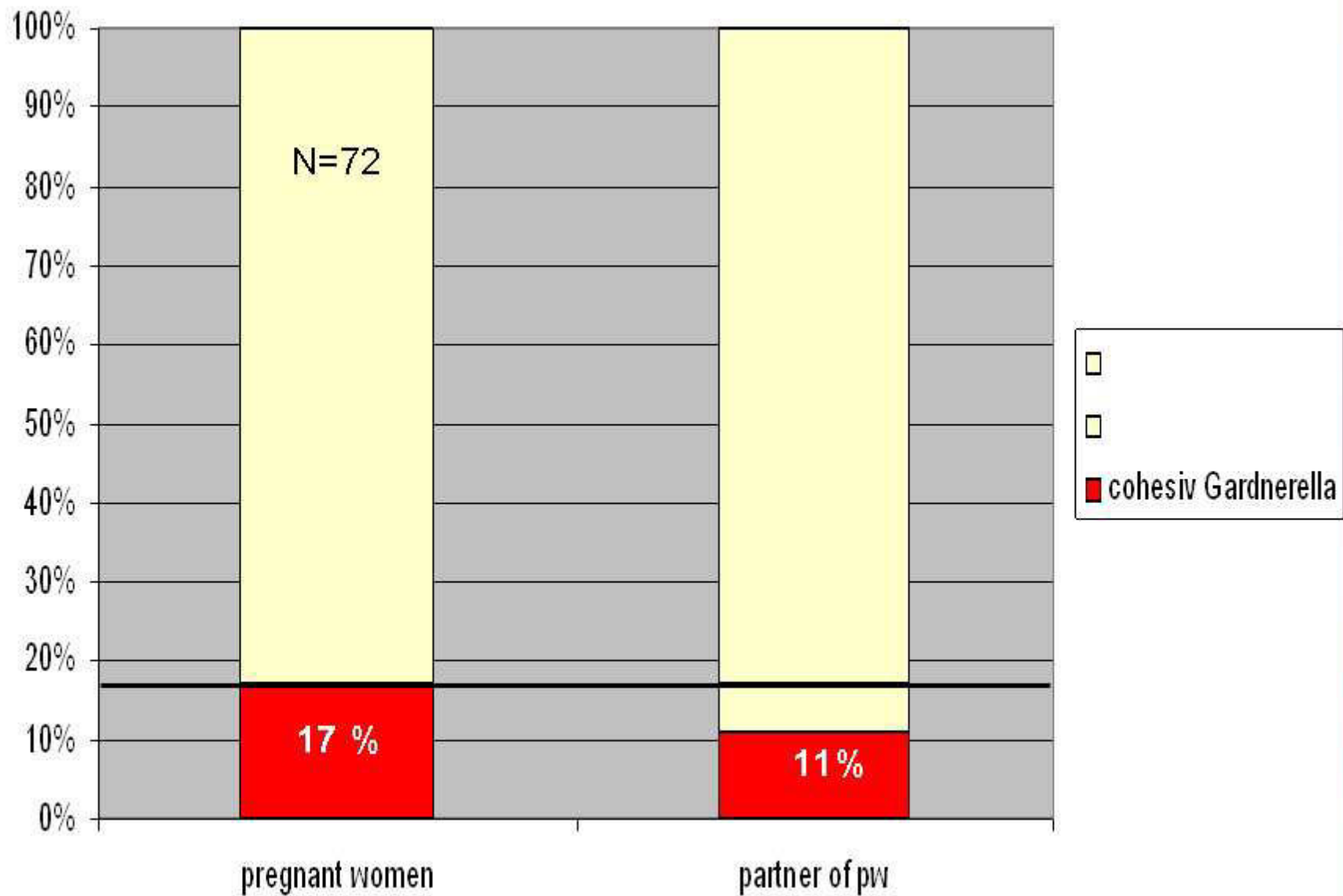


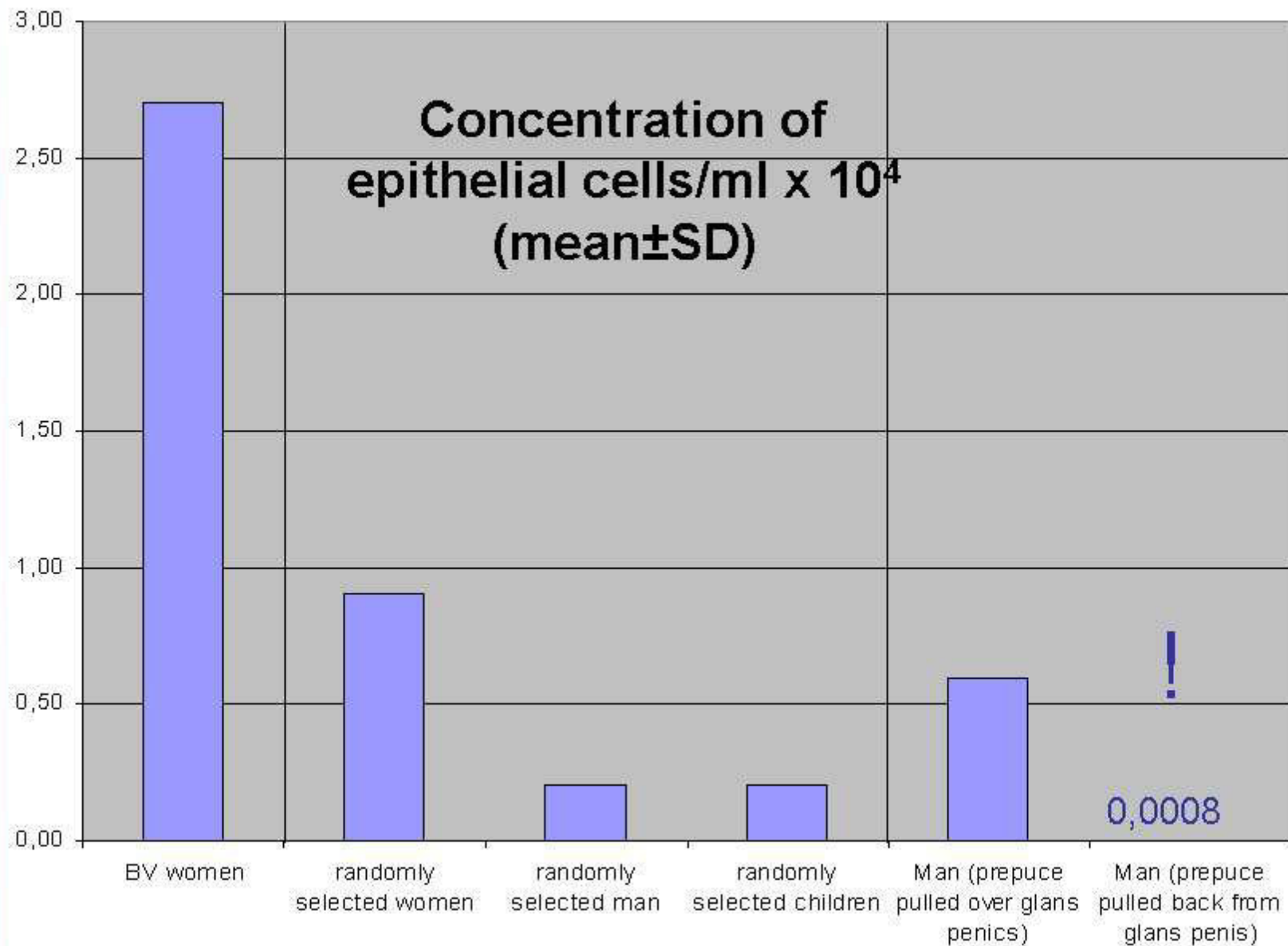
Urinesediment from male (Gardnerella Probe)



Randomly selected urine samples from hospitalized patients







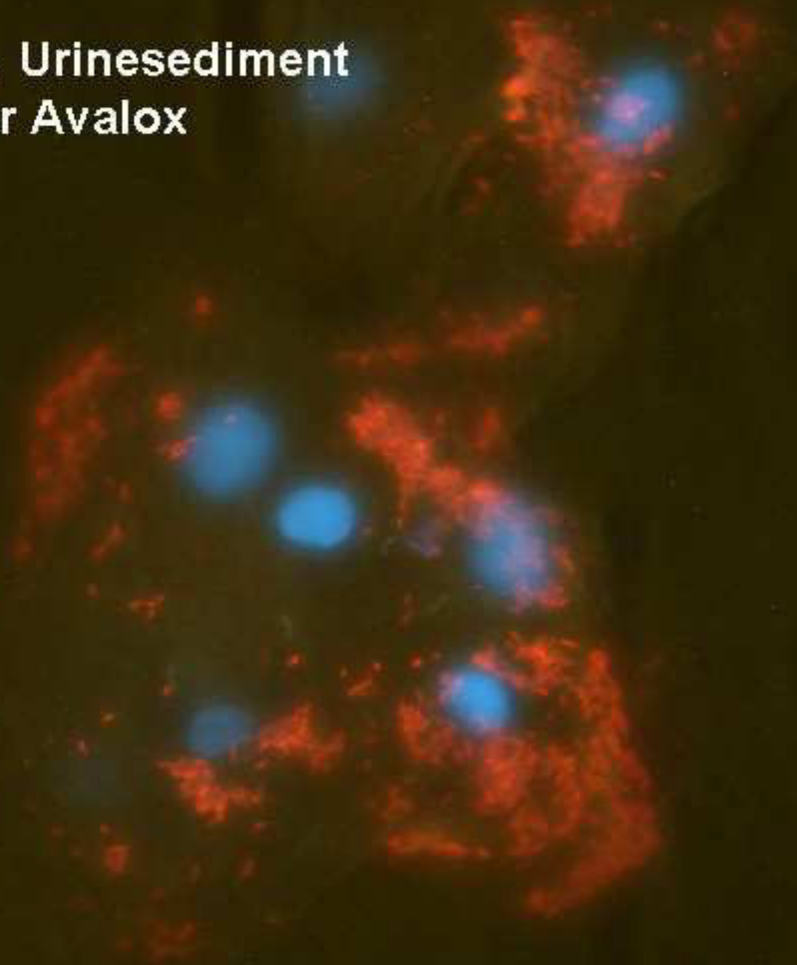
## Fertilisation





C

Patient. NG, Urinesediment  
3. week after Avalox



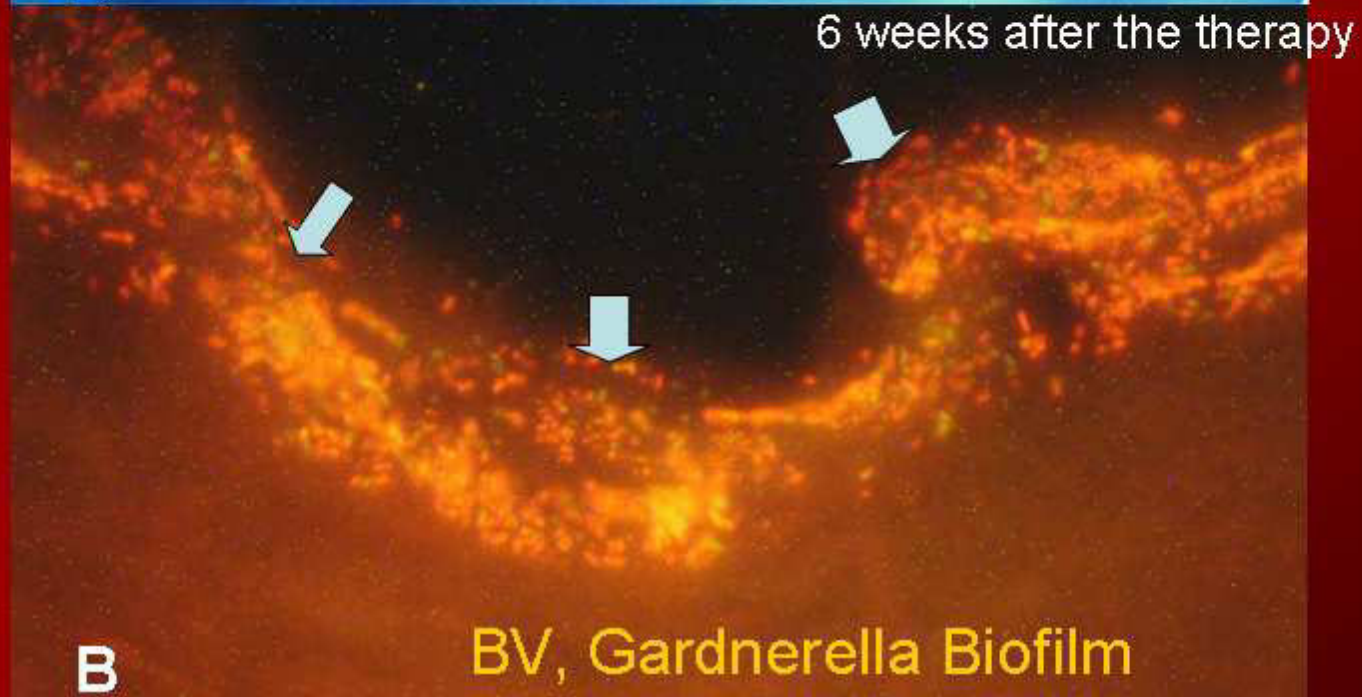
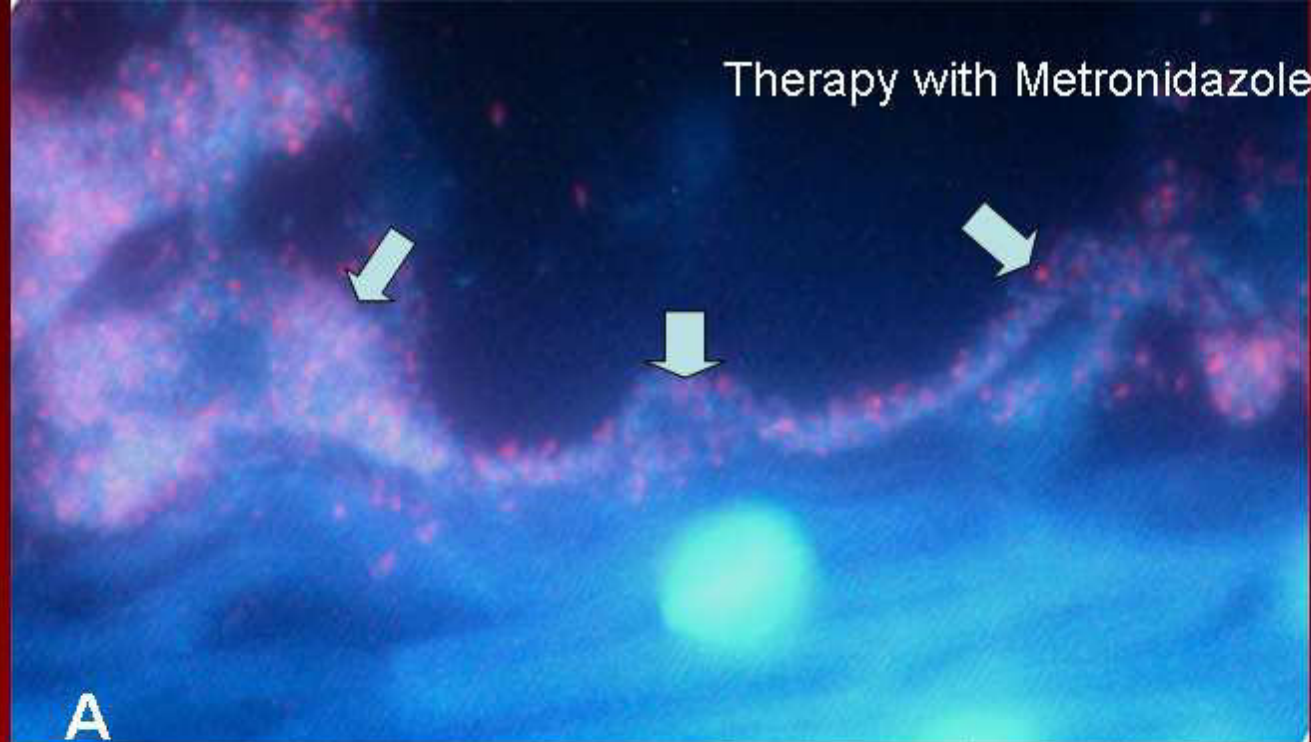
**Antibiotics**

**Metronidazole**

**Avalox (Moxifloxacin)**

**Antiseptics (Octenisept)**





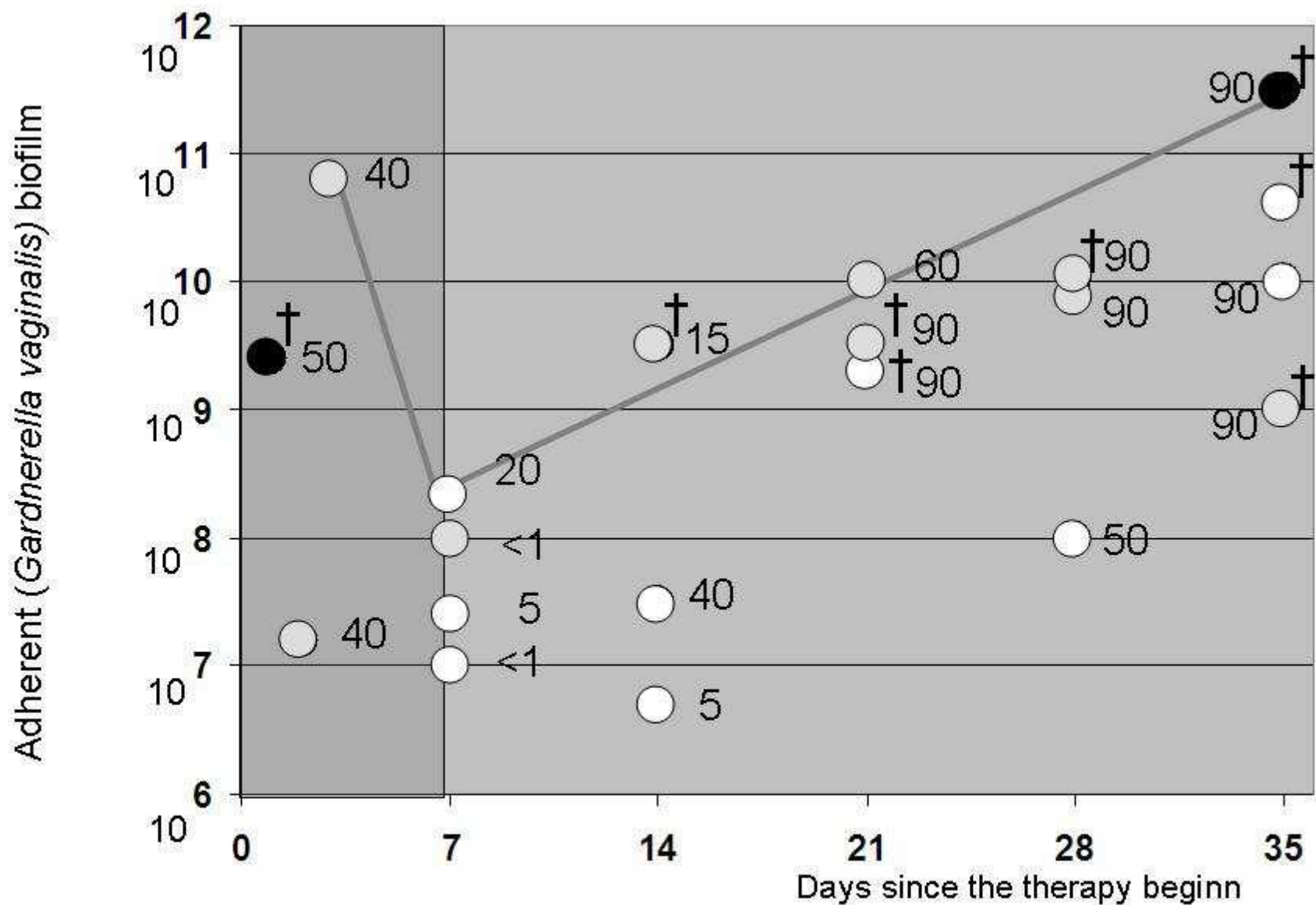
# FISH analysis of mucosal biofilms



Eub338  
A10b  
Esa42a  
Gama2a  
DhaC  
Ec1531  
Y16s-69  
Srh385  
Sgd  
Hsp-1  
Hsr1430  
HGC  
LCC  
Sfb  
Erec  
Lach  
Ehal  
Chis150  
Clit135  
Lab158  
Stre93  
Enc131  
EfaeC  
Ato291  
Con653  
Ecy1  
Phasco  
Veil  
Rbro, Rfla  
UroA, UroB  
Ser1410  
Bif164  
CF119a  
Bac303  
Bfra602  
Bdis656  
Fprau  
Dss658  
Arch915

with r-RNA based probes

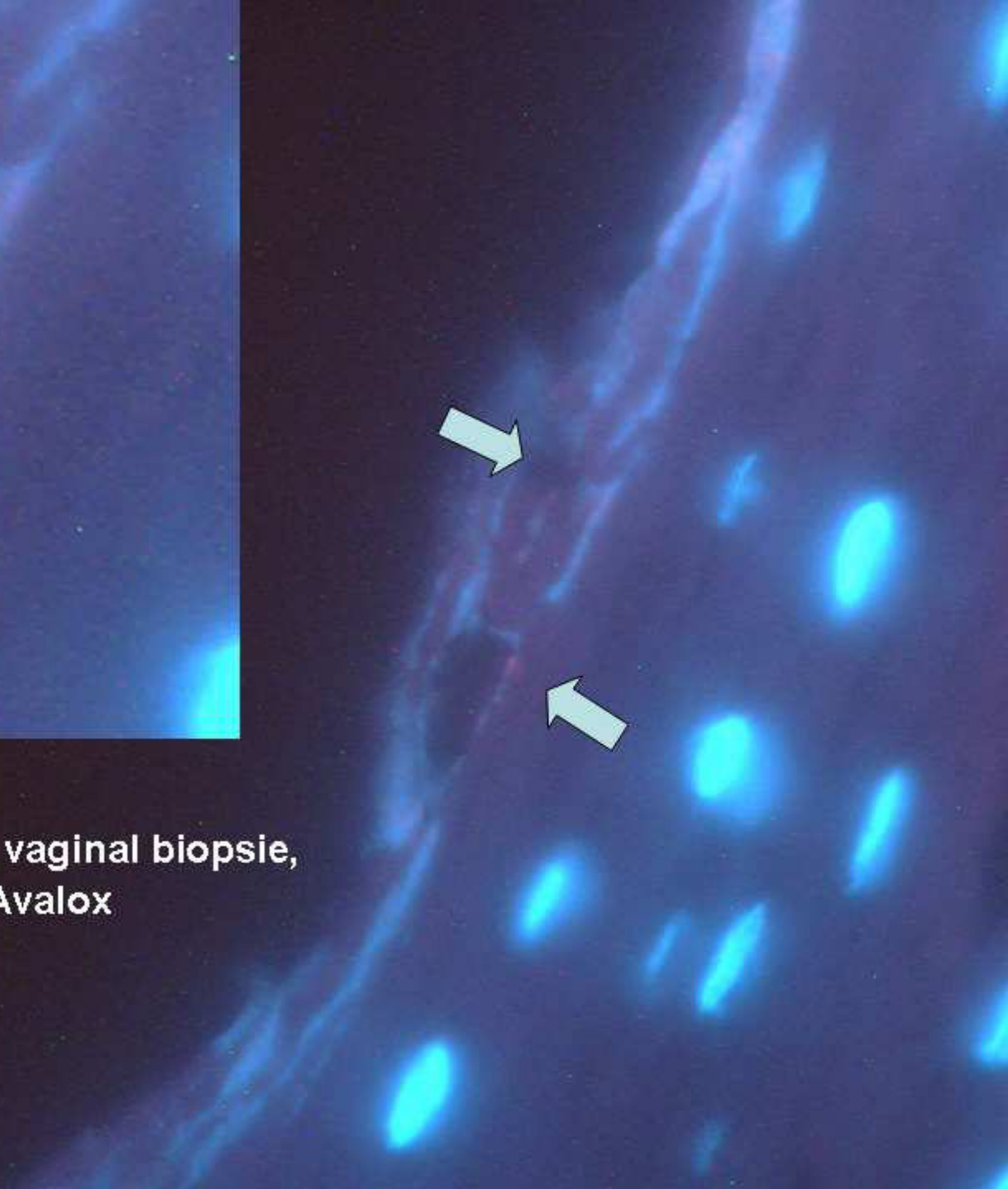
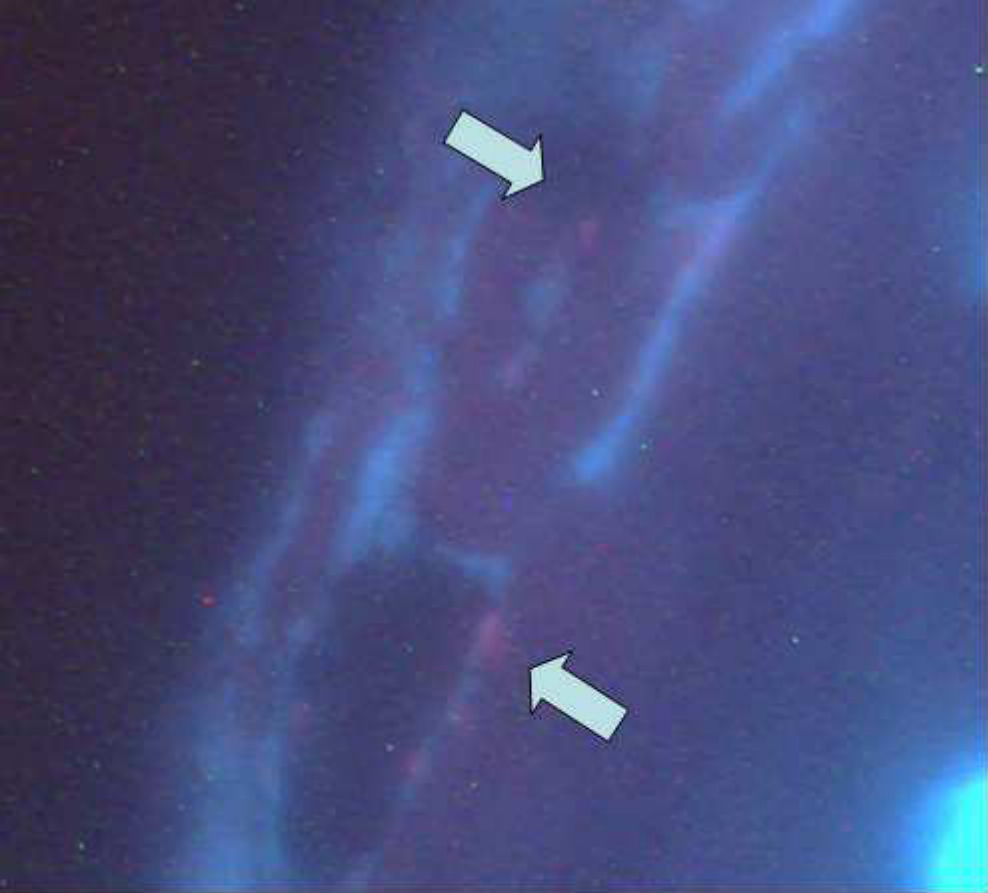




● pH ≥ 6, clue cells    ○ 5 ≥ pH < 6, disturbed flora    ○ pH < 5 no abnormality

1-90 Percent of DAPI stained bacteria, which positively hybridize with universal bacterial probe



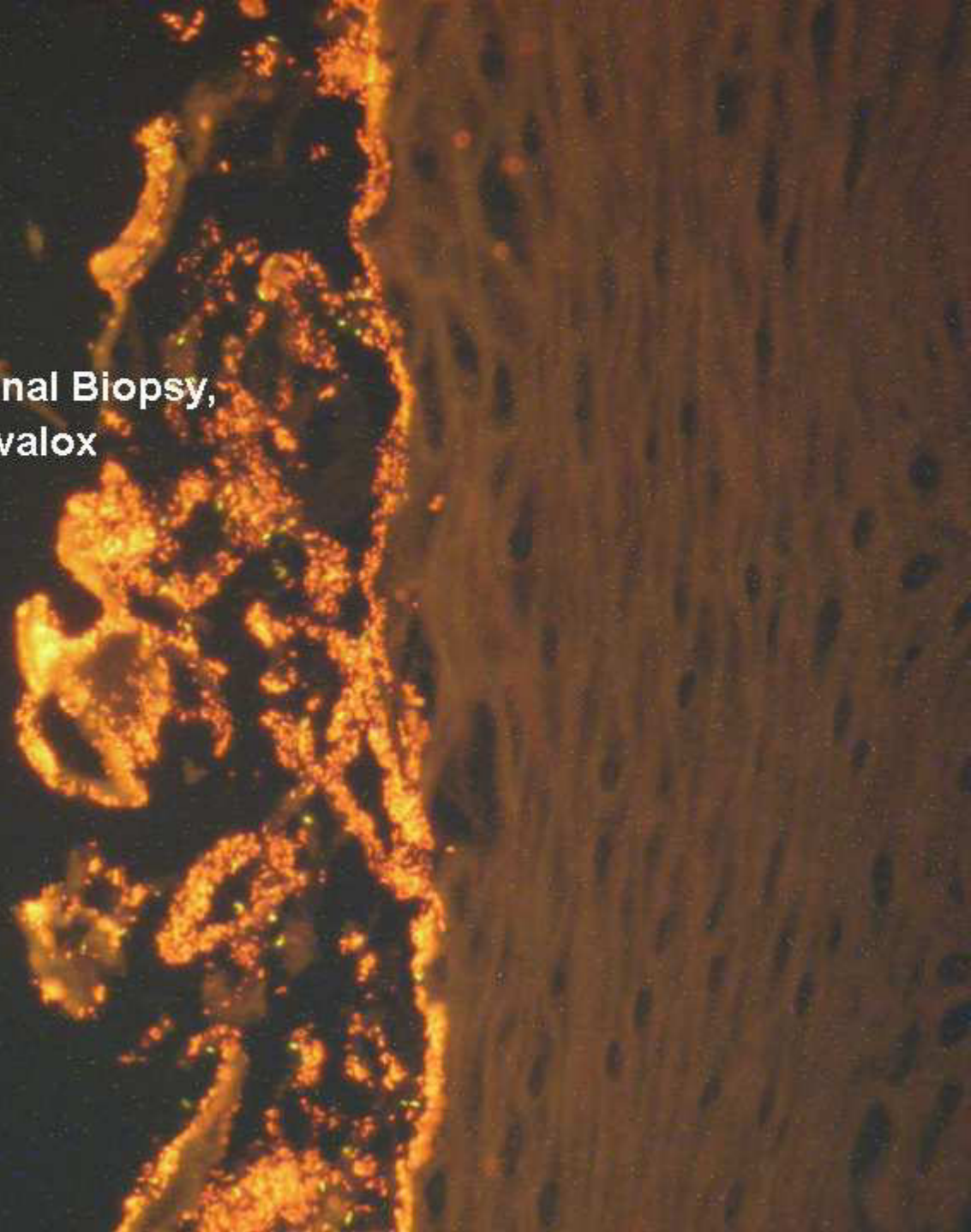


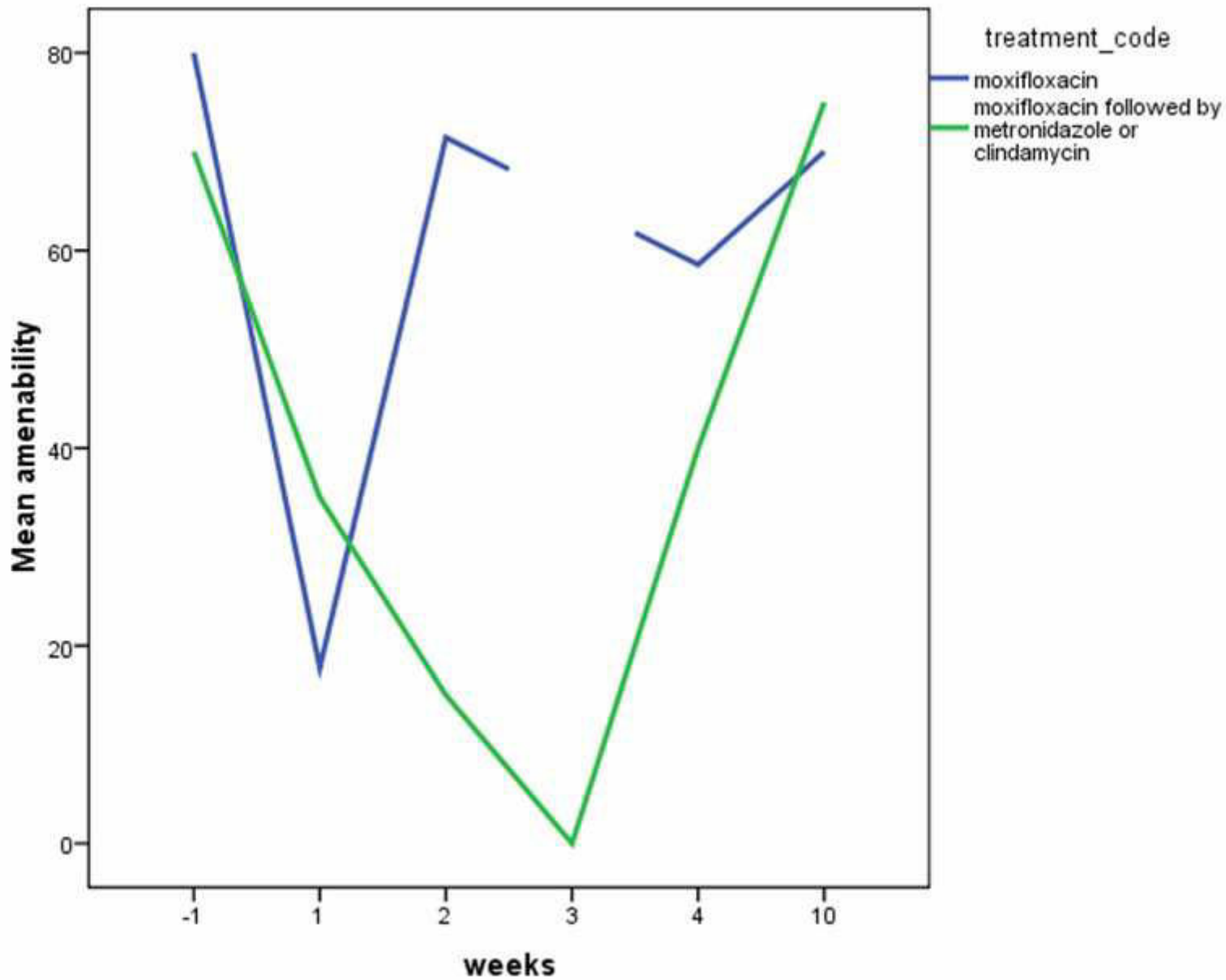
**B**

Patient NG, vaginal biopsy,  
5th day of Avalox

D

Patient NG, vaginal Biopsy,  
12. week after Avalox







## Impact of octenisept on StPM *Gardnerella* biofilms

	Response	Relapse within one to 6 month	No response	Cumulative non-response rate
Initial therapy for 7 days (N=24)	21	14	3	13%
2. therapy repeated for 28 days (N=17)	11	4	6*	25%
3. therapy repeat for 28 days followed by intermittent weekly applications for 2 month (N=4)	1	0	3*	38%

Occurrence of bacteria in uterus/tubes in women with and without vaginal StPM-*Gardnerella* biofilm

	Samples of uterus/tubes positive for bacteria
Vaginal StPM <i>Gardnerella</i> biofilm négative (N=50)	13/50 (26%)
Vaginal StPM-Gard positive (N=18)	12/18 (67%) $P < 0.005$
including StPM Gard pos. non pregnant	4/9 (44%)
Including StPM Gard pos. pregnant	8/9 (89%)

Structured polymicrobial *Gardnerella* biofilm (StPM)  
is an acting subject for bacterial vaginosis





[www.charite.de/arbmk1](http://www.charite.de/arbmk1)



Occurrence of vaginal StPM *Gardnerella* biofilms in patients with missed abortion and non-pregnant women undergoing tubal surgery or uterine curettage

<b>Group:</b>	<b>Vaginal StPM Gardnerella biofilms</b>	<b>P compared to group A</b>
<b>A. Missed abortion (curettage)</b>	<b>9/20 (43%)</b>	
<b>B. Nonpregnant women with curettage of the uterus or tubal surgery</b>	<b>9/48 (19%)</b>	<b>P=0.05</b>
<b>C. Randomly selected women hospitalized for different nongynaecologic diseases <sup>*ref</sup></b>	<b>13/100 (13%)</b>	<b>P=0.002</b>
<b>D. Randomly selected pregnant women <sup>*ref</sup></b>	<b>12/72 (17%)</b>	<b>P=0.012</b>
<b>E. Samples from randomly selected outpatients from the general practice <sup>*</sup></b>	<b>58/262 (22%)</b>	<b>P =0.04</b>

A microscopic image showing a cross-section of vaginal epithelium. The tissue is stained, revealing a regular, organized pattern of cells. The cells are arranged in a stratified layer, with a distinct boundary between the epithelial layer and the underlying connective tissue. The overall appearance is that of healthy, non-inflamed tissue.

Healthy vaginal epithelium, no bacteria detectable







