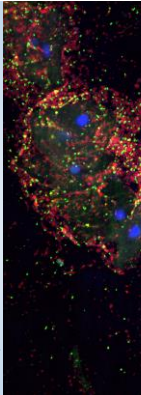


The impact of vaginal bacterial biofilm on intravaginal rings

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World STI/HIV congress 2015, Brisbane - 15/09/2015



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Introduction: Ring Plus study

- Multipurpose vaginal rings in development
- Interaction with vaginal microbiome?
- Rwanda: high unmet need for contraception


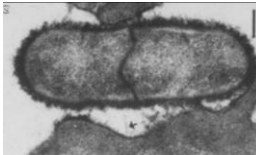
—> Contraceptive vaginal ring (Nuvaring®) in Rwanda

- Acceptability?
- Safety?
 - Effect on the vaginal microbiome
 - **Biofilm formation**
 - Inflammation

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Introduction: biofilm

- A coherent cluster of bacterial cells in a matrix – more tolerant to antibiotics and host immune system
- Vaginal biofilm in bacterial vaginosis: Poster P07.16
- Biofilm on medical devices:
 - Urinary/vascular catheters
 - Orthopedic implants
 - **Vaginal rings?**

G. vaginalis attached to McCoy cell *in vitro*
Scott, Journal of general microbiology, 1987

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Introduction: contraceptive vaginal ring

- Nuvaring®
 - Approved for use in 2001 in 32 countries (1.5 million users)
 - Etonogestrel(120 µg/d)/ethinyl estradiol(15µg/d)
 - Ethylene-vinyl acetate (pEVA)
 - Regimen: 3 weeks of use + 1 week of withdrawal bleeding
- Earlier studies: good safety profile and no effect on vaginal microbiome BUT
 - Cultures
 - Study population: healthy vaginal microbiome
 - No biofilm assessment

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Methods: Ring Plus study

120 participants, follow-up for 3 months

- Vaginal sampling at baseline and after contraceptive ring removal (N=527)
- Contraceptive rings: 3 parts (N=404)

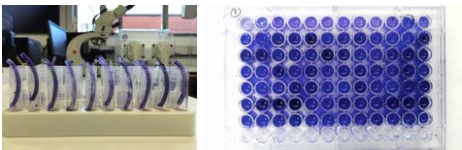
Laboratory method	Goal	Contraceptive rings	Vaginal samples	Reference
Crystal violet (CV) assay	Measuring biomass	X		O'Toole, J Vis Exp 2011
Fluorescence In Situ Hybridisation (FISH)	Visualisation biofilm	X	X	Hardy, PLoS ONE 2015
qPCR	Quantification bacteria	X	X	Jespers, BMC Microbio 2002
Nugent score	BV diagnosis		X	Nugent, J Clin Microbio 1991

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Method: CV assay

Adapted CV assay for microtiter plates: staining and measuring of the biomass

- Staining of ring with 3 ml of 0.1% CV
- Solubilising of CV with 3 ml of 30% acetic acid
- Measuring of absorbance at 550 nm in duplo

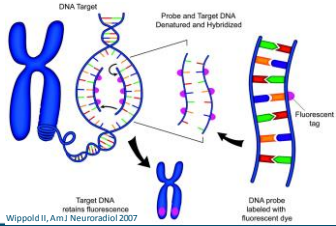


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Method: FISH

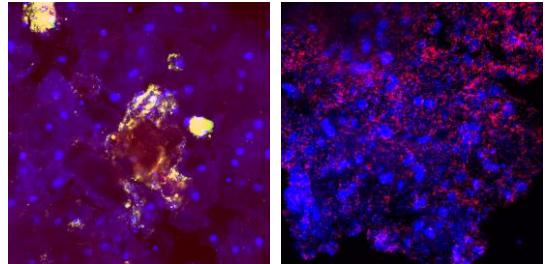
22 contraceptive rings and all vaginal slides with PNA probes:

BacUni-1: universal [Perry-O'Keefe] Gard162: *G. vaginalis* [Machado]
 Lac663: lactobacilli [Machado] AtoITM1: *A. vaginae* [Hardy]



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Results: FISH contraceptive rings



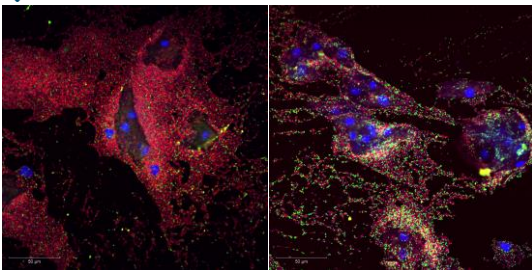
CSLM of contraceptive ring with vaginal epithelial cells (DAPI - blue) and *G. vaginalis* (Gard162-Alexa 647, red) and *A. vaginae* (AtoITM1- Alexa 488, green)

CSLM of contraceptive ring with vaginal epithelial cells (DAPI - blue) and lactobacilli (Lab663-Alexa 647, red)



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Results: FISH vaginal samples 1/2



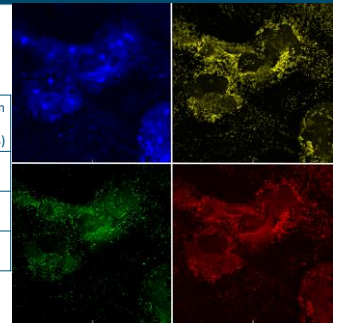
CSLM of vaginal slide with vaginal epithelial cells (DAPI - blue) and *G. vaginalis* (Gard162-Alexa 647, red) and *A. vaginae* (AtoITM1- Alexa 488, green)



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Results: FISH vaginal samples 2/2

	Absent N (%)	Present, no biofilm N (%)	Biofilm N (%)
FISH all bacteria	0 (0)	230 (49.7)	233 (50.3)
FISH <i>G. vaginalis</i>	172 (37.1)	100 (21.6)	191 (41.3)
FISH <i>A. vaginae</i>	268 (57.9)	69 (14.9)	126 (27.2)

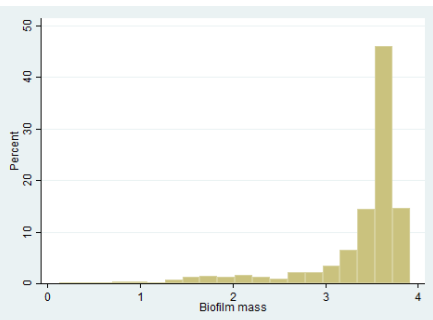


Vaginal epithelial cells (DAPI - blue), all bacteria (BacUni-1-Alexa555, yellow), *G. vaginalis* (Gard162-Alexa 647, red) and *A. vaginae* (AtoITM1- Alexa 488, green)



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Results: CV assay contraceptive rings



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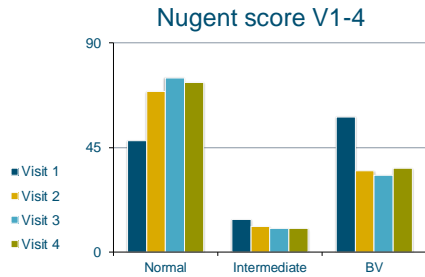
Results: qPCR contraceptive rings & vaginal samples

	Present N (%)		Mean \pm SD (log ₁₀) for present category	
	Vaginal ring N=404	Vaginal swab N=518	Vaginal ring	Vaginal swab
<i>Lactobacillus</i> spp.	376 (93.1)	NT	6.25 \pm 0.98	NT
<i>G. vaginalis</i>	231 (57.2)	389 (75.1)	6.04 \pm 1.16	6.97 \pm 1.37
<i>A. vaginae</i>	151 (37.4)	247 (47.7)	6.66 \pm 0.89	7.21 \pm 1.51



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Results: Nugent score vaginal samples



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Risk factors for biomass on contraceptive ring

FISH vaginal samples	Presence <i>G. vaginalis</i> biofilm	$p < 0.001$	+0.235
	Presence <i>A. vaginae</i> biofilm	$p = 0.006$	+0.214
qPCR vaginal samples	Increase in <i>G. vaginalis</i>	$p = 0.150$	No effect
	Increase in <i>A. vaginae</i>	$p = 0.002$	+0.09
qPCR contraceptive ring	Increase in <i>G. vaginalis</i>	$p < 0.001$	+0.12
	Increase in <i>A. vaginae</i>	$p = 0.003$	+0.53
Nugent	Diagnosis of bacterial vaginosis	$p = 0.002$	Inter +0.100 BV +0.261



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Conclusion

- Growth on contraceptive rings are common
 - vaginal cells
 - vaginal microbiome
- Dysbiosis vaginal microbiome & vaginal biofilm
 - denser biomass on contraceptive rings
- Could this ring biomass lead to:
 - Persistence or deterioration of vaginal microbiome?
 - Hampering of release of active product?



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Acknowledgement

- STI reference laboratory, ITM, Antwerp
- PRU, Kigali
- PhD advisors
 - Dr. Vicky Jespers (ITM)
 - Dr. Tania Crucitti (ITM)
 - Prof. Mario Vaneechoutte (UGent)
- Funders
 - EDCTP
 - CHAARM
 - Dormeur Investment services
 - ISSTR



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