Culture Based Techniques in the Omics Era

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Sharon Hillier PhD
Magee-Womens Research Institute
University of Pittsburgh School of Medicine
Department of Ob/Gyn/RS and Microbiology and Molecular Genetics
Pittsburgh, PA

Overview

1. Regulatory vs scientific goals for evaluation of the vaginal microflora in microbicide studies
2. MTN-004 study of VivaGel
3. Assessment of microbiota on ring vs vaginal swab
4. Use of “culturomics” in studies of microbicides
5. Final thoughts

2014 FDA Guidance on Safety Evaluation of Microbicides

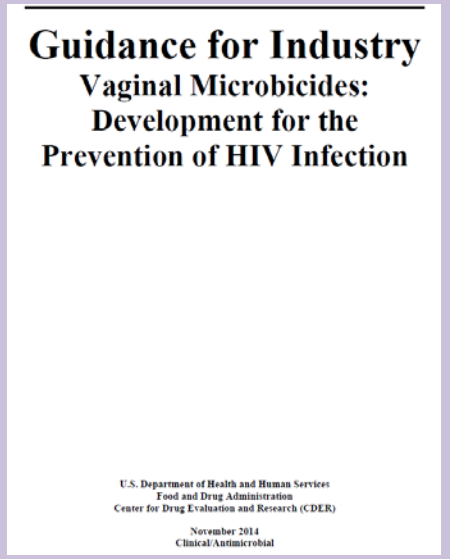
Sponsors should perform assessments for microbicide effects on vaginal pH, balance of vaginal microflora, and the frequency of other STIs. Significant shifts in local microflora may have clinical implications because the normal vaginal microflora is thought to play a role in preventing HIV-1 infection and other STIs (Myer, Kuhn, et al. 2005). Certain types of microflora imbalance or decreases in particular flora species can also increase the likelihood of bacterial vaginosis, urinary tract infections including urosepsis, and pelvic infections.

Focus on selected microbiota:

- *Lactobacillus* (marker of health; preclinical studies require that microbicide candidates be neutral to lactobacilli)
- *G vaginalis* (increases associated with BV)
- *E coli* (increases associated with UTI)
- *Staphylococcus aureus* (associated with toxic shock)
- *Candida* (associated with yeast infections)

Balance of microflora assessed using the Nugent criteria from a Gram stained vaginal swab

Total cost: $80 per participant visit



Detection of Selected Microbiota vs Study of the Microbiome

Selected Microbiota

- Can rely on culture based techniques or qPCR for selected microorganisms
- Detects microbes at low population density which is relevant for detection of *E coli, Staph aureus, Candida*
- Provides estimate of quantity present
- Provides no information on community state

Microbiome

- Provides comprehensive view of microbial communities and how they change
- Detects a fuller range of cultivable and noncultivable microbiota
- Does not detect low density pathogens of interest to regulators
- Complex data

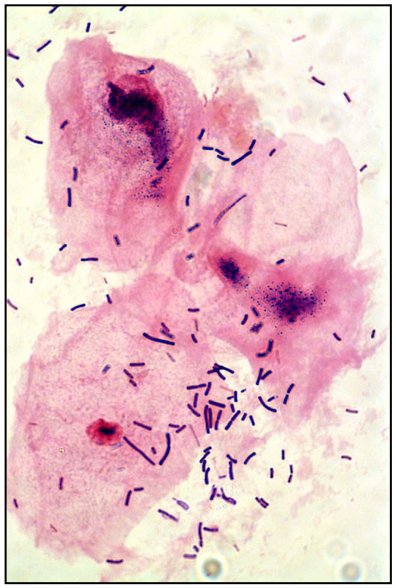
The MTN-004 Study

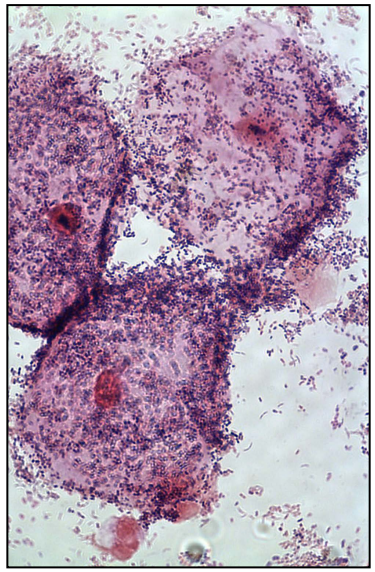
- VivaGel: polyanonic dendrimer based gel containing SPL7013 as the active ingredient
- Phase I, double blinded, randomized, controlled comparison trial with 14 days of twice daily exposure to either Viva Gel, VivaGel Placebo, or HEC Placebo.
- 61 healthy, non-pregnant, sexually active women aged 18-24
- STIs excluded at baseline; sexually abstinent
- Vaginal swabs were collected at enrollment, 1 week and 2 weeks after daily use, and 1 week after completion of product use; 58 women had all four visits available for microbiological analyses

Impact of VivaGel and Placebo on Nugent Pattern

Nugent score	Enrollment	2 weeks on product	1 week off product
0-3	58%	53%	50%
4-6	26%	24%	28%
7-10	16%	19%	22%

No major impact of VivaGel use on vaginal flora patterns over time
Limitation: Nugent scores relies on relative proportion of three bacterial morphotypes





Impact of VivaGel and Placebo on Microbiota Density

Microorganism	Placebo VivaGel (n=21)		VivaGel (n=21)	
	Change in Colony Count [Mean log_{10} CFU/ml (95% CI)]	P-value	Change in Colony Count [Mean log_{10} CFU/ml (95% CI)]	P-value
Any *Lactobacillus* species	- 0.5 (-1.2 – 0.3)	0.26	-0.7 (-1.2 – -0.1)	0.01
Gardnerella vaginalis	-1.4 (-2.3 – -0.6)	0.001	-1.2 (-1.9 – -0.5)	0.001
Enterococcus species	0.3 (-0.2 – 0.9)	0.27	1.1 (0.4 – 1.9)	0.002
Group B *Streptococcus*	0.0 (-0.2 – 0.1)	0.9	1.2 (0.1 – 2.2)	0.03
Any coliform	0.0 (-0.7 – 0.7)	0.99	1.2 (0.3 – 2.0)	0.005
Any anaerobic GNR	-0.9 (-1.5 – -0.2)	0.01	-0.7 (-1.3 – -0.1)	0.02
Pigmented ana GNR	-0.8 (-1.4 – -0.2)	0.01	-0.4 (-0.7 – -0.04)	0.03
Non-pigmented ana GNR	-0.8 (-1.4 – -0.1)	0.02	-0.7 (-1.3 – -0.1)	0.02

Use of both VivaGel active and VivaGel placebo associated with increases in gut microbiota

Impact of VivaGel and Placebo on Microbiota Density

Microorganism	Placebo VivaGel (n=21)		VivaGel (n=21)		HEC Gel (n=16)	
	Change in Colony Count [Mean log_{10} CFU/ml (95% CI)]	P-value	Change in Colony Count [Mean log_{10} CFU/ml (95% CI)]	P-value	Change in Colony Count [Mean log_{10} CFU/ml (95% CI)]	P-value
Any *Lactobacillus* species	- 0.5 (-1.2 – 0.3)	0.26	-0.7 (-1.2 – -0.1)	0.01	-0.3 (-0.8 – 0.2)	0.23
Gardnerella vaginalis	-1.4 (-2.3 – -0.6)	0.001	-1.2 (-1.9 – -0.5)	0.001	0.1 (-0.8 – 1.0)	0.77
Enterococcus species	0.3 (-0.2 – 0.9)	0.27	1.1 (0.4 – 1.9)	0.002	-0.2 (-0.7 – 0.2)	0.36
Group B *Streptococcus*	0.0 (-0.2 – 0.1)	0.9	1.2 (0.1 – 2.2)	0.03	-0.5 (-1.0 – 0.05)	0.08
Any coliform	0.0 (-0.7 – 0.7)	0.99	1.2 (0.3 – 2.0)	0.005	-0.4 (-1.0 – 0.2)	0.25
Any anaerobic GNR	-0.9 (-1.5 – -0.2)	0.01	-0.7 (-1.3 – -0.1)	0.02	0.0 (-0.6 – 0.6)	1.0
Pigmented ana GNR	-0.8 (-1.4 – -0.2)	0.01	-0.4 (-0.7 – -0.04)	0.03	0.2 (-0.1 – 0.6)	0.24
Non-pigmented ana GNR	-0.8 (-1.4 – -0.1)	0.02	-0.7 (-1.3 – -0.1)	0.02	-0.1 (-0.7 – 0.6)	0.85

HEC placebo did not significantly alter these same microbes

Impact of VivaGel on Frequency of Selected Microbiota

Microorganism	Placebo VivaGel N=21			Viva Gel N=21			HEC Gel N=16		
	off product	on product	P	off product	on product	P	off product	on product	P
Lactobacillus	9.5	4.5	0.03	9	7	0.22	8	7.5	0.81
Gardnerella vaginalis	11	7	0.02	8	3	0.002	9	8	0.49
Enterococcus species	10	12	0.16	9.5	13	0.01	5.5	5	0.77
Group B *Streptococcus*	2.5	2.5	1.0	8	11.5	0.1	6	3	0.001
Any Anaerobic GNR	13	9	0.03	10.5	10	0.82	10.5	9.5	0.6
Pigmented Ana. GNR	6.5	2	0.008	6.5	0.5	0.002	5.5	6.5	0.49
Non-pigmented GNR	13	9	0.03	10.5	10	0.82	9.5	8.5	0.57

Statistically significant decreases in the frequency of G vaginalis and anaerobic gram negative rods in the Viva Gel group and an increase in Enterococcus

What Happened Next?

- Assessment of VivaGel formulation (both placebo and active gel) to identify gel components which caused disruptions
- Product not further evaluated by MTN
- Dendrimer reformulated and evaluated for treatment of bacterial vaginosis!
- “simple” cultivation based methods detected microbiota changes which triggered reformulation of product.

Use of Cultivation to Assess Ring vs Vaginal Swab Microbiota

PLOS ONE | 2015 Aug 12;10(8):e0134460. .

RESEARCH ARTICLE

Effects of a One Year Reusable Contraceptive Vaginal Ring on Vaginal Microflora and the Risk of Vaginal Infection: An Open-Label Prospective Evaluation

Yongmei Huang[1¤], Ruth B. Merkatz[1]*, Sharon L. Hillier[2], Kevin Roberts[1], Diana L. Blithe[3], Régine Sitruk-Ware[1], Mitchell D. Creinin[4]

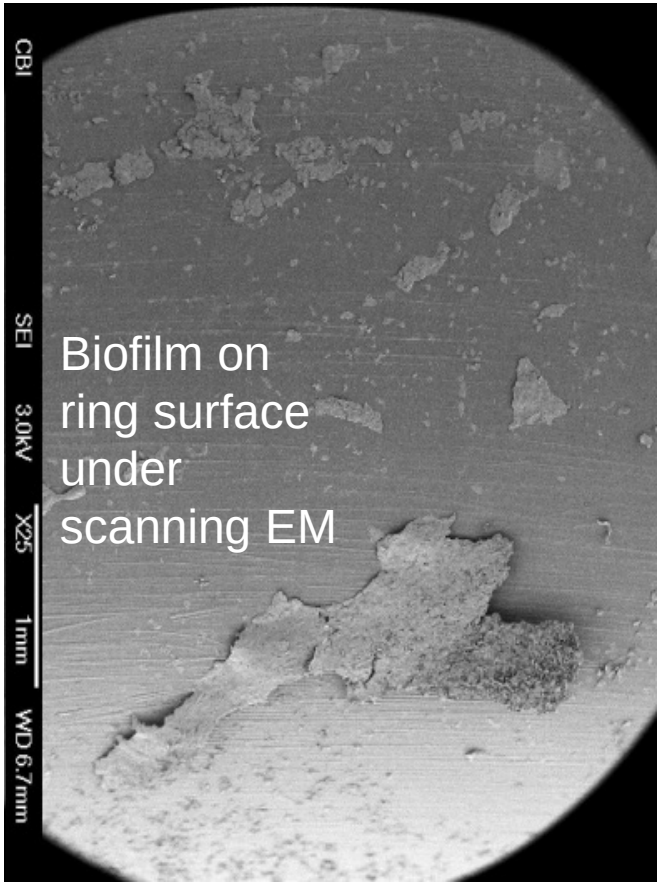
1 Population Council, Center for Biomedical Research, New York, United States of America, **2** Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh and the Magee-Womens Research Institute, Pittsburgh, United States of America, **3** Contraceptive Discovery and Development Branch, National Institute of Child Health and Human Development, Bethesda, United States of America, **4** Department of Obstetrics and Gynecology, University of California Davis, Sacramento, United States of America

Major questions posed by the FDA:

- Did extended ring use promote growth of *Staph aureus*?
- Were the microbes on the ring surface the same as those in the vagina?
- Did extended use of the same ring promote vaginitis (BV and yeast)?

12 Month Vaginal Ring Study

- 120 women enrolled into a CVR Phase III trial microbiology sub-study for up to 1-year of cyclic product use.
- Vaginal swabs were obtained for wet mount microscopy, Gram stain and culture at baseline, 6 and 12 months.
- The CVR was removed from the vagina at the last study visit and cultured and the results compared to the vaginal swab sample from that visit.
- Prevalence of BV and yeast vaginitis were similar over the year of ring use.



PLoS One. 2015 Aug 12;10(8):e0134460. doi: 10.1371/journal.pone.0134460

Ring vs. Vaginal Swab

	Vaginal culture vs. Ring culture (N=72)		
	Vaginal culture n (%)	Ring culture n (%)	Concordant pairs n (%)
H_2O_2-positive *Lactobacillus*	65 (90.3)	66 (91.7)	67 (93.1)
H_2O_2-negative *Lactobacillus*	16 (22.2)	12 (16.7)	66 (91.7)
Gardnerella vaginalis	16 (22.2)	16 (22.2)	68 (94.4)
Enterococcus faecalis	12 (16.7)	17 (23.6)	59 (81.9)
Staphylococcus aureus	4 (5.6)	3 (4.2)	67 (93.1)
Escherichia coli	9 (12.5)	6 (8.3)	65 (90.3)
Candida albicans	15 (20.8)	18 (25.0)	69 (95.8)
Other Yeast	5 (6.9)	4 (5.6)	71 (98.6)
Anaerobic GNR	40 (55.6)*	26 (36.1)	56 (77.8)

These data were useful in supporting the use of the same ring over 12 months

Final Thoughts

- There are distinct strengths of both targeted and broader approaches (microbiome) to evaluation of microbicide effects.
- Need to weigh regulatory needs to describe certain microbiota as safety measure vs what is scientifically interesting and choose methods accordingly
- Culturomics has revealed great cultivable diversity in the vaginal microbiota and may be complementary to pyrosequencing.

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