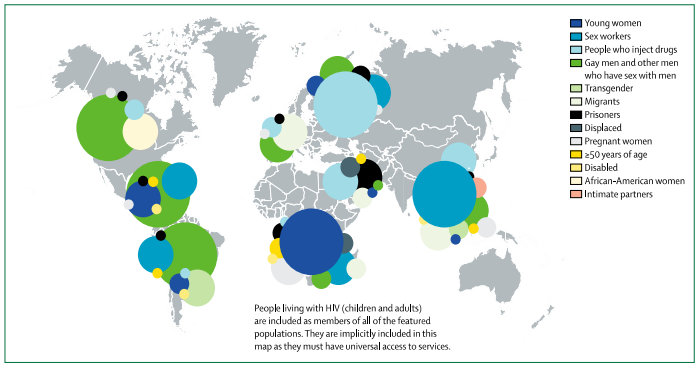
Disclosures

No (potential) conflict of interests

Adolescent Girls and Young Women Continue to Face a Disproportionately High Risk of HIV Infection



Estimated 37 million individuals living with HIV globally in 2017, 45% affecting women

New HIV infections in sub-Saharan Africa represent 65% of the 1.8 million global infections

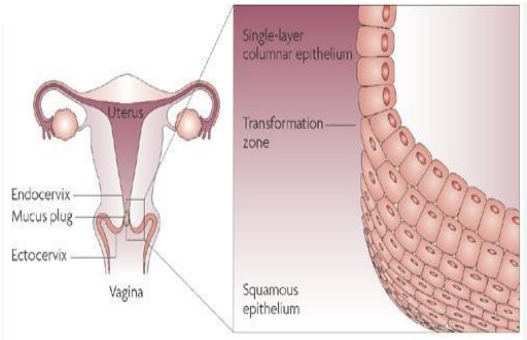
Every week 7000 adolescent girls and young women became infected with HIV in 2017

Most HIV Infections in Women Occur by Entry via the Female Reproductive Tract (FRT)

>80% of new HIV infections in women occur by HIV entry via the FRT

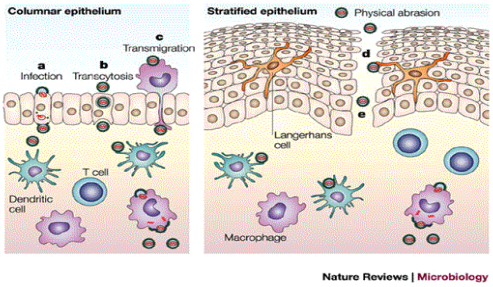
HIV can enter mainly through the endocervix ectocervix and vagina

FRT: Probability of HIV transmission per exposure event is usually low relative to rectal/parenteral 1 in 1000 (semen)



Hladik and Hope 2009 Current HIV/AIDS Reports 6: 20 Buve et al 2014 AIDS July
Powers et al 2008 Lancet Infect Dis 8: 553 Boily et al 2009 Lancet Infect Dis 9:118

Antimicrobial Defense Mechanisms in the FRT



Endocervix

Ectocervix and Vagina (apparent preference)

Physical Defense
Mucous
Ciliary clearance
Epithelial cells

Biological Defense

Immune Immune cells (including epithelial cells respond to) MAMPs/PAMPs to produce immune mediators: antiviral and pro-inflammatory (paradoxically promote HIV infection in women)

Microbiota Communities of commensal bacteria (along with fungi and viruses)

Competition, bacteriocins, organic acid metabolites (i.e. lactic acid –produced by vaginal lactobacilli associated with HIV protection)

Shattock and Moore 2003 Nat Micro Reviews Carias et al 2013 J Virol 87: 11388
Wira et al 2011 Am J Rep Immunol Stieh et al 2014 Plos Path 10:e1004440

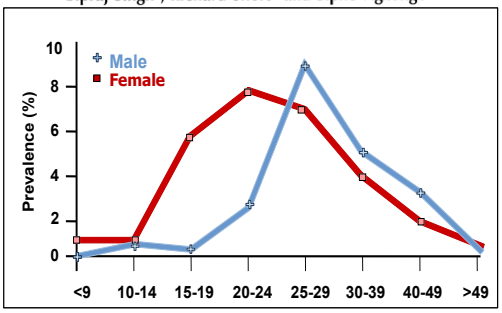
Disproportionate Burden of the HIV Epidemic in Young Women in sub-Saharan Africa

AIDS 1992

Seroprevalence of HIV infection in rural South Africa

AIDS 1992, 6:1535-1539

Quarraisha Abdool Karim, Salim S. Abdool Karim, Bipraj Singh*, Richard Short† and Sipho Ngxongo‡



Older male sexual partners

Biological factors
Genital inflammation (subclinical)

STIs (including asymptomatic)

Twice as likely to be living with HIV compared to their male counterparts

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UNAIDS 2010; 2014, 2015, 2018 Passmore et al 2016 Curr Opin HIV AIDS 11:156
Mlisana et al 2012 JID 206:6

Vaginal Microbiome Affects HIV Risk in Young Women in Sub-Saharan Africa

INFECTIOUS DISEASE

Vaginal microbiome affects HIV risk

Unusual bacteria in vagina help explain high infection rates in South African women

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Cohen 2016 Science 353:6297 Masson et al 2015 Clin Infect Dis 61: 260
Anahtar et al 2015 Immunity 42: 965 Gosmann et al 2017 Immunity 46:1

Vaginal Microbiome of Asymptomatic Reproductive-Age Women Dominated by *Lactobacillus* spp.(USA)

Group	Bacterial communities (CST)	
I	*Lactobacillus crispatus*	< pH 4.0*
II	*Lactobacillus gasseri*	pH 5.0
III	*Lactobacillus iners*	pH 4.4
IVA*	Modest *Lactobacillus sp.*, *Anaerococcus*, *Corynebacterium*, *Finegoldia* and *Streptococcus* (high diversity)	pH 5.3
IVB*	No *Lactobacillus sp.* detected, *Atopobium*, *Prevotella*, *Sneathia*, *Mobiluncus*, *Peptoniphilus* and several other taxa (high diversity)	pH 5.3
V	*Lactobacillus jensenii*	pH 4.7

L crispatus — most protective against STIs including HIV
acidifies vagina to lower pH by lactic acid

L. iners — least protective and less stable - transitions to CST IV
similar to bacterial vaginosis (BV) – associated with adverse sexual and reproductive health outcomes

*African Amercian and Hispanic – racial

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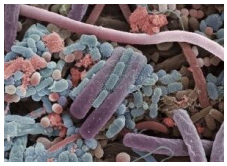
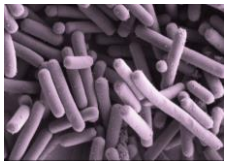
Gajer et al., 2012 STM 4:132ra52; Ravel et al., 2011 PNAS 108:4680
Marrazzo 2013 JID; Sha et al 2005 J Infect Dis 191:25; Mitchell et al 2013 AIDS Res Hum Retroviruses 29:1300

Diverse Vaginal Microbiota: Bacterial Vaginosis (BV)

- Common vaginal condition in reproductive age women
- Increase in load and diversity of obligate and facultative anaerobes and depletion of beneficial *Lactobacillus* spp.
- Affects 29% of women of reproductive age (USA)
- Up to 55% women in sub-Saharan Africa (high HIV prevalence)
- Australia: non indigenous 12%; indigenous 30%

BV





LB

BV Diagnosis:
Amsel criteria (clinical criteria/symptomatic) 3 of 4, discharge, fishy smell, clue cells, pH>4.5

Nugent Score (gold standard): gram stain, weighted score of relative abundance of morphotypes - 0 – 3 LB dominated; 4 - 6 Intermediate; 7 – 10 BV

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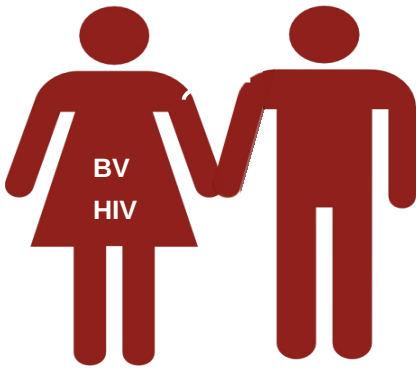
Kenyon C et al 2013 Am J Obstet Gynecol 209:505

Bacterial Vaginosis (BV) is a Major Risk Factor for HIV

RISK OF HIV INCREASES 2-FOLD[1]



RISK OF HIV INCREASES 3-FOLD[2]

BV is an extremely heterogeneous condition
Nugent score underestimates the presence of diverse vaginal microbiota

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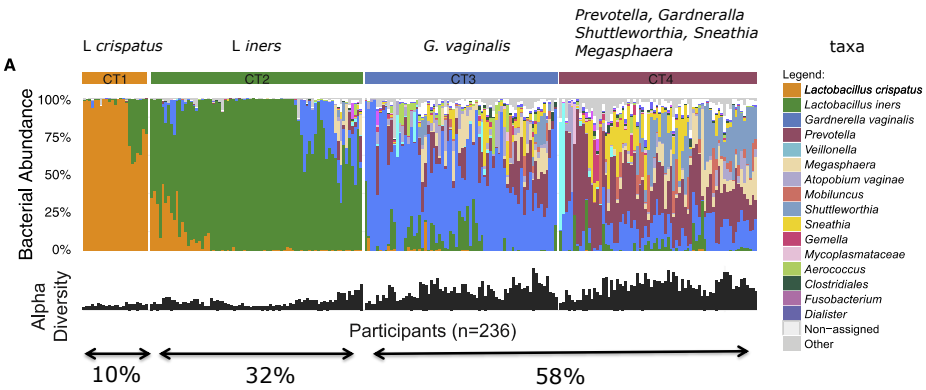
[1] Atashili et al 2008 AIDS 22:1493
[2] Cohen et al., 2012 PLoS Med 9:e1001251

Vaginal Microbiome of Young Healthy South African Women Dominated by High Bacterial Community Diversity



Prospective observational study (19 – 23 years of age HIV neg)
FRESH – Females rising through Education, Support and Health Cohort

Many women in CT4 were BV negative by Nugent

75% no identifiable STI
Excluded sex acts, dry sex

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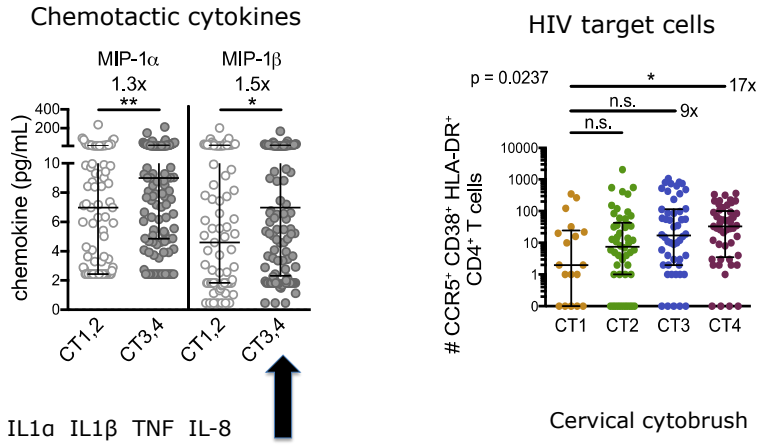
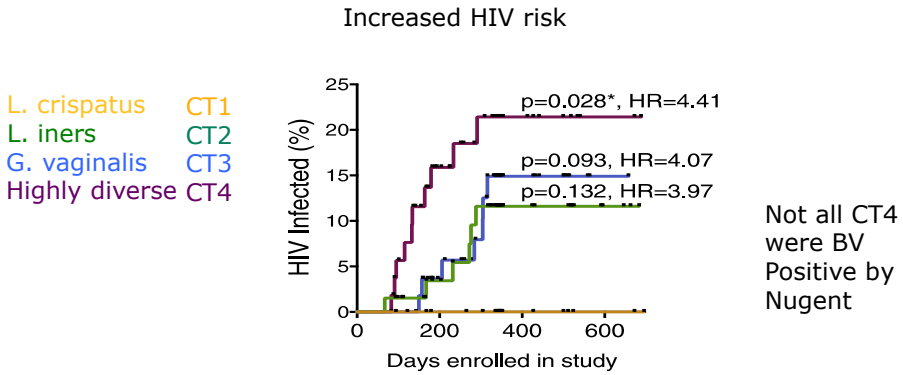
Gosmann et al 2017 Immunity 46:1

High Diversity Bacterial Communities Associated with Increased Genital Inflammation



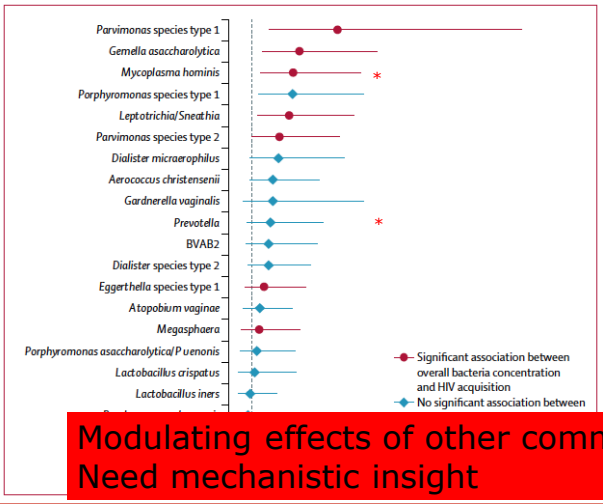
High Cervicovaginal Bacterial Diversity (CT4) Increases Risk of HIV Acquisition by 4.4-fold



Lactobacillus spp. (non iners) associated with protection against HIV

Identification of Key Vaginal Bacteria and Increased Risk of HIV Acquisition in African Women from Five Cohorts

Concentration Dependent Associations with Increased Odds for HIV in Women



5 prospective cohort studies for HIV risk

Sex workers, pregnant/postpartum women serodiscordant relationships

Diverse regions of Africa

50 case and 50 controls

16S rRNA sequencing, relative abundance – selected 20 taxa (entire cohort)

Load of individual bacteria by quantitative PCR

Bacterial diversity (BV)

Modulating effects of other community members? Need mechanistic insight

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McClelland et al 2018 Lancet Infect Dis 18: 554

Summary: Evidence for Genital Inflammation Increasing HIV Risk in Women

Genital inflammation = increase in proinflammatory cytokines/chemokines

Symptomatic and asymptomatic STIs (lab-diagnosed)

South Africa only 12% of women with lab-diagnosed discharge causing STIs were symptomatic

Vaginal Microbiota

- Bacterial vaginosis (Nugent)
- Depletion of beneficial *Lactobacillus* spp
- Highly diverse bacterial communities (16S rRNA gene sequencing)
- Load (absolute levels) of certain bacteria quantitative PCR

Clear associations with HIV risk
Need mechanistic insights on the contributions of bacterial communities and key taxa to design effective interventions to reduce HIV risk in women

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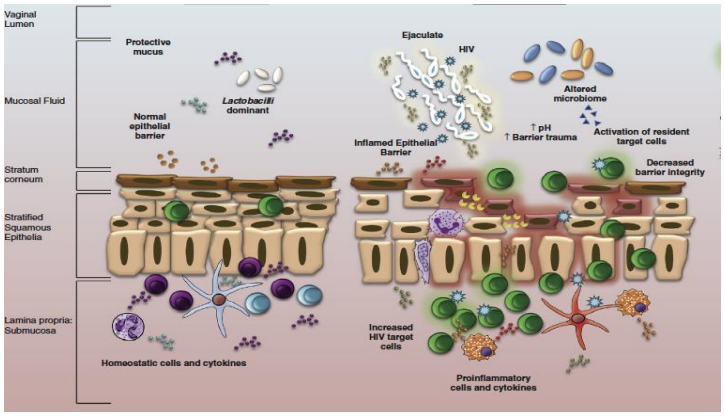
Vaginal Microbiome: Activation and Recruitment of Target cells and Disrupting the Mucosal Barrier

Lactobacillus-dominated Highly Diverse Microbiota (e.g. BV)



Recruitment/ activation of HIV target cells
Proinflammatory cytokines/chemokines

Disruption of protective cervicovaginal mucous
BV bacteria: sialidases, mucinases

Disruption of epithelial barrier
Neutrophils-proteases
Cytokines

If genital inflammation increases HIV risk does it attenuate topical PrEP efficacy in women?

Topical/Oral Pre-Exposure Prophylaxis (PrEP) for Preventing HIV Evaluated for Efficacy in Women

Topical PrEP

Antiretroviral based topical PrEP formulated in a gel or ring to prevent or reduce the sexual transmission of HIV when applied to the vagina:

1% Tenofovir Gel
Dapivirine Ring

Oral PrEP

Tenofovir (TFV)-based (Truvada)
FDA/TGA approved

Most of the HIV prevention clinical studies have been performed with TFV based topical gels and oral PrEP

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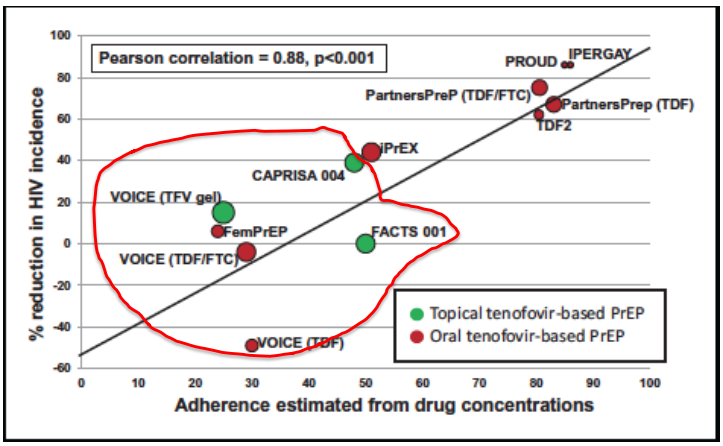
Shattock and Rosenberg 2012 Cold Spring Harb Perspect Med 2(2)
Patterson et al 2011 Sci Transl Med 3(112)

Inconsistent Topical and Oral PrEP Efficacy in Women Largely Attributable to Poor Adherence

Correlation between % of Participants Samples with Detectable Drug and PrEP Effectiveness



Oral PrEP consistently shown efficacy in men

Inconsistent efficacy in in women

Partners PrEP -discordant couples

TDF2 -heterosexuals

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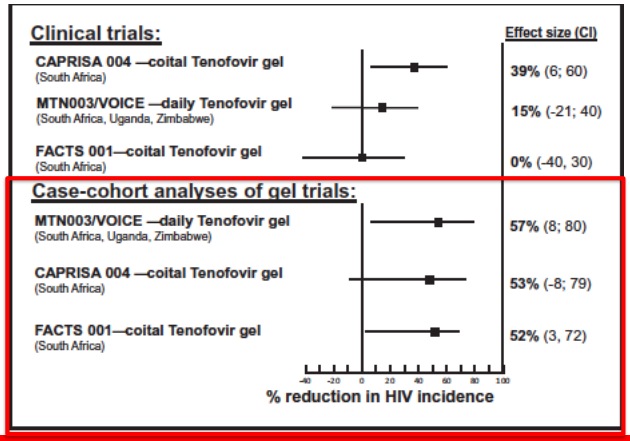
Abdool Karim et al 2017 Curr Opin HIV AIDS 12
Janes et al 2018 AIDS Res Hum Retro 34:645

Case Control Analysis of Three TFV Gel Trials in Women with Detectable Drug Levels show Modest Efficacy



Modest HIV protection 50 – 60% if product adherence is high

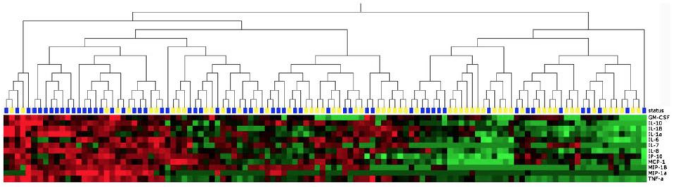
Evidence for Biological Factors Attenuating Topical PrEP Efficacy

Burnet Institute *Abdool Karim et al 2017 Curr Opin HIV AIDS 12*

Genital Inflammation Undermines Effectiveness of 1% TFV Gel in Preventing HIV Acquisition in Women

Genital Inflammation (≥3 of 9 cytokines), upper quartile

IL1a, IL1b, IL6, TNF, IL8, IP-10, MCP-1, MIP1a, MIP1b



CAPRISA 004 Trial
Overall efficacy 34%

Post hoc analysis

N=774

	HIV Protection TFV	HIV Protection TFV highly adherent
Genital inflammation	57% (95% CI:7-80%)	75% (95% CI: 25-92%)

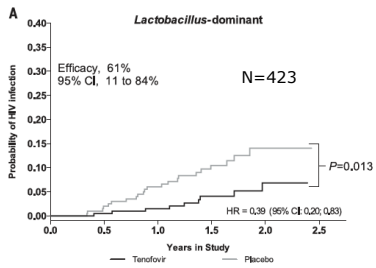
Women without genital tract inflammation largely account for the protective effect of tenofovir gel in CAPRISA 004

Burnet Institute *McKinnon et al 2018 Nature Medicine 24:491*

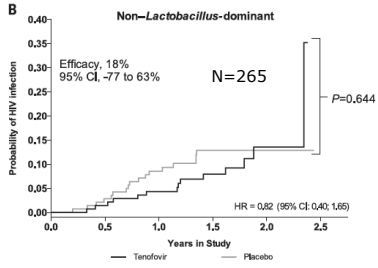
Highly diverse vaginal microbiota metabolise antiretrovirals to potentially decrease topical PrEP efficacy

CAPRISA 004: Diminished Tenofovir Efficacy in Women with Vaginal Microbiota NOT Dominated with Lactobacilli

Lactobacillus-dominant



Non-Lactobacillus-dominant



N=688

TFV gel reduced HIV incidence c/w placebo gel

61% (p=0.013)

18% (p=0.644)

Similar clinical, behavioural, demographic characteristics at baseline as well as similar sexual behaviour and gel adherence during trial
Similar proportion of women with L crispatus in gel vs placebo group in LD category

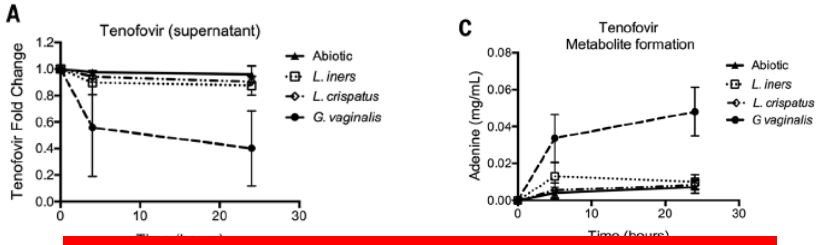
TFV gel is less effective against HIV in women with non-Lactobacillus dominant vs women with Lactobacillus-dominant microbiota

Klatt et al 2017 Science 356:938

CAPRISA 004: Tenofovir Metabolised to Adenine by *G. vaginalis* but not *L crispatus* or *L iners*

CVL samples	**Genital TFV Concentration** (upper quartile)
LD Women	8020 ng/ml
Non-LD Women	24.3 ng/ml (p = 0.0077) *n=270*

(no difference in adherence)



LD = h

BV associated microbiota metabolise TFV reducing extracellular drug availability

Klatt et al 2017 Science 356:938

Tenofovir in Topical but not Oral PrEP Metabolised by Vaginal microbiota

Topical TFV PrEP metabolism supported by *in vivo* study

Women with BV associated *G. vaginalis* had decreased levels of TFV diphosphate in cervical tissues and plasma following 2 hours of directly observed product application vs women with lactobacillus-dominant microbiota

Oral PrEP

Women in the Partners PrEP Study (oral daily TFV-based PrEP)

No difference in PrEP efficacy for women with bacterial vaginosis vs women with lactobacillus-dominant microbiota (Based on Nugent Score, *G. vaginalis*, absence of *Lactobacillus* spp morphotypes)

Diverse vaginal microbiota appears to decrease TFV levels from topical but not oral TFV-based PrEP

Hillier et al 2017 CROI Heffron et al 2017 Lancet HIV 4:e449 Carlson et al 2017 JID
Taneva et al 2018 JCI Insight 3: e99545

Summary- Vaginal Microbiota Attenuates PrEP Efficacy in Women

Diverse vaginal microbiota (e.g. BV) but not *Lactobacillus* spp attenuates efficacy of topical tenofovir based-PrEP but not oral PrEP

Genital TFV levels negatively correlate with BV (*G. vaginalis)*

Studies are in progress to determine if applicable to other topical PrEP modalities i.e. dapivirine

Development of novel ring and film-based products should investigate whether diverse vaginal microbiota reduces their efficacy

How do lactobacilli protect against HIV?

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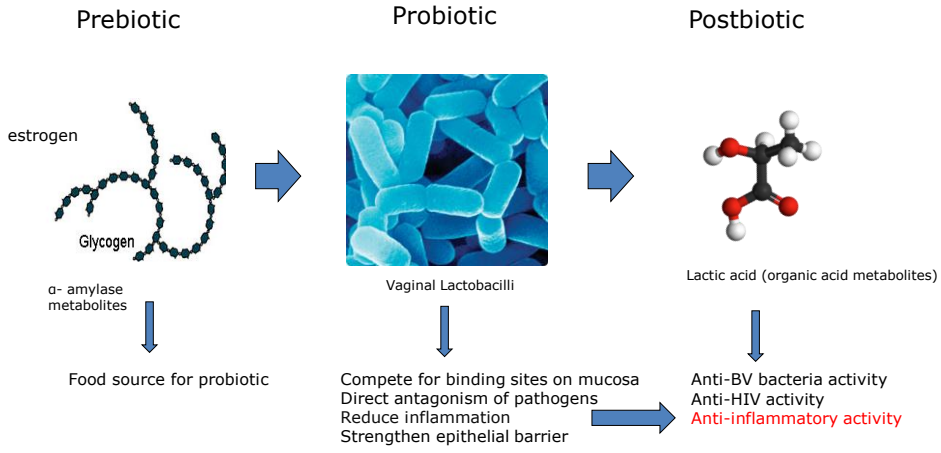
Protective Vaginal Microbiota Produces more Lactic Acid

Acid	Lactobacillus (mM)	High diversity (BV) (mM)
	pH <4.5	**pH ≥4.5**
Lactic	~120[1,2]	≤20[4]
Acetic	2-4[3]	≤120[1, 3]
Propionic	<1	2-4[3]
Butyric	<1	2-4[3]
Succinic	<1[4]	≤20[4]

L. crispatus acidifies vagina to lower pH[5]

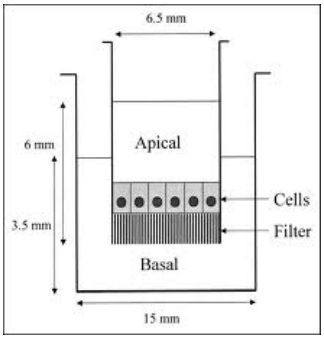
Does lactic acid have a protective role in the FRT?

Lactic acid – an Effector Metabolite Produced by Vaginal Lactobacilli?



[1]O'Hanlon et al 2011 BMC Infect Dis 11; [2]O'Hanlon et al 2013 PLoS One 8:e80074; [3]Aldunate et al 2013 J Antimicrob Chemother; [4]Palomino et al 2017 Front Microbiol 8 907 [5]Graver and Wade 2011 Clin Microbiol Antimicob 10:8; [6]Gong et al 2014 PLoS ONE 9:e107758; [7]Isaacs et al 2013 Antimicrob Agents Chemother 57: 3806; [8]Hearps et al 2017 Mucosal Immunol 10:1480; [9]Tyssen et al 2018 mSphere Jul 5; 3(4)

Experimental System for Evaluating Immune Modulatory Effects of Lactic acid



Human Epithelial Cells:

Vaginal: VK2/E6E7
Ectocervix: Ect1/E6E7
Endocervix: End1/E6E7

Primary ectocervical cells
Organotypic cervicovaginal tissue model

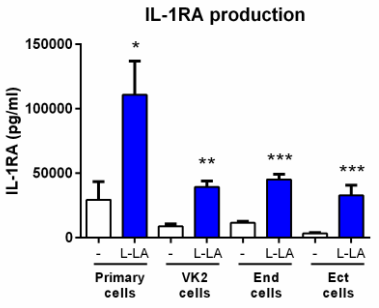
Add LA ± TLR agonist apically SFKM:

TLR1/2 [Pam(3)CSK(4)] (HIV gp120, BV)
TLR3 (polyIC - PIC)
TLR4 (LPS) (HIV gp120, BV)

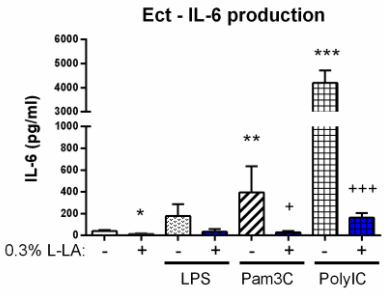
Soluble immune mediators relevant to HIV infection: cytokine bead array/luminex

Lactic Acid (LA) is Anti-inflammatory on Cervicovaginal Epithelial Cells

Anti-inflammatory Cytokine



Pro-inflammatory Chemokine

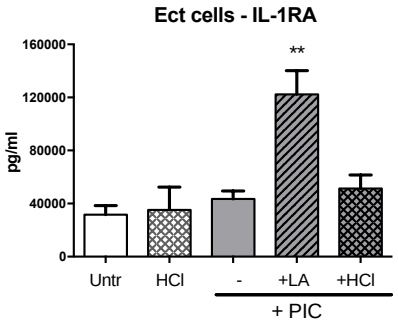


Similar increase in IL-1RA with TLR agonists: polyIC, LPS, Pam3C
Mops up IL1a and IL1b – increase HIV

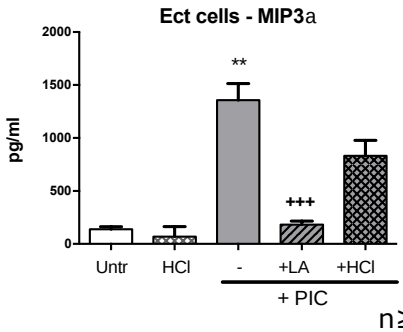
Similar effects IL-8, TNF, RANTES, MIP3α

Low pH Alone does not Reproduce Lactic Acid's Anti-inflammatory Effects

Anti-inflammatory Cytokine



Pro-inflammatory Chemokine

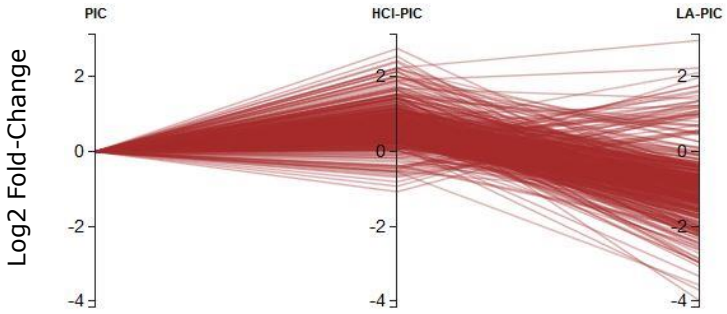


n≥ 5

LA is not simply a low pH effect and it is mediated by as little as 1 hour treatment

RNA-Seq – Distinct Gene Expression Profile for Lactic acid vs HCl relative to PolyIC (DEGUST)

Relative to PIC

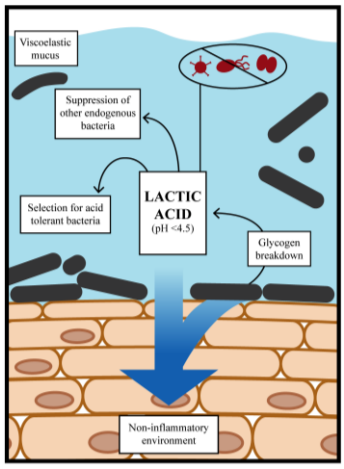


Parallel coordinates plot

FDR <0.05
Log FC 0.5

Lactobacillus Dominant Vaginal Microbiota to Decrease HIV Susceptibility and Promote PrEP Efficacy – Adjunct to ARV PrEP

Lactobacillus spp.



Topical PrEP

HIV STIs

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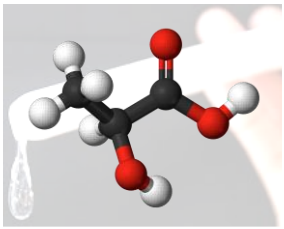
Aldunate et al 2015 Frontiers in Physiology 6:164

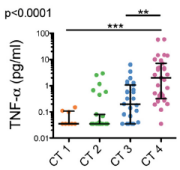
Translation: POC Study in Women with BV

Bespoke LA Vaginal Gel Physiological Levels and pH



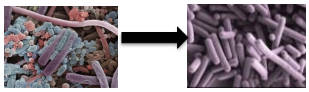
ACH2

Sustained release formulations

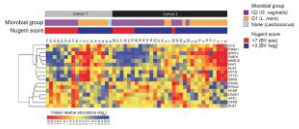


Inflammation

US Patent: Anti-inflammatory/BV



Vaginal microbiome



Vaginal proteome

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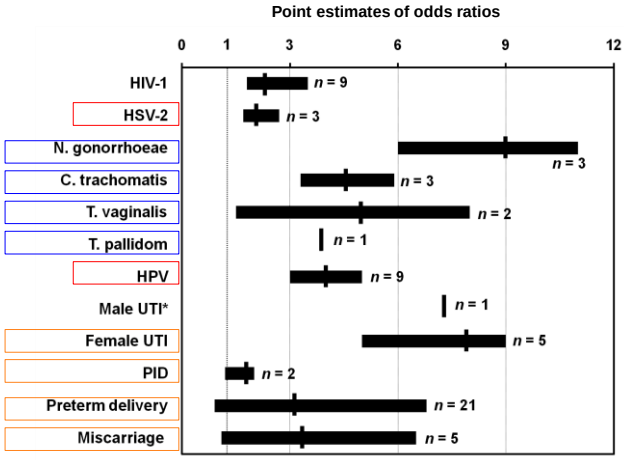
High Vaginal Bacterial Diversity (BV) is Associated with Adverse Sexual and Reproductive Health Outcomes



64 prospective studies

BV or not on day of trial entry

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Compiled by Richard Cone and Thomas Moench

Acknowledgments

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