

Effect of the vaginal environment on infected leukocytes

Richard Cone

Biophysics, Johns Hopkins University
ReProtect, Inc.

“Trojan Horse” workshop, Boston, 10/19/2013

Effect of the **multiple** vaginal environments on infected leukocytes

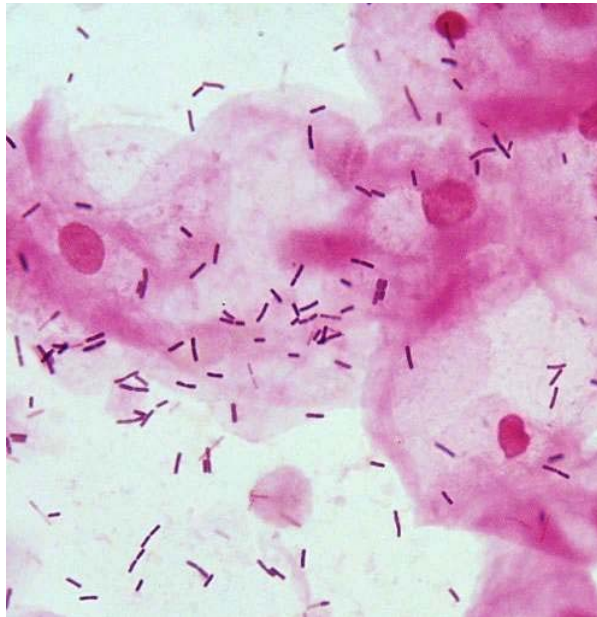
Richard Cone

Biophysics, Johns Hopkins University
ReProtect, Inc.

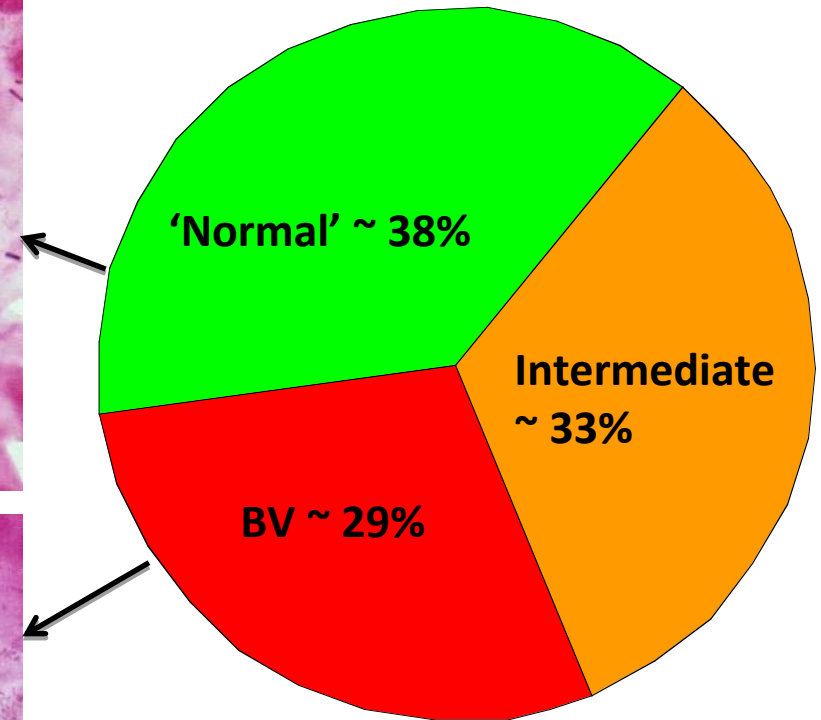
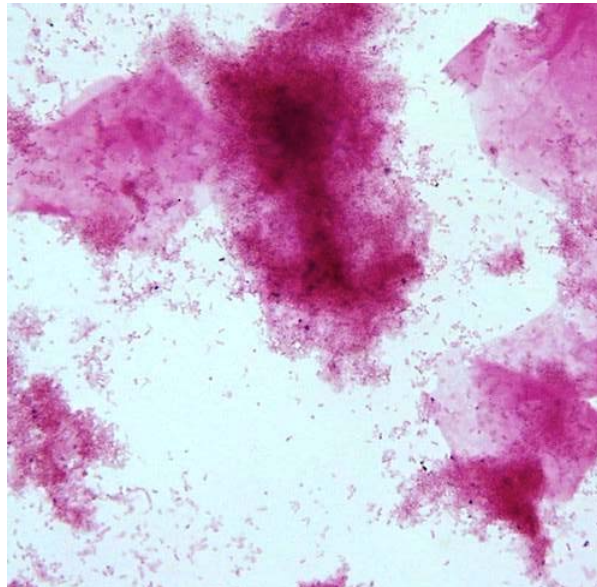
“Trojan Horse” workshop, Boston, 10/19/2013

Only a minority of women have a 'normal' lactobacillus dominated vaginal microbiota

pH 3.5
1% Lactic acid

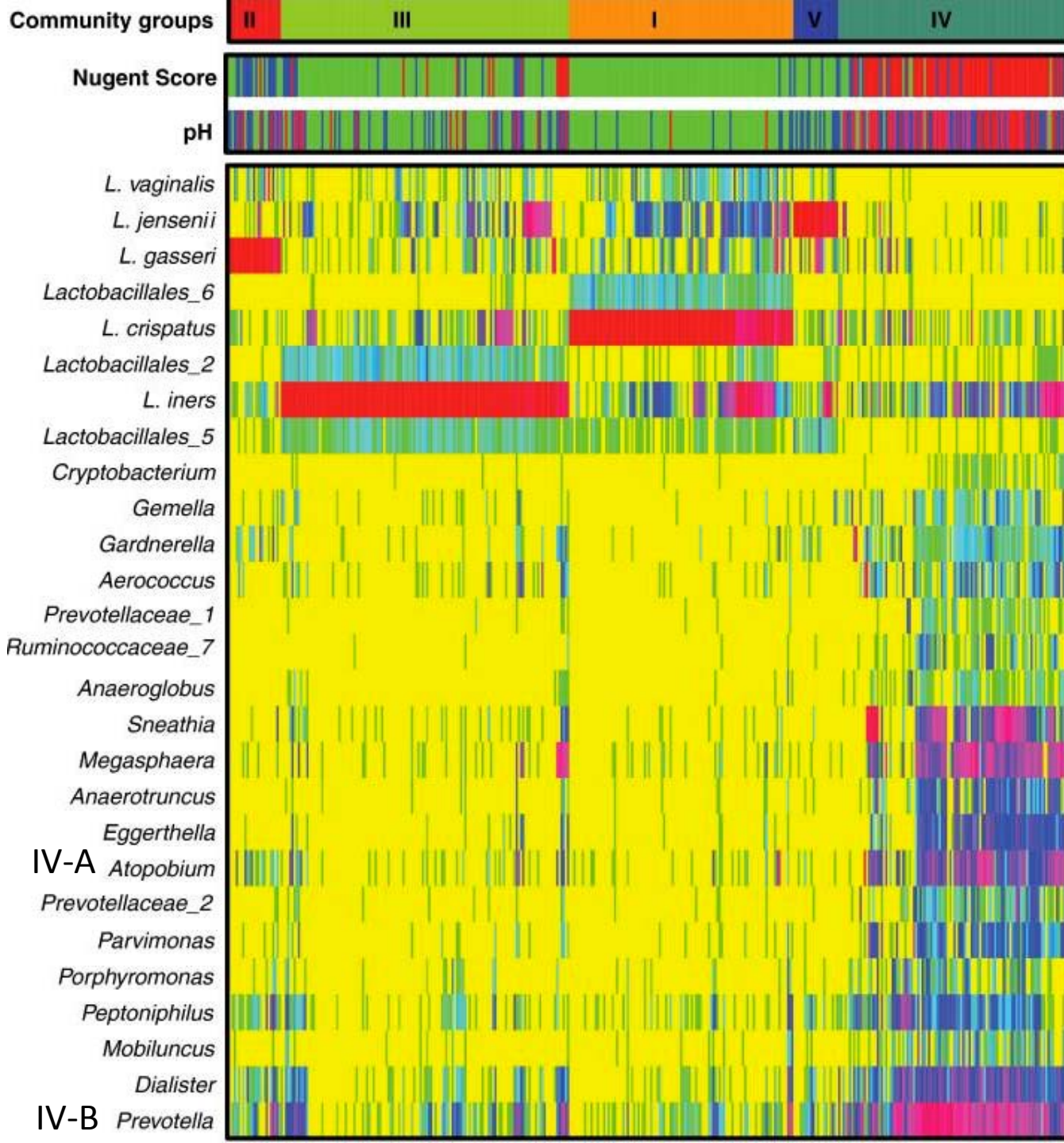


pH 5-6
0.3% Acetic acid
Succinic acid
Butyric acid
Putrescine
Cadaverine
Tyramine
(fishy odor)



% of U.S. women
on any given day

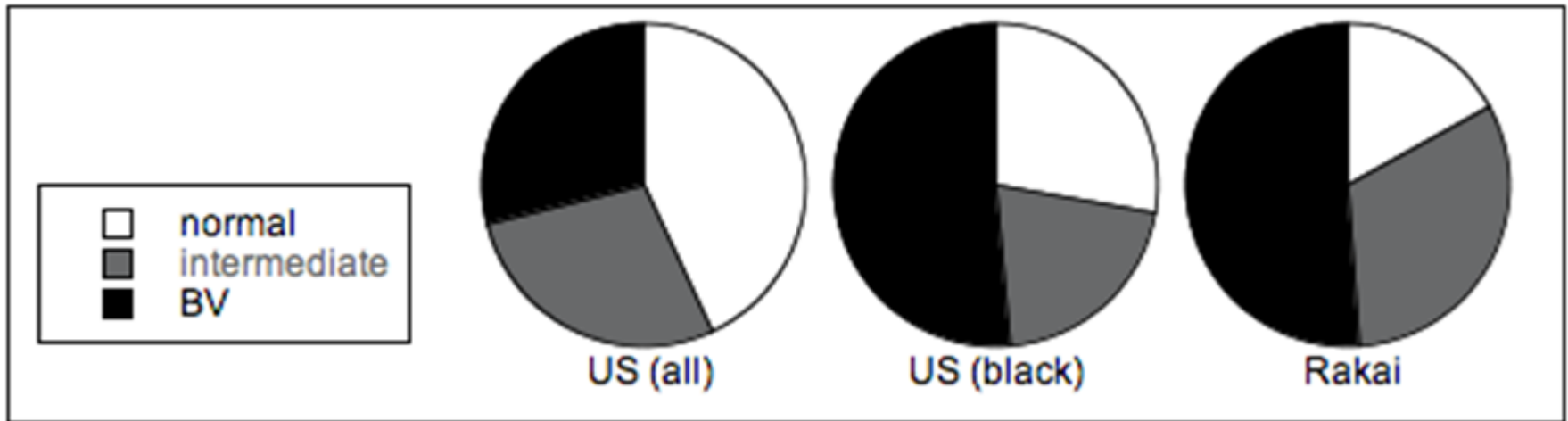
Allsworth and Peipert, 2007



Ravel et al
PNAS 2011

BV is strongly associated with increased risk of infections and poor birth outcomes:

Populations with high prevalence of BV are at high risk of HIV:



BV increases several factors that may increase susceptibility to infections, e.g., inflammatory cytokines, and it also eliminates protection by Lactic Acid!.

Most prevalent vaginal microbiota communities:

Pre-menopause, *L. crispatus*, *L. iners*, and IV ('BV')

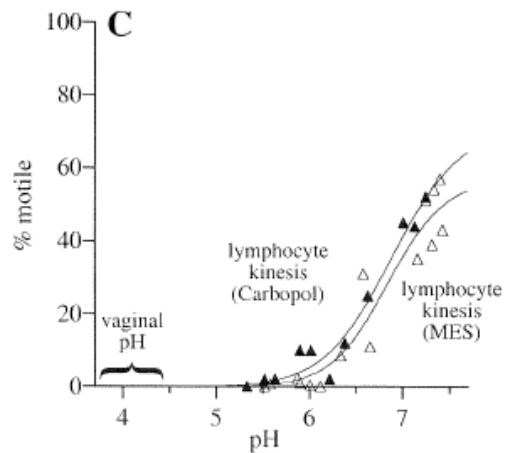
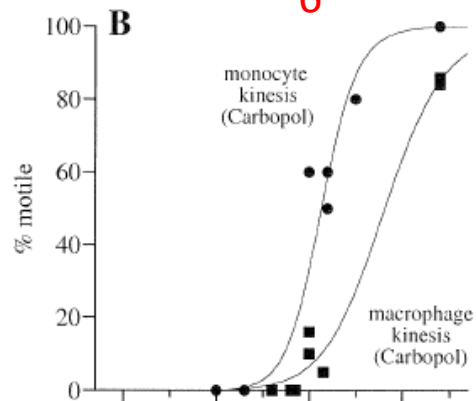
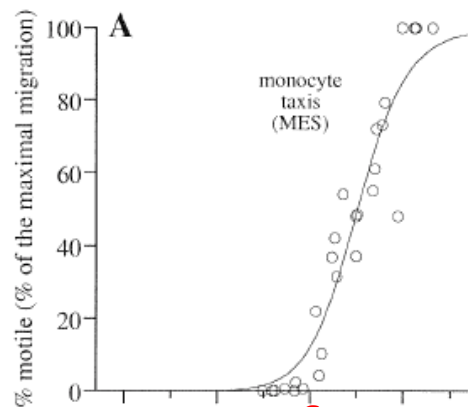
Peri-menopause, IV-A (Prevotella) and *L. gasseri*

Post-menopause: IV-A

Vulvovaginal atrophy post-menopause: IV-A

Risk of VVA 25-fold higher with IV-A vs *L. crispatus*

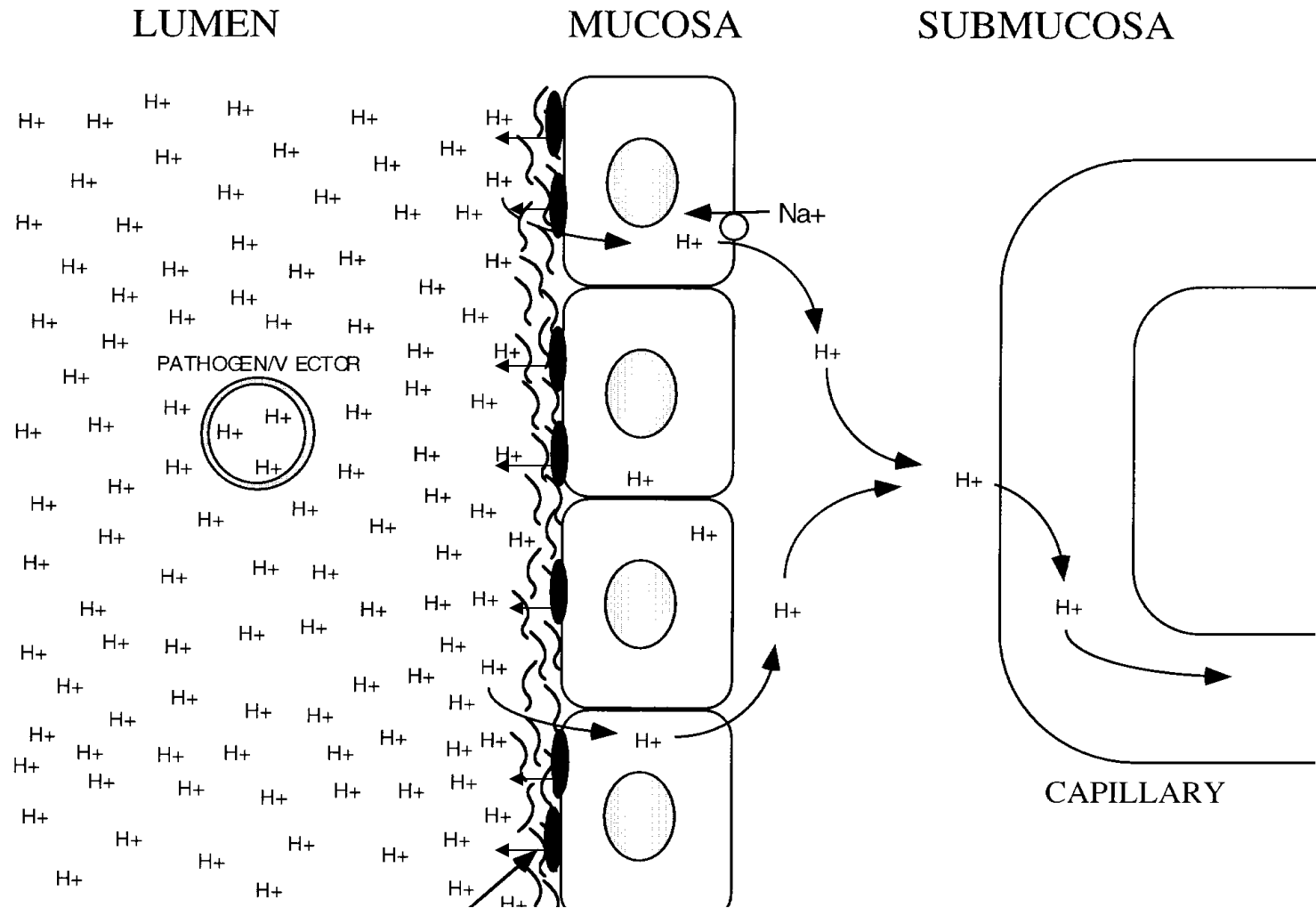
Brotman et al, Menopause, 2013



Low pH immobilizes and kills human leukocytes and prevents transmission of cell-associated HIV in a mouse model

Olmsted, Khanna, et al, BMC Infect Dis 2005

VAGINAL ACIDITY INACTIVATES CELLS AND PATHOGENS WITHOUT INJURING MUCOSA



Lactobacilli acidify the vagina with lactic acid

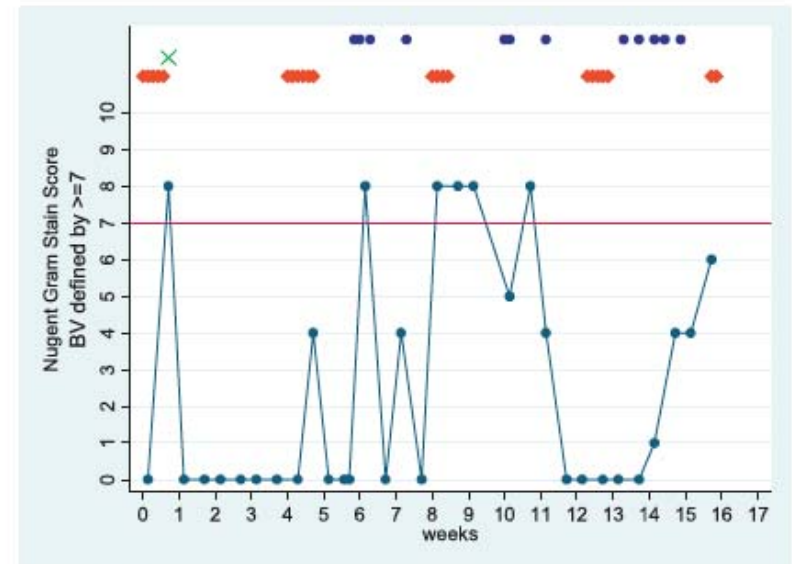
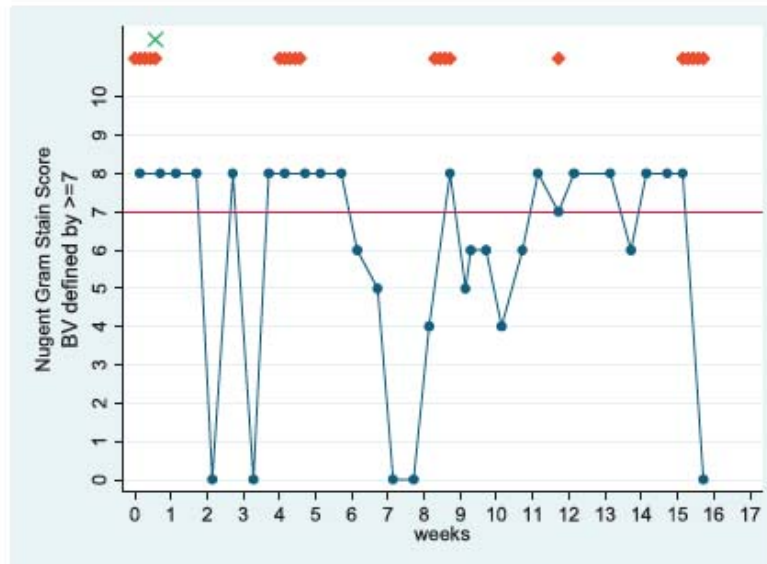
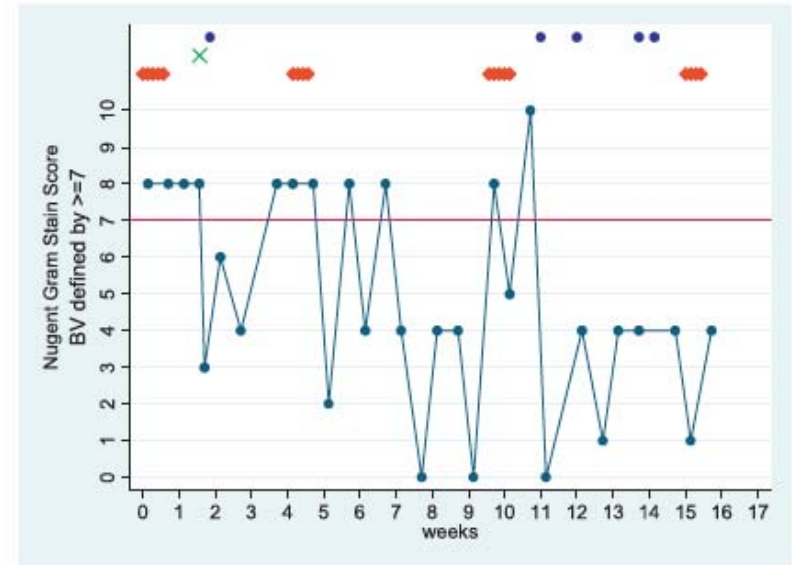
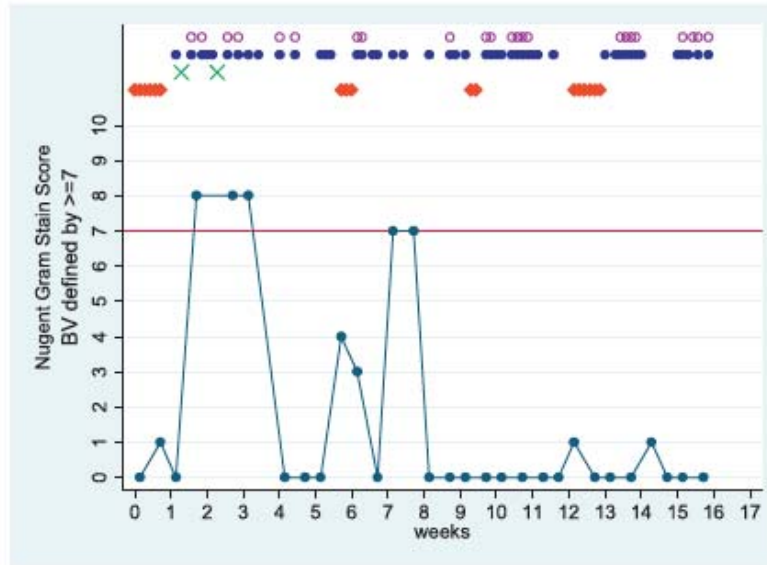
Rapid fluctuation of vaginal microbiota (over 4 months)

Brotman, Ravel Cone, et al STI 2010

BV

Inter-
mediate

LB



- Nugent score
- receptive oral sex
- penile / vaginal intercourse
- ◆ menses
- × vaginal douche

Temporal Dynamics of the Human Vaginal Microbiota

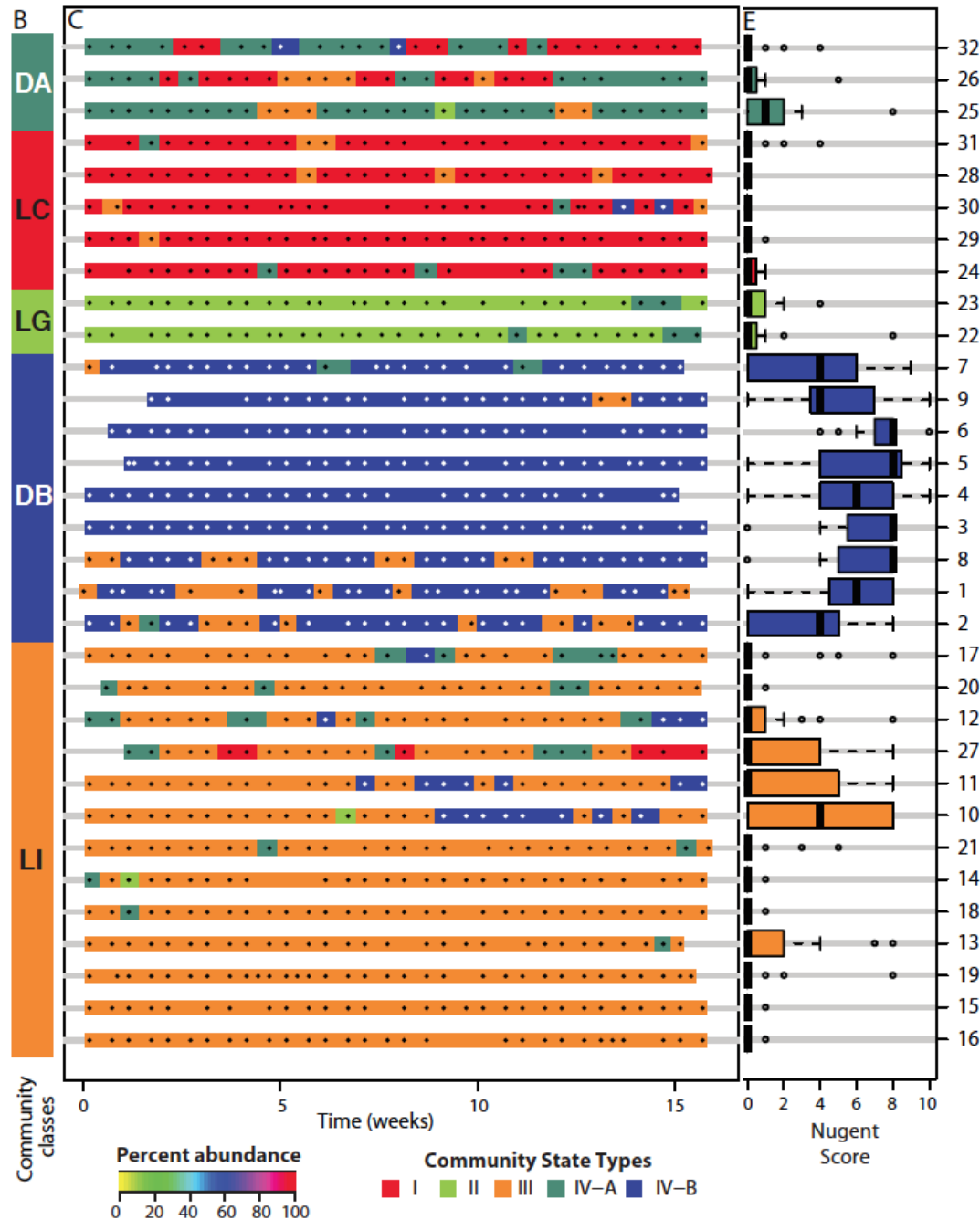
Communities

IV-A

L. crispatus

IV-B

L. iners

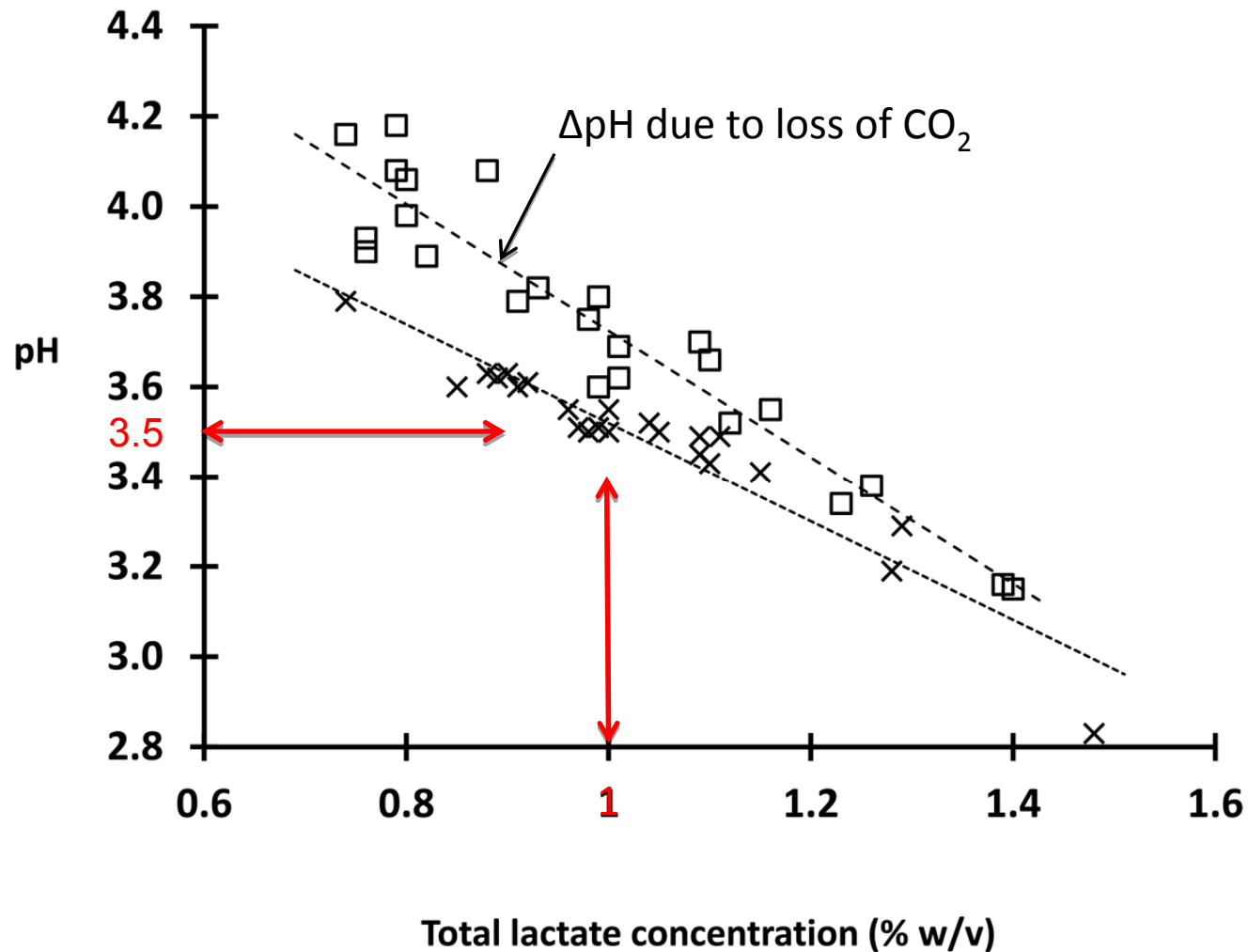


Nugent Scores

Gajer, Brotman et al,
Sci Transl Med, 2012

**Vaginal pH and microbicidal lactic acid concentration when
L. crispatus dominates the microbiota (Nugent Scores 0-3)**

Our observations are based on Johns Hopkins students: *L. crispatus* and *L. iners*



L. crispatus acidifies vaginal pH to $\sim 3.5 \pm 0.3$ O'Hanlon et al PLoS One 2013
and produces a ~racemic mixture of D- and L lactic acid.

When vaginal isolates of *L. crispatus* are cultured, they acidify the medium to essentially the same pH and D-/L- ratio as the vagina from which they were obtained. Boskey Cone Whaley Moench, Human Reproduction 2001

Essentially all our samples from **Hopkins students** are *L. crispatus* and *L. iners*.

We have collected over 250 Hopkins student samples with Nugent scores 0-3

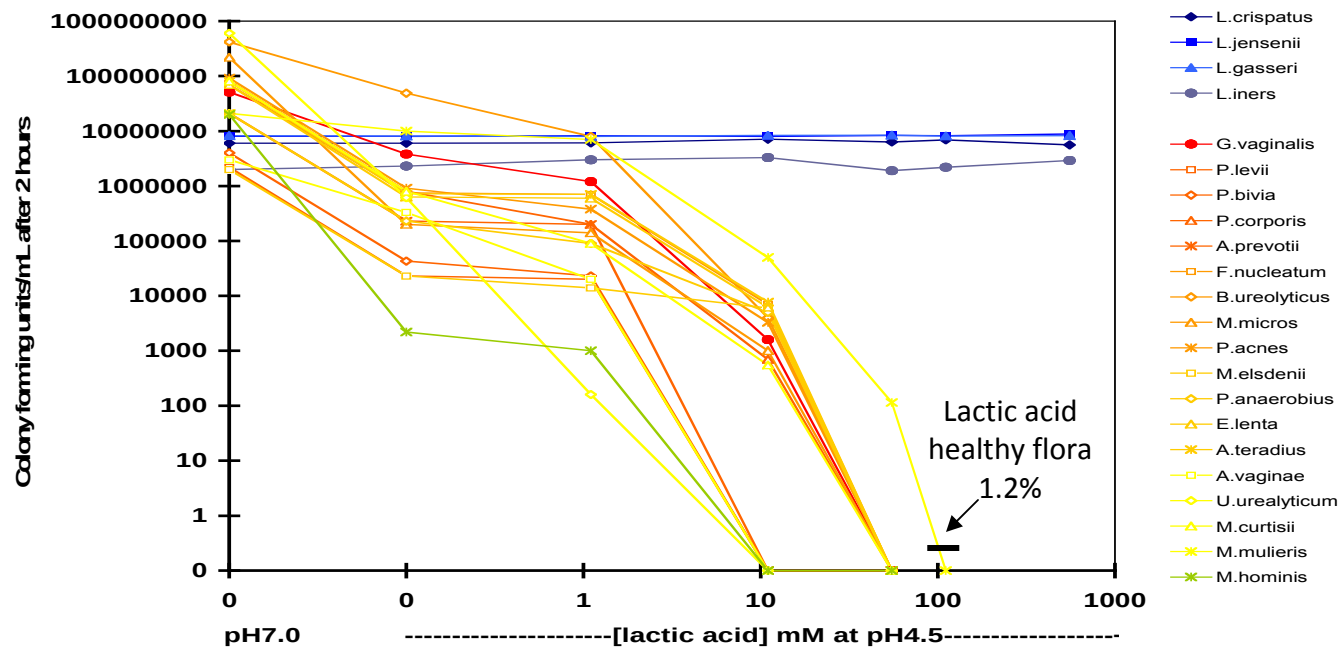
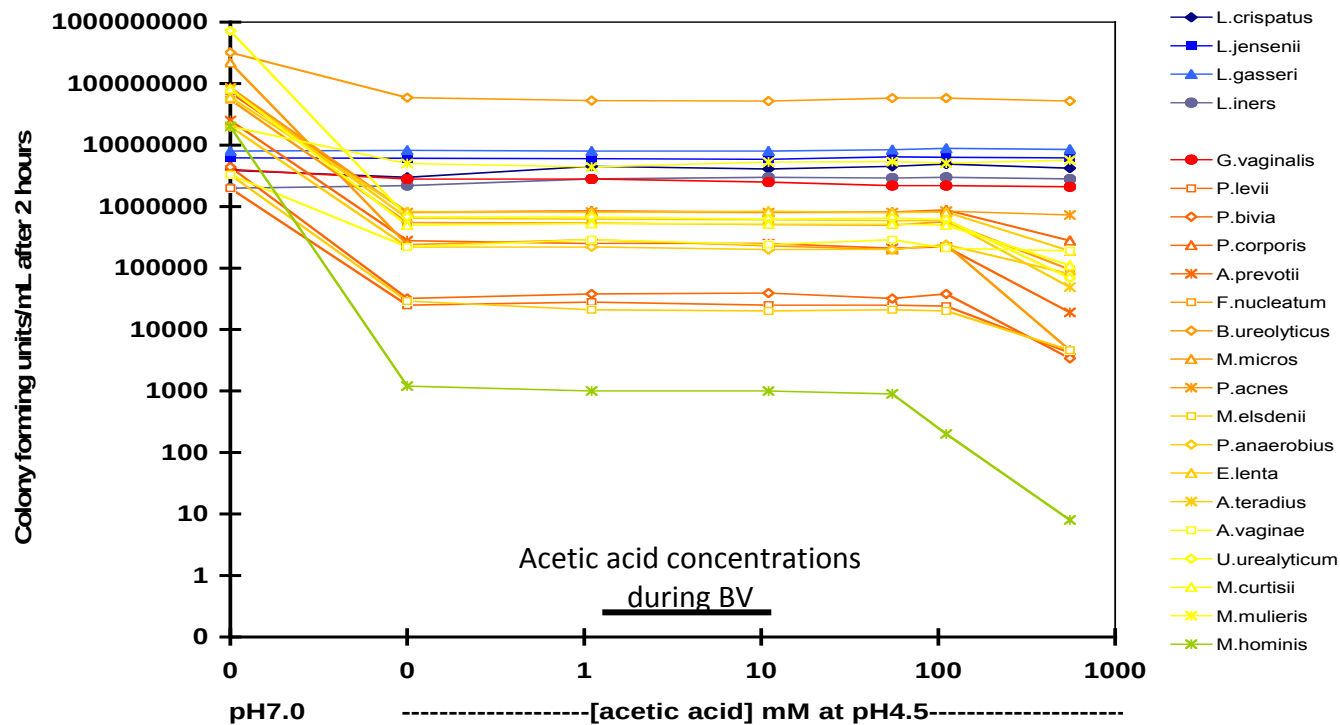
At least 70 Of these students were black,

We had only one BV sample, and the donor was white. O'hanlon *in prep*.

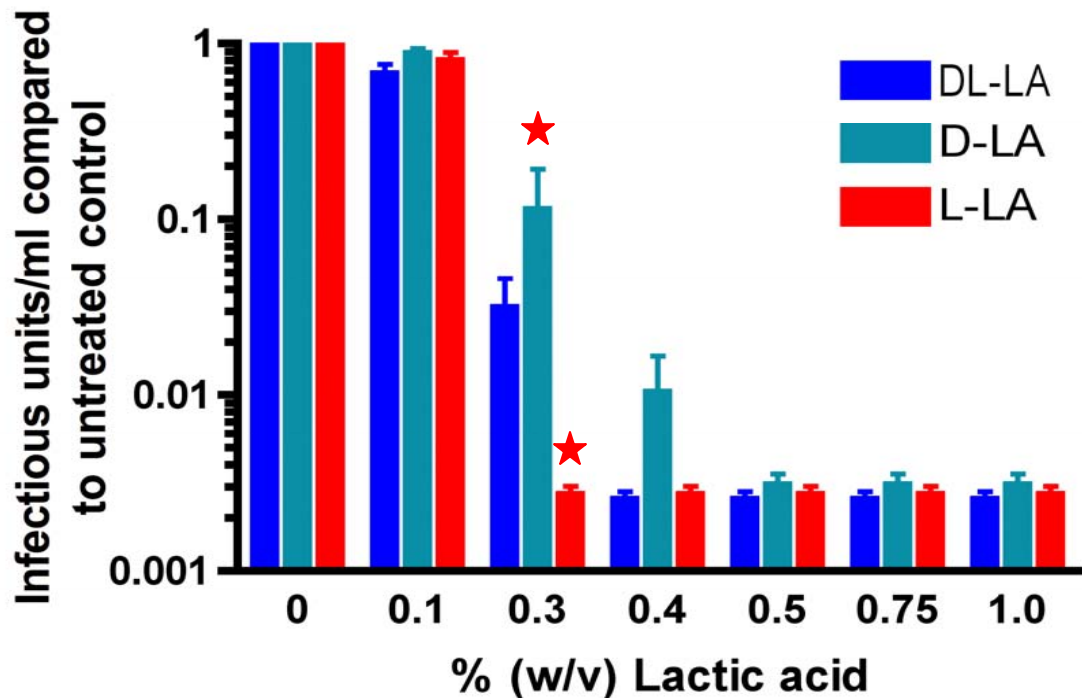
Most of our low Nugent score samples from the **Baltimore STD clinic** are *L. iners*.

L. iners produces only the L-isomer. Witkin et al, mBio, 2013

L. iners in vitro is less susceptible to the diamines produced by BV-bacteria than *L. crispatus*. So *L. iners* “survives” BV better? Li Han Lai JHU Masters Thesis 2010



L-Lactic acid is more potent than D- and DL-LA in inactivating HIV_{Ba-L}



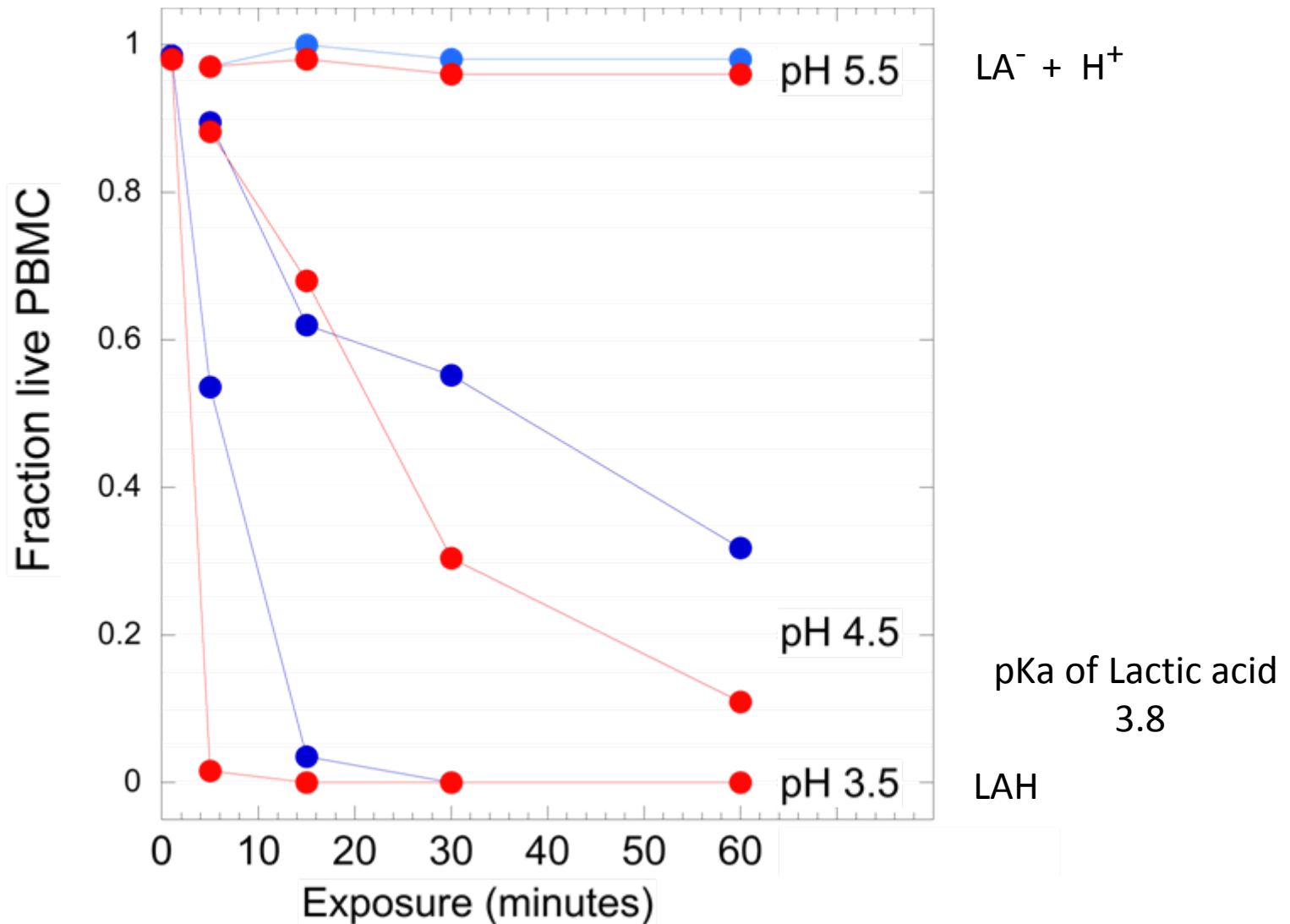
★ p=0.05 (n=3)

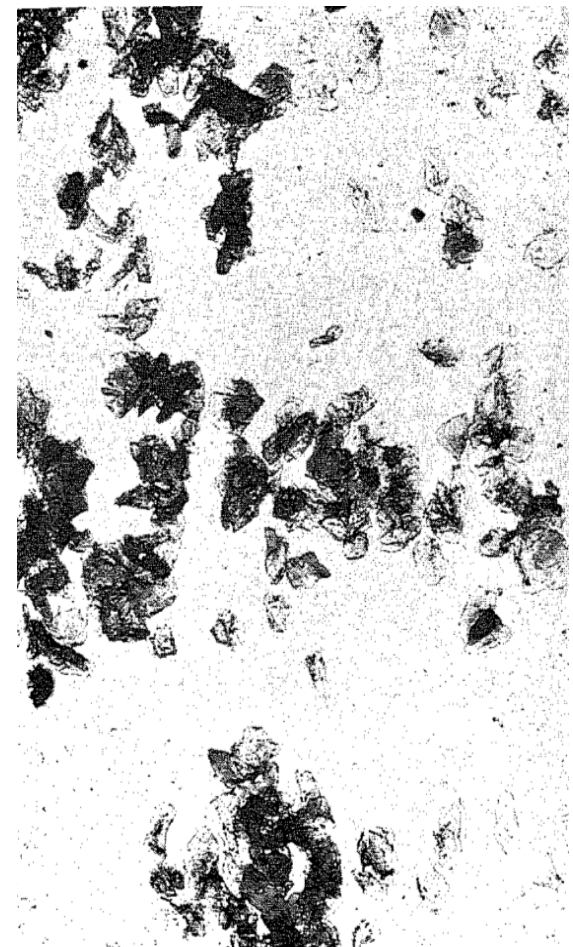
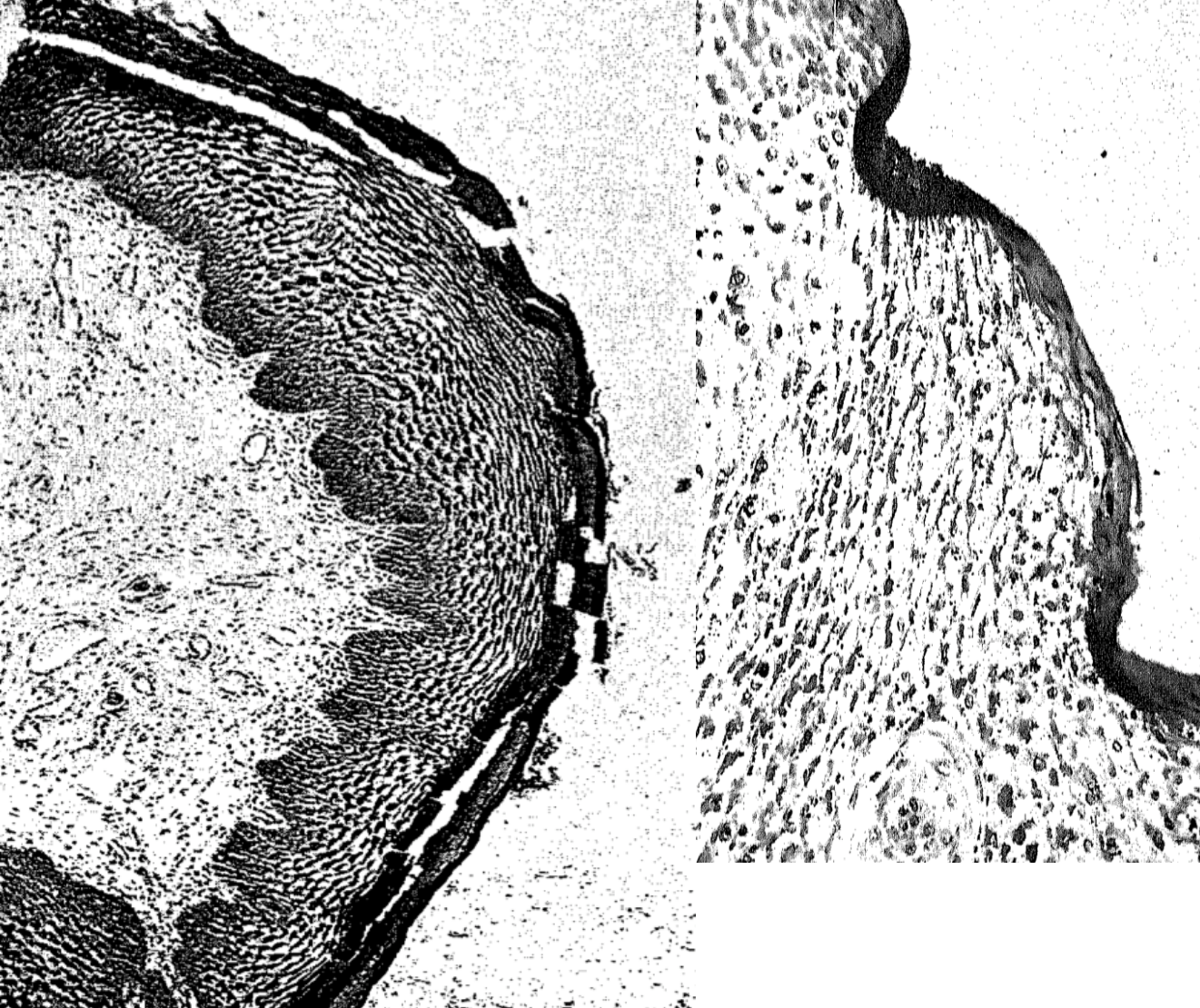
37°C/30 min, dilute and neutralize, infectious virus TZM-bl

Vaginal concentrations of lactic acid potently inactivate HIV

Aldunate, Tyssen, Johnson, Zakir, Sonza, Moench, Cone, Tachedjian, J. Antimicrob Chemother 2013

Acid inactivation of PBMC at 37°C in RPMI media
acidified with HCL or 0.5% Lactic Acid



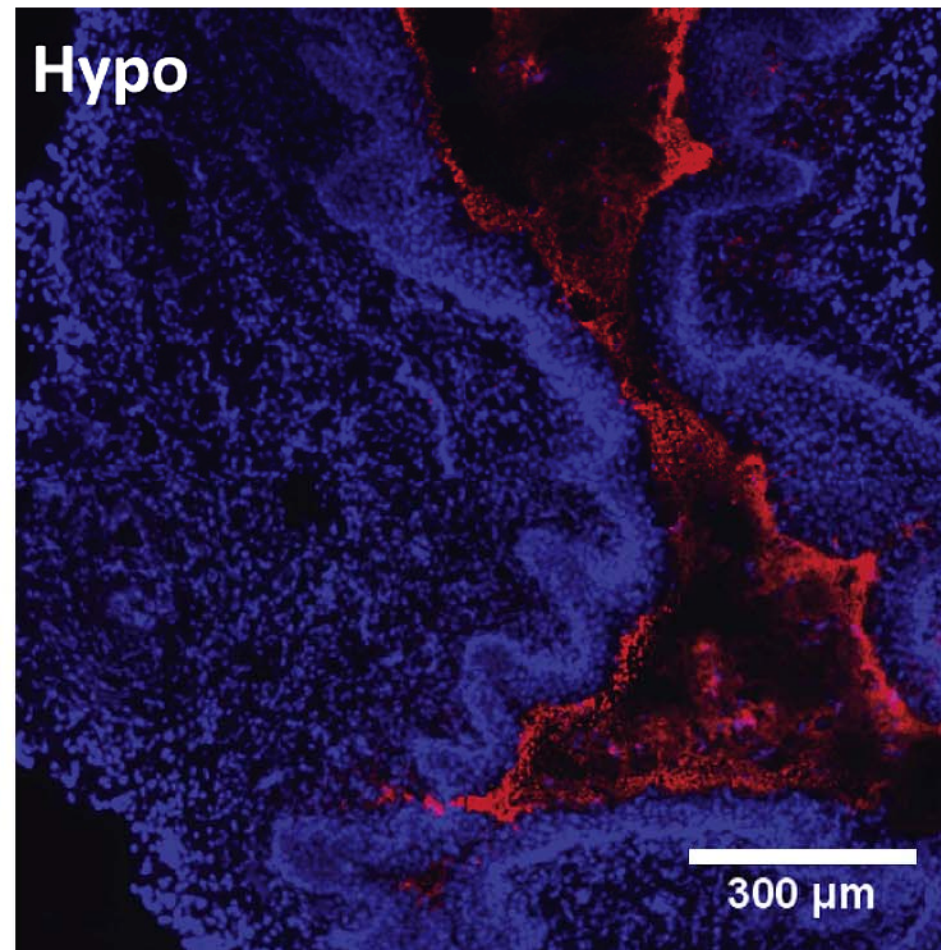
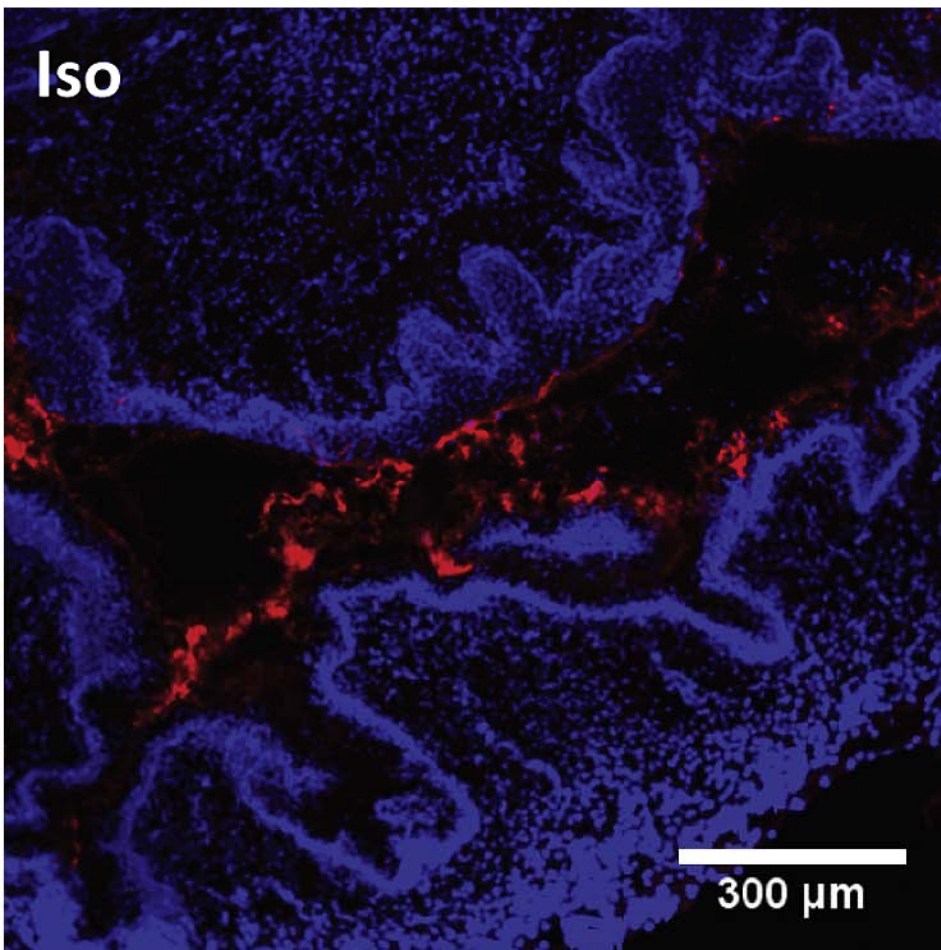


Human vaginal epithelium and shed cells stained for glycogen

Enhanced vaginal drug delivery through the use of hypotonic formulations that induce fluid uptake

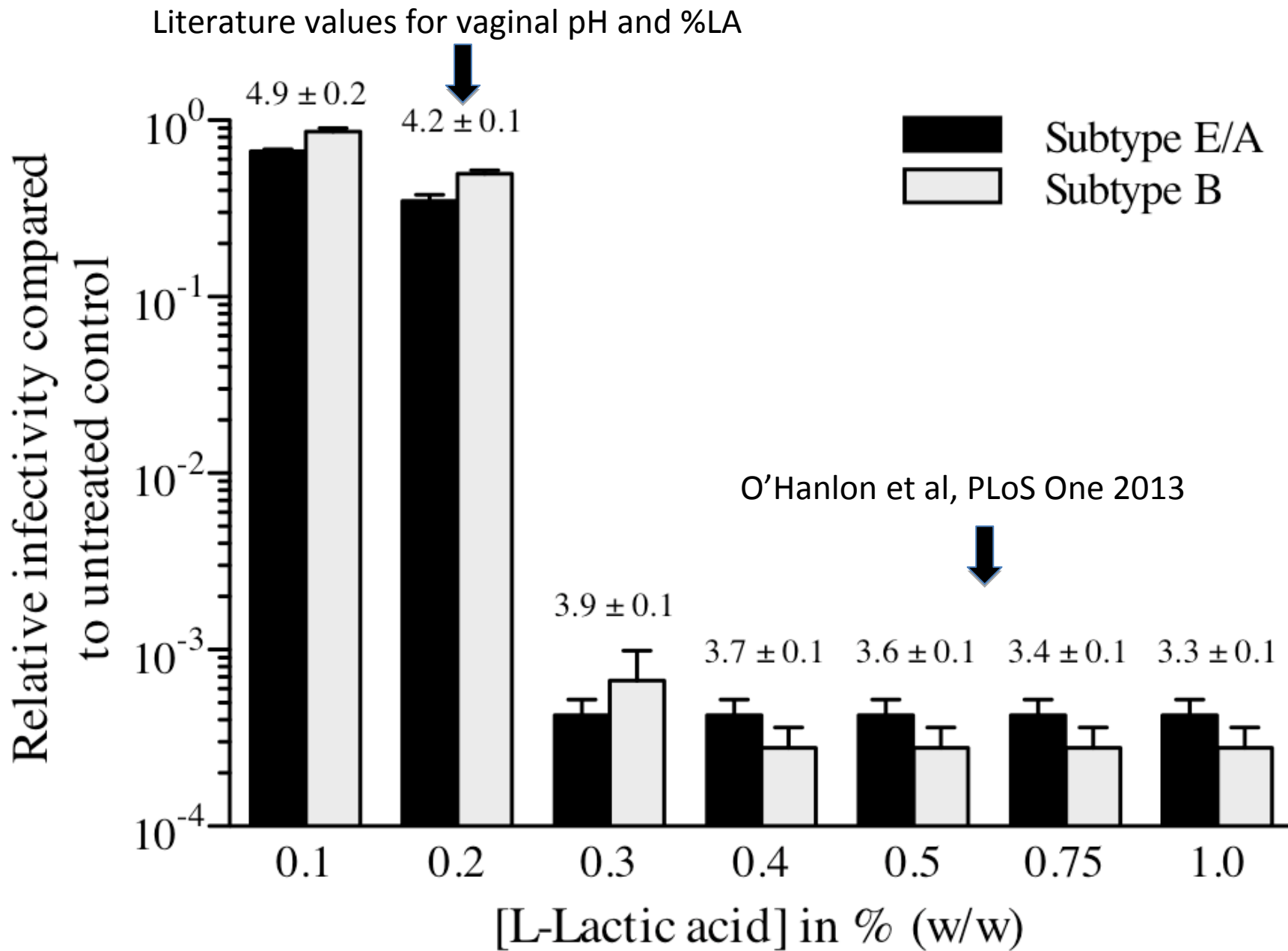
Ensign, Hoen, Maisel, Cone, and Hanes

Biomaterials 2013

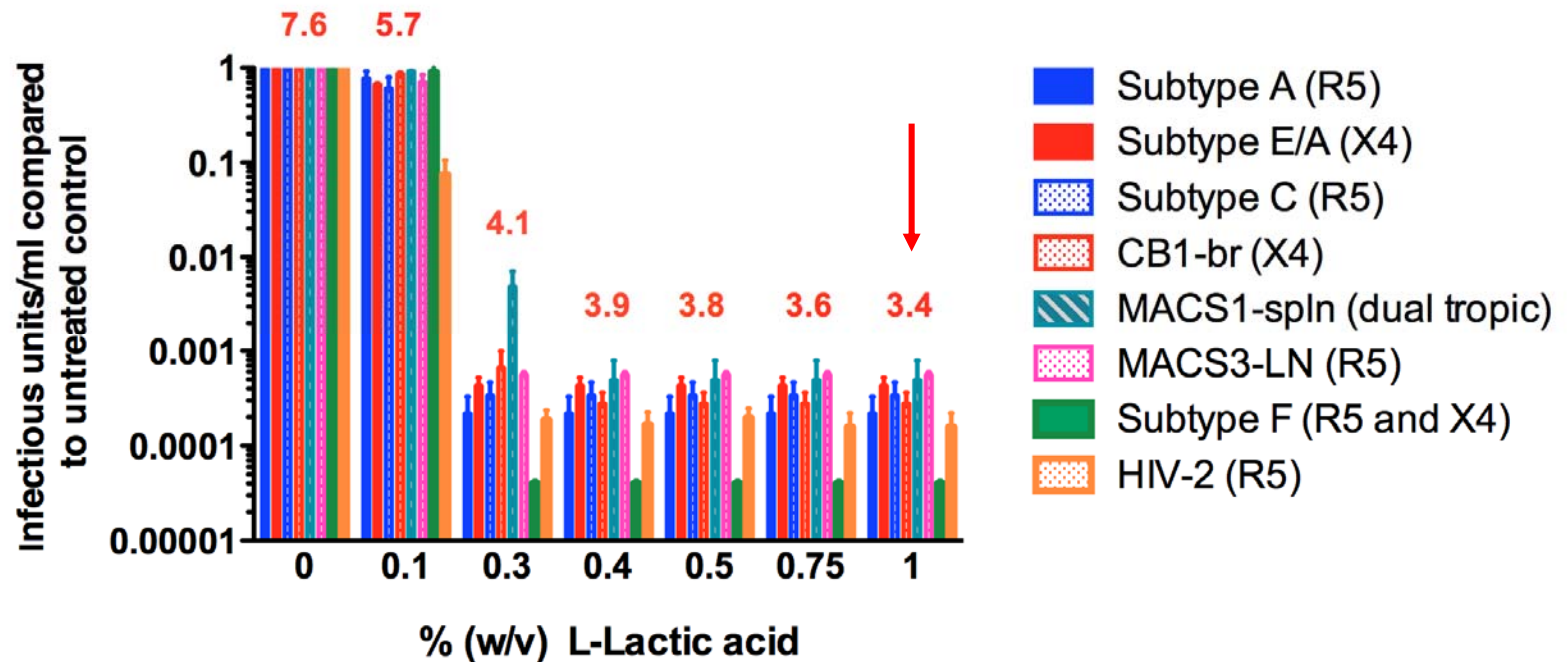


Mucus penetrating particles 100 nm in diameter delivered acyclovir more effectively than free drug for preventing HSV infections in the mouse vagina.

“Advective” transport caused by osmotically driven absorption of water from the lumen.



L-LA inactivates different HIV-1 subtypes, X4 and R5 strains, patient isolates and HIV-2



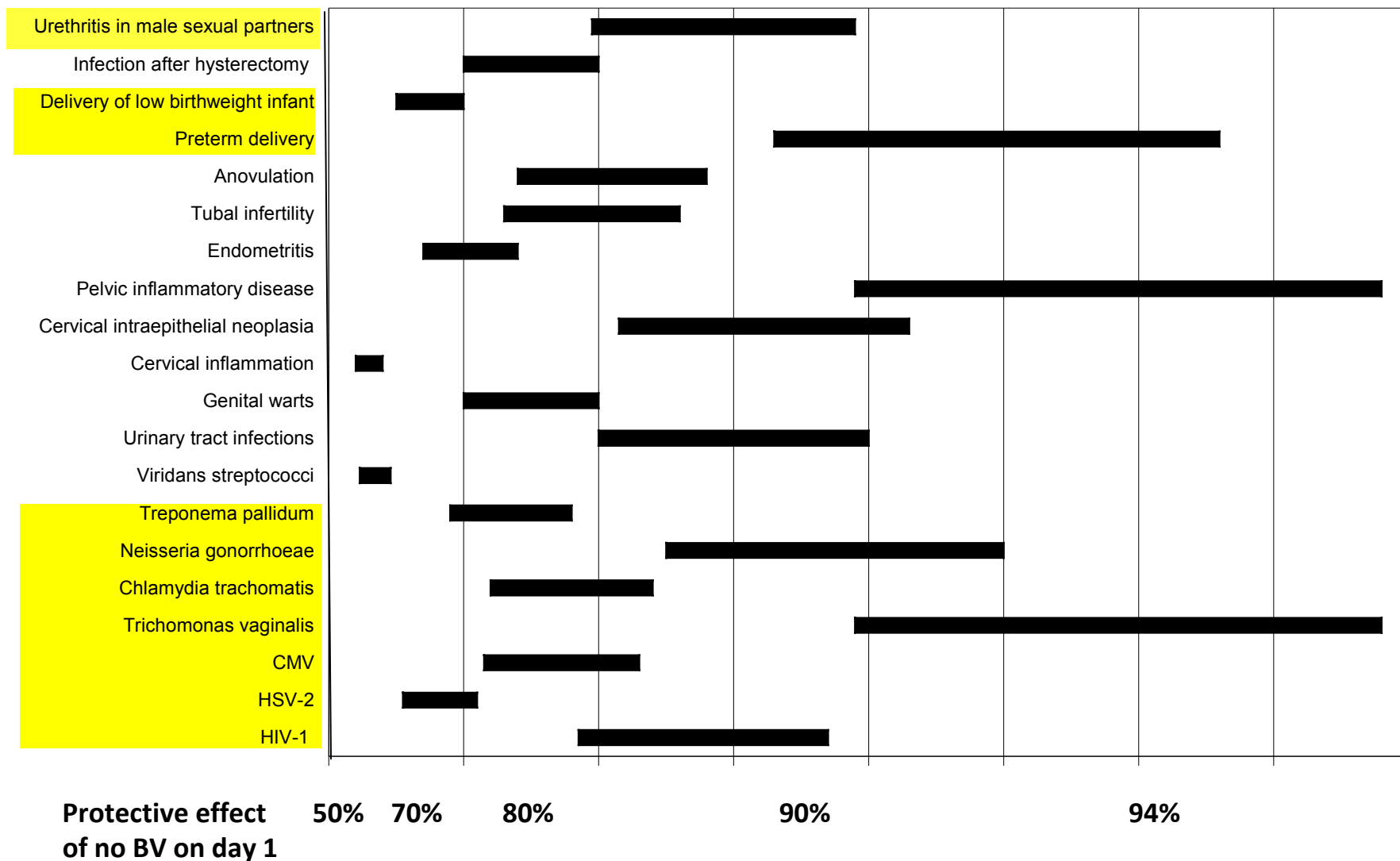
Inactivation by LA is pH dependent
and appears irreversible

37°C/30 min, dilute and neutralize, infectious virus TZM-bl

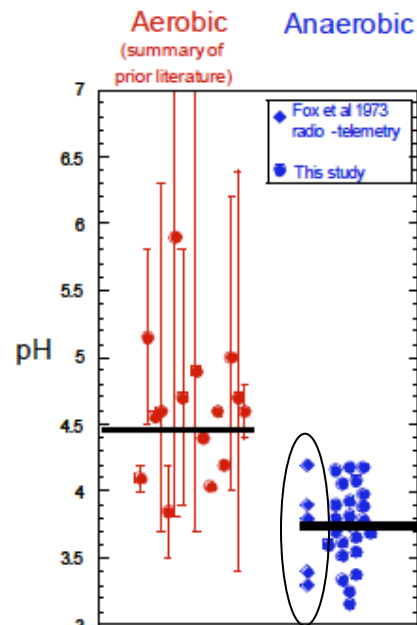
Vaginal concentrations of lactic acid potently inactivate HIV

Aldunate, Tyssen, Johnson, Zakir, Sonza, Moench, Cone, Tachedjian, , J. Antimicrob Chemother 2013

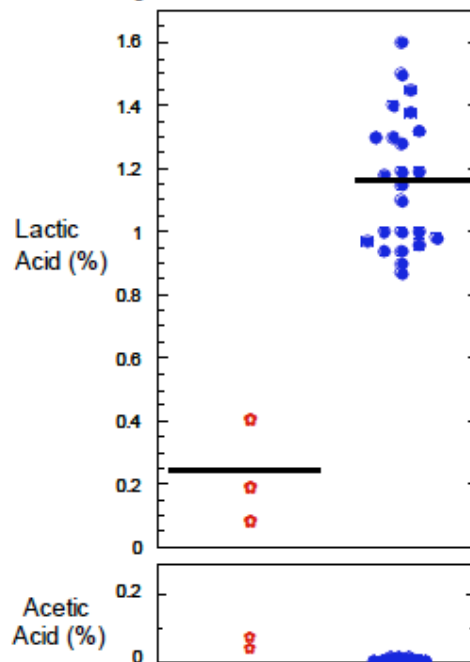
Protective effect if 'healthy' or 'intermediate flora' present at day of trial entry



Summary of Aerobic versus Anaerobic observations:



Combo(+) =
3% SPL7013
BufferGel
0.6% lactic acid
0.09% acetic acid



Microbicide production by vaginal lactobacilli: vaginal acidity (pH) and lactic acid are more potent than previously reported.

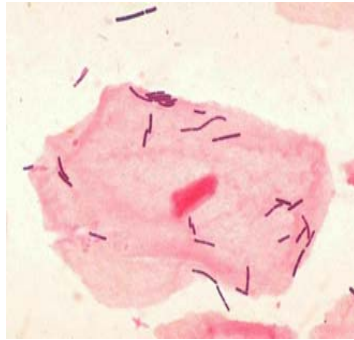
Deirdre E. O'Hanlon*, Thomas R. Moench, MD†, Suzanne Harrold*, Richard A. Cone, PhD*†
•Thomas C. Jenkins Department of Biophysics, Johns Hopkins University; †ReProtect Inc., Baltimore MD

Microbicides 2008, New Delhi, Poster TA-23

Only a minority of women have a 'normal' vaginal microbiota

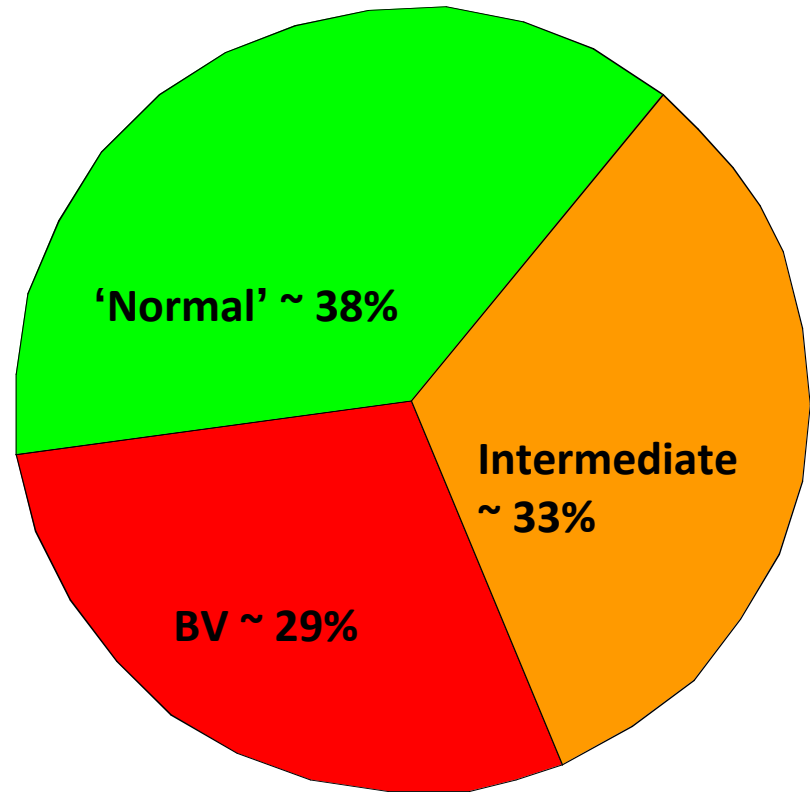
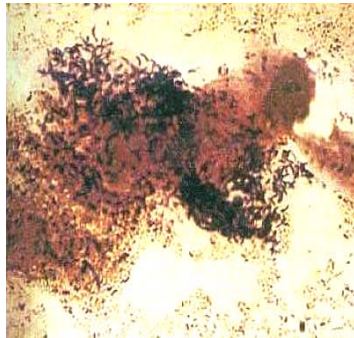
'Normal':

Viscoelastic Mucus
Light gram positive
monoculture
pH 3.5
1% Lactic acid



BV:

Variable discharge
Heavy polyculture
pH ≥ 4.5
Succinic acid
Acetic acid
Butyric acid
Putrescine
Cadaverine
Tyramine
(fishy odor)

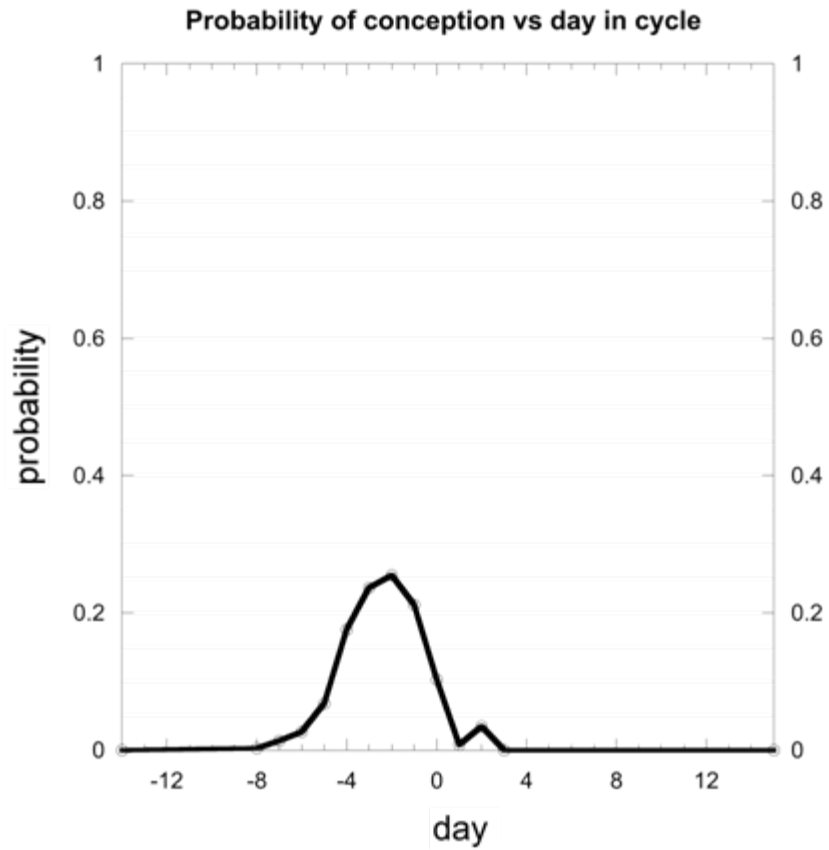


% of U.S.women
on any given day

Allsworth JE, Peipert JF Prevalence of bacterial vaginosis: 2001-2004 National Health and Nutrition Examination Survey data. Obstet Gynecol. 2007 Jan;109(1):114-20. (3,727 women)



The rate at which human sperm are immobilized and killed by mild acidity
Olmsted, Dubin, Cone and Moench
Fertil Steril 2000

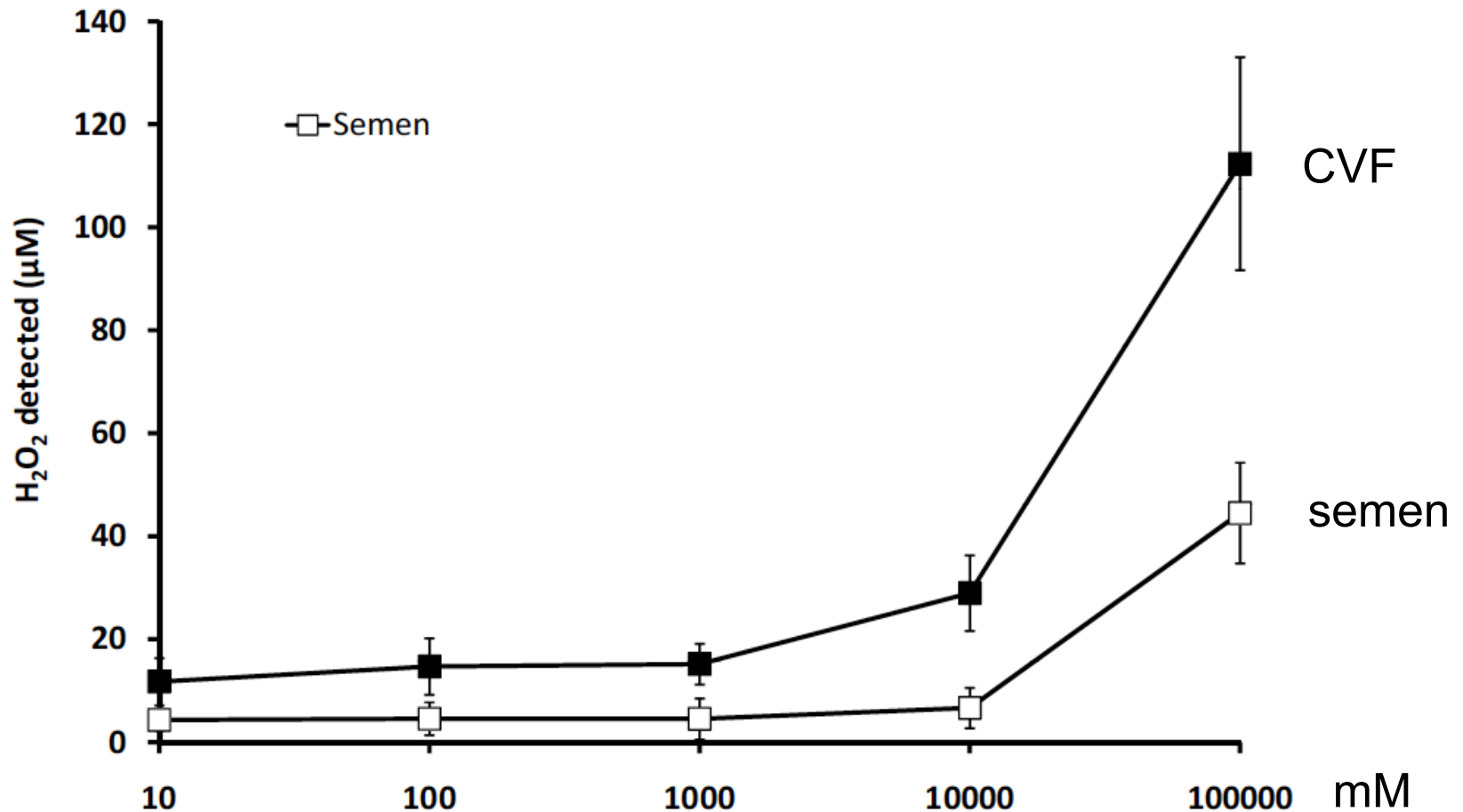


Probability per intercourse: ~1.4%

Cervicovaginal fluid (CVF) and semen block the microbicidal activity of (the virtually undetectable) hydrogen peroxide produced by vaginal lactobacilli

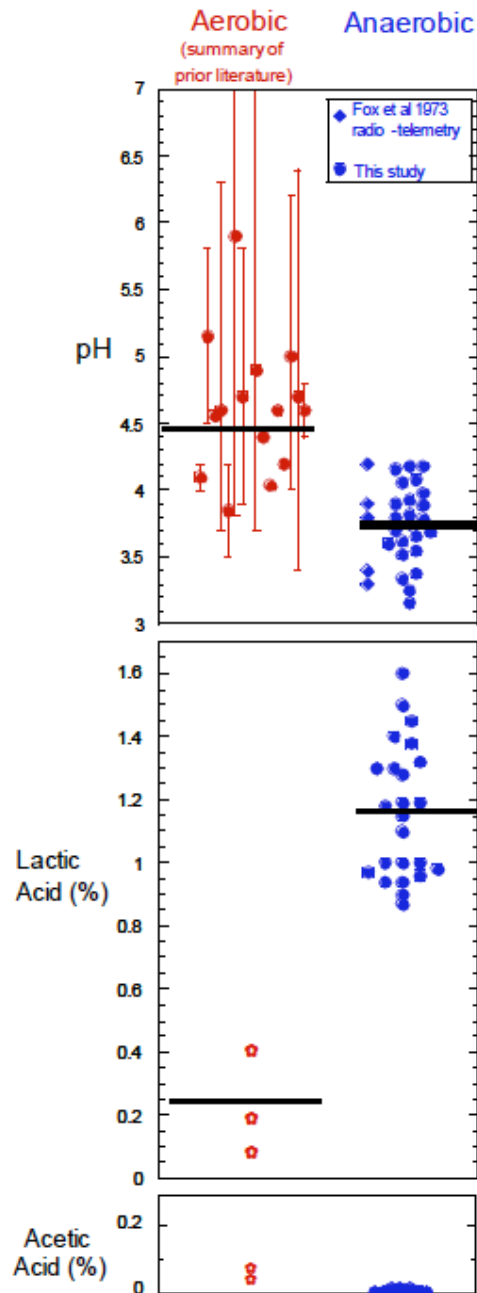
O'Hanlon, Lanier, Moench and Cone

BMC Infect Dis 2010



μM concentration of H_2O_2 detected by Amplex Red[®] assay in CVF (n = 8) and semen (n = 6) samples, versus the mM concentration of exogenous H_2O_2 added to the samples.

Summary of **Aerobic** versus **Anaerobic** observations:



Microbicide production by vaginal lactobacilli: vaginal acidity (pH) and lactic acid are more potent than previously reported.

Deirdre E. O'Hanlon*, Thomas R. Moench, MD†, Suzanne Harrold*, Richard A. Cone, PhD*†

*Thomas C. Jenkins Department of Biophysics, Johns Hopkins University; †ReProtect Inc., Baltimore MD

Microbicides 2008, New Delhi, Poster TA-237