Using soluble immune mediators to study the mucosal milieu

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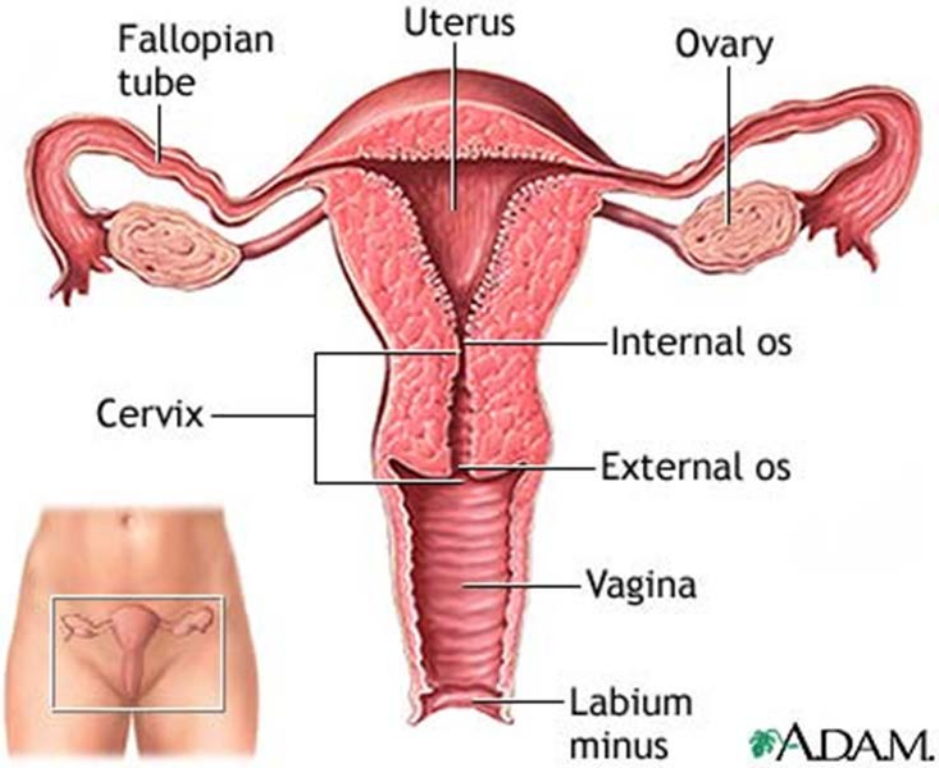
Best Practices in Mucosal Sampling Workshop
Washington, DC May 1, 2014

What to sample?

- Genital tract secretions
  - Easily obtained
  - Swabs have no dilution, but minimal volume
  - Cervicovaginal lavage (CVL) will dilute, but larger volume
- Genital tract tissue
  - More invasive
  - Variability in immune cells
  - Reproducibility/sample bias
  - Vaginal vs cervical tissue



MTN-001

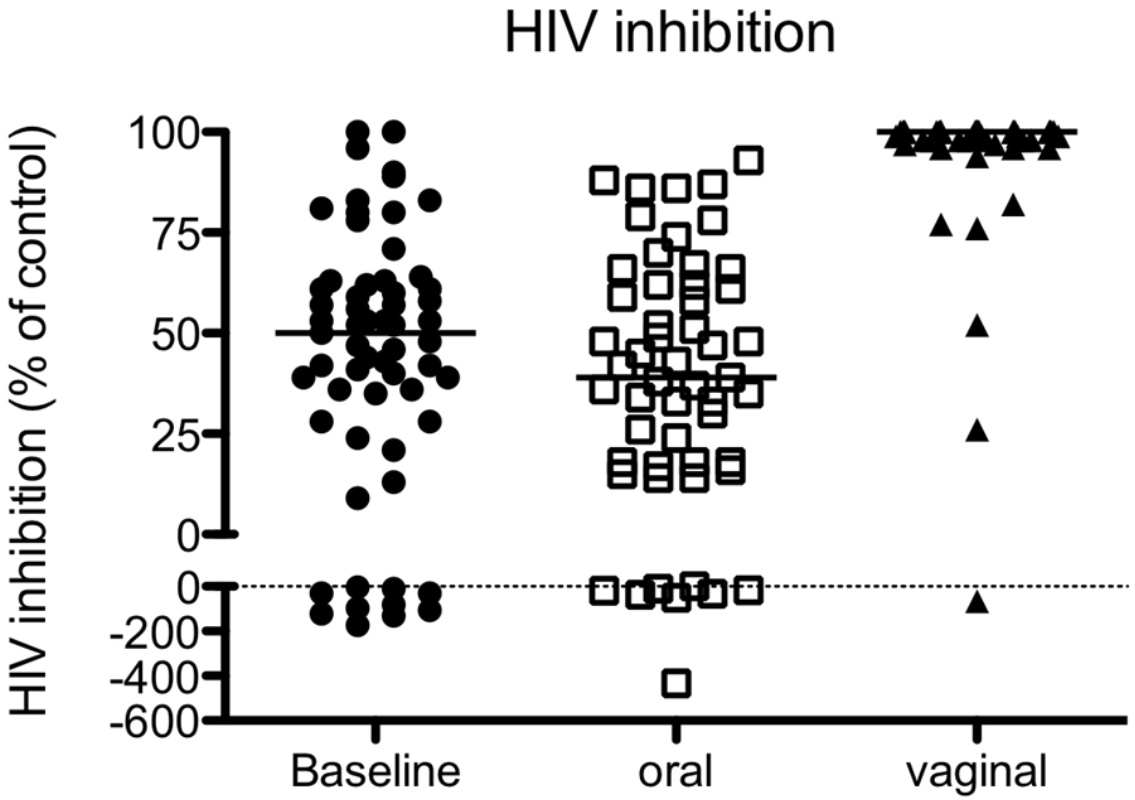
- Phase 2 Adherence and Pharmacokinetics Study of Oral and Vaginal Preparations of Tenofovir
- Participants randomized to regimen sequence:
  - 6 wks daily oral TDF
  - 6 wks daily vaginal TFV gel
  - Both
- CVL collected at baseline & each 6 week period
- US sites: Pittsburgh, Bronx NY, Cleveland, Birmingham
- African sites: Bothas Hill and Unkomass SA; Makerere Uganda

Hendrix, C.W., et al. PLOS ONE 2013

Hypotheses & Objectives

- Primary:
  - Antiviral activity of CVL will correlate with drug levels
  - CVL antiviral activity will be greater following vaginal gel used compared to oral PrEP
- Secondary:
  - Baseline activity may correlate with concentrations of antimicrobial peptides, cytokines, chemokines and/or flora
  - Mucosal environment at baseline may differ between U.S. and African women
- Implications
  - Will activity and drug levels of CVL correlate with clinical trial outcomes?
  - Can CVL antiviral activity provide biomarker of potential PrEP efficacy?

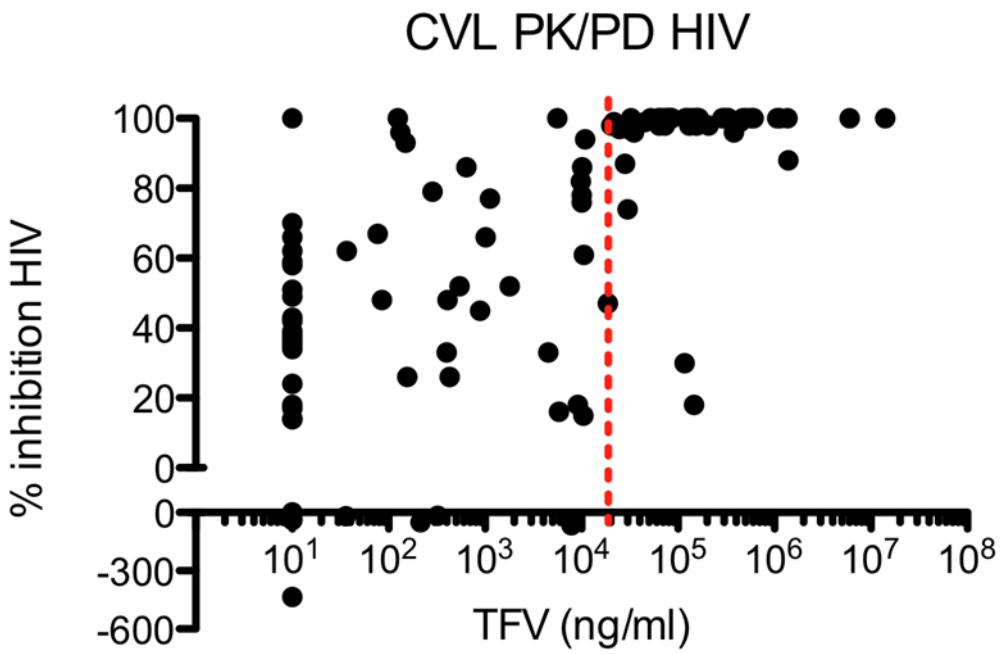
CVL anti-HIV activity



- 49/55 participants had >90% inhibitory activity after vaginal gel compared to 1/53 on oral.
- "Risk" of having anti-HIV activity of ≥90% was 47.2 times higher on gel than no product.

Herold, BC et al., *J AIDS* 2014

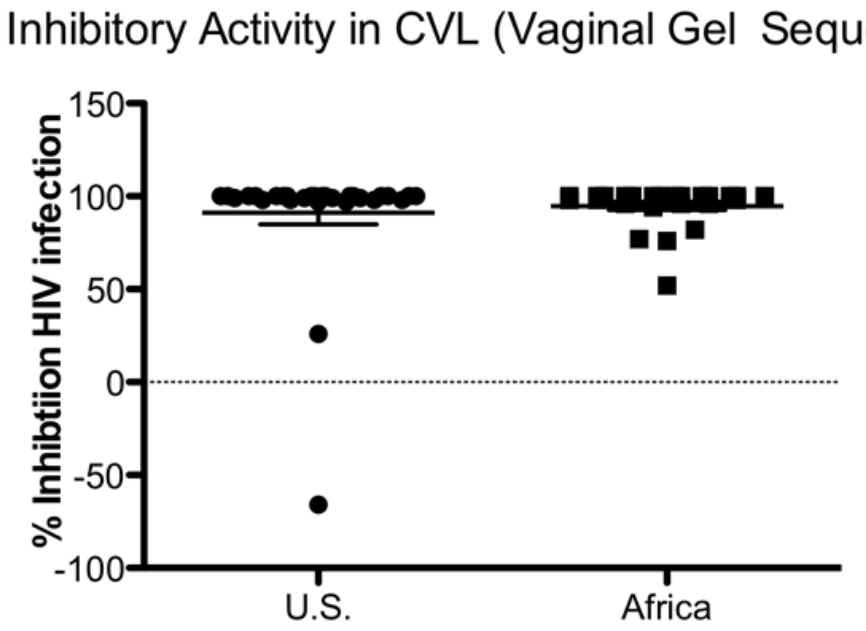
Correlation of PK/PD



- HIV inhibitory activity correlated significantly with [TFV]; Spearman rho=0.74; but NOT with other immune mediators
- For each 1 $\log_{10}$ ↑ in [TFV], the likelihood of HIV activity ≥90% ↑ 1.50X (95% CI: 1.29, 1.74, p<0.001)

Herold, BC et al., *J AIDS* 2014

Were there PK/PD differences between U.S. and African women?



P = N.S.

Herold, BC et al., *J AIDS* 2014

Summary

- Women have variable levels of innate anti-HIV activity (baseline)
- Oral TFV did not increase anti-HIV activity beyond baseline
- Topical TFV did increase anti-HIV activity
  - Higher TFV levels significantly correlated to better anti-HIV activity
- No significant difference in anti-HIV activity between U.S. and African women when topical gel was used

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Baseline CVL testing

- Total protein (BCA)
- Cytokines/chemokines (luminex): IL-1β, IL-6, IP-10, IL-8, MIP-1α, MIP-3α
- Innate factors (ELISA): SLPI, Lf, MPO, HβD1, HβD2, HβD3, HNP1-3, MPO
- Antimicrobial activity
  - Anti-HIV
  - Anti-HSV
  - Anti-E. coli

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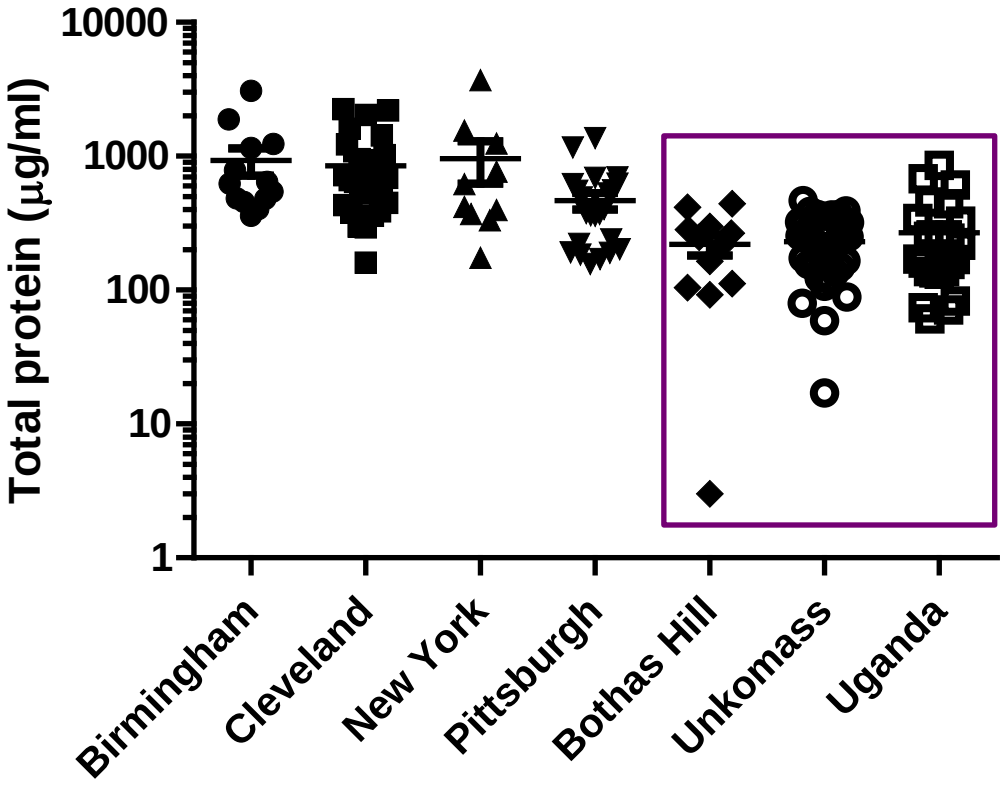
CVL protein content



Significant ($p < .001$) differences found between U.S. and African women

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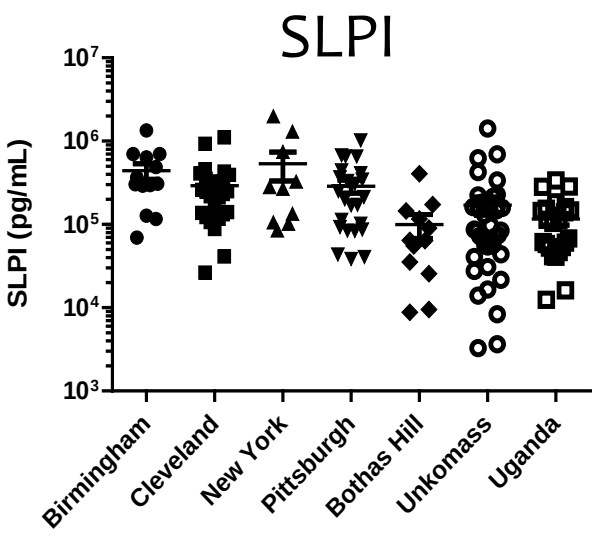
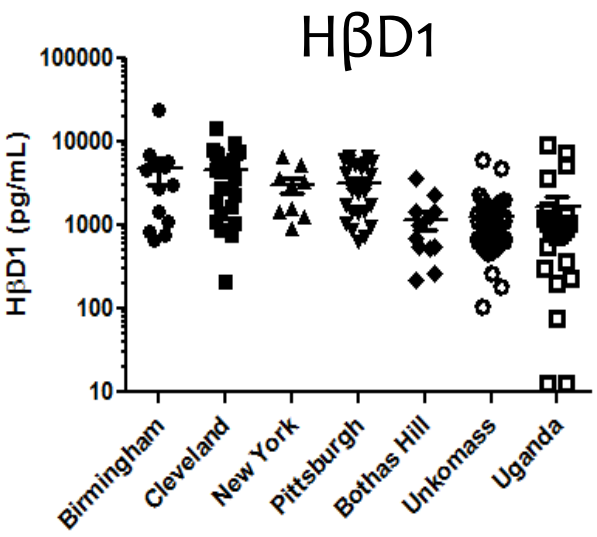
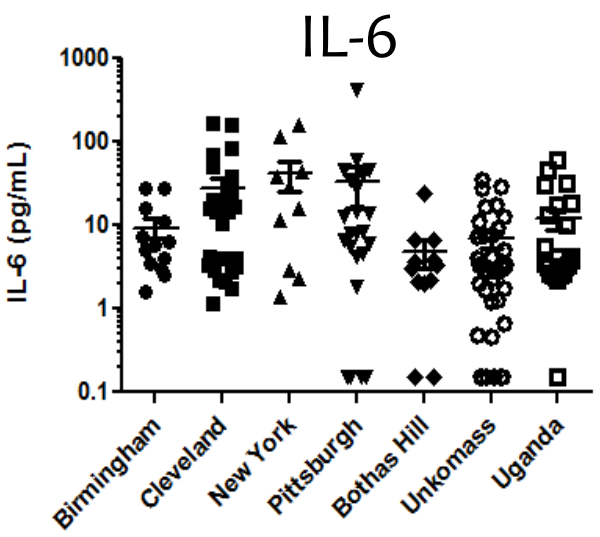
Soluble mediators







Significant (p = .001) differences found between U.S. and African women

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Differences in soluble mucosal immunity between U.S. and African women

Variable	U.S. ♀ % (n) or mean (SD) n=73	African ♀ % (n) or mean (SD) n=73	Univariable p-value	Multivariable p-value (controlling for log_{10} total ptn)
Log_{10} total ptn	2.76 (0.31)	2.28 (0.35)	<0.001	
Log_{10} MPO	2.72 (0.93)	2.38 (0.82)	0.02	0.3
Log_{10} SLPI	5.37 (0.40)	4.90 (0.50)	<0.001	0.001
Log_{10} Lactoferrin	3.04 (0.52)	2.56 (0.60)	<0.001	0.1
Log_{10} HβD-1	3.45 (0.39)	2.92 (0.50)	<0.001	<0.001
Log_{10} HβD-2	3.27 (0.53)	3.02 (0.84)	0.04	0.9
Log_{10} HβD-3	3.12 (0.45)	2.72 (0.51)	<0.001	0.1
Log_{10} HNP1-3	4.54 (0.65)	4.37 (0.67)	0.1	0.3
Log_{10} IL-1β	0.94 (0.88)	0.54 (0.78)	0.005	0.2
Log_{10} IL-6	0.94 (0.71)	0.51 (0.67)	<0.001	0.07
Log_{10} IP-10	2.24 (0.83)	1.84 (0.77)	0.004	0.4
MIP-1α > LL	51% (37)	53% (39)	0.9	0.3
Log_{10} IL-8	2.93 (0.53)	2.56 (0.56)	<0.001	0.9
Log_{10} MIP-3α	1.71 (0.78)	1.33 (0.82)	0.006	0.6
E. coli activity %	31.74 (35.73)	16.81 (22.73)	0.003	0.8

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Differences in soluble mucosal immunity between U.S. white and black women

Variable	U.S. White ♀ % (n) or mean (SD) n=35	U.S. Black ♀ % (n) or mean (SD) n=35	Univariable p-value	Multivariable p-value (controlling for log_{10} total ptn)
Log_{10} total ptn	2.69 (0.27)	2.83 (0.35)	0.07	
Log_{10} MPO	2.99 (0.78)	2.45 (1.02)	0.02	0.001
Log_{10} SLPI	5.39 (0.39)	5.36 (0.42)	0.3	0.5
Log_{10} Lactoferrin	3.03 (0.52)	3.05 (0.53)	0.9	0.6
Log_{10} HβD-1	3.51 (0.40)	3.37 (0.38)	0.1	0.04
Log_{10} HβD-2	3.28 (0.42)	3.32 (0.42)	0.7	0.9
Log_{10} HβD-3	3.04 (0.40)	3.21 (0.44)	0.1	0.3
Log_{10} HNP1-3	4.62 (0.63)	4.45 (0.69)	0.3	0.09
Log_{10} IL-1β	0.84 (0.91)	1.04 (0.90)	0.4	0.7
Log_{10} IL-6	1.02 (0.81)	0.86 (0.63)	0.4	0.08
Log_{10} IP-10	2.19 (0.61)	2.23 (1.00)	0.8	0.7
MIP-1α > LL	59% (20)	43% (15)	0.2	0.1
Log_{10} IL-8	2.93 (0.50)	2.94 (0.58)	1.0	0.3
Log_{10} MIP-3α	1.80 (0.82)	1.62 (0.76)	0.4	0.1
E. coli activity %	32.46 (37.77)	32.20 (33.06)	0.3	0.2

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Summary

- U.S. women had significantly higher levels of all variables except HNP1-3 (trend) and MIP-1α as compared to African women.
- In multivariable regression models controlling for differences in $\log_{10}$ total protein only SLPI and HβD-1 remained significantly different (IL-6 trend) between U.S. and African women
- Differences between U.S. white and black women were modest
- U.S. white women had significantly higher levels of MPO and HβD-1 (IL-6 trend) controlling for differences in $\log_{10}$ total protein compared to U.S. black women

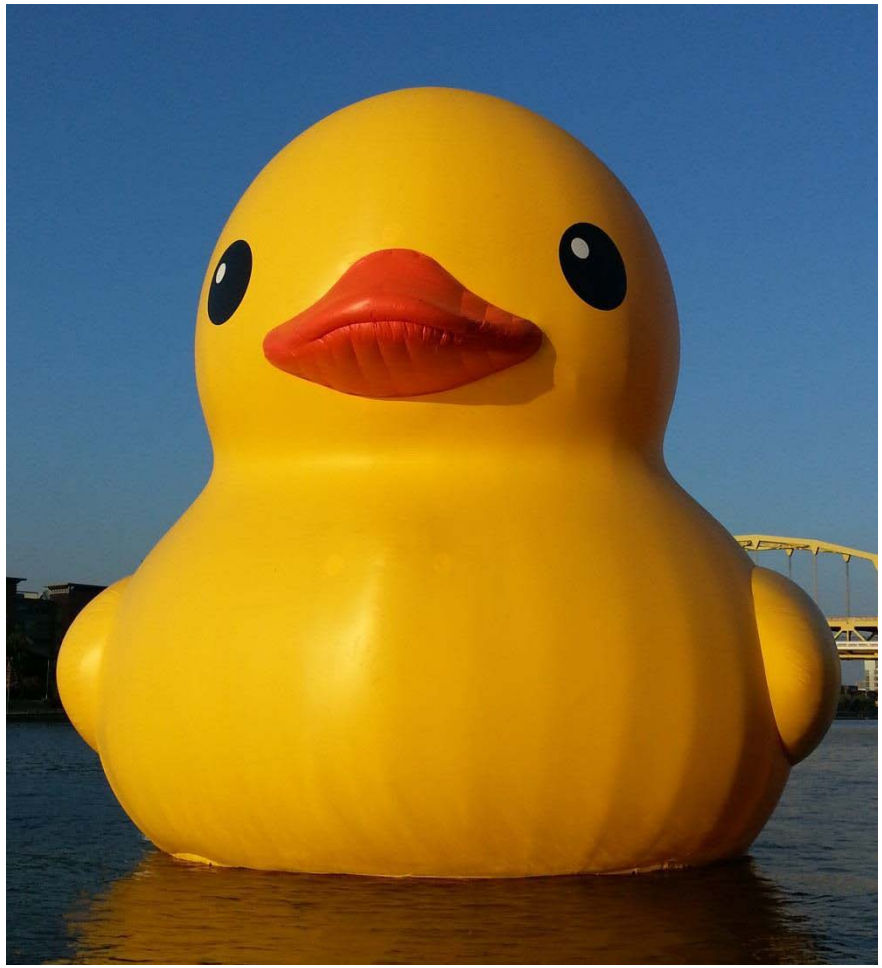
Conclusions

- Measurable PD activity in mucosal fluids correlated to topical drug levels (not oral PrEP) irrespective of nationality
- U.S. women have a higher "set point" of soluble innate immune factors than African women
  - Consistent with findings from Cohen et al. AIDS 2010
- "Normal ranges" of the soluble mediators need to be determined based on location of study participants

Acknowledgements

MTN Biomedical Sciences working group

- Peter Anderson
- Charlene Dezzutti
- Craig Hendrix
- Betsy Herold ←
- Sharon Hillier
- Jeanne Marrazzo
- Ian McGowan
- Barbra Richardson ←
- Barbara Shacklett



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