Mucosal Assays in HIV Prevention Trials: Vaginal Microbicide Trials

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Use of Mucosal Assays in Microbicide Trials
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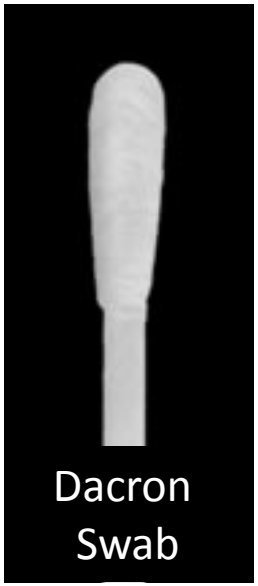
Today's discussion

- Mucosal assays that have been used in microbicide trials to collect female genital tract specimen
- Mucosal assays for future trials

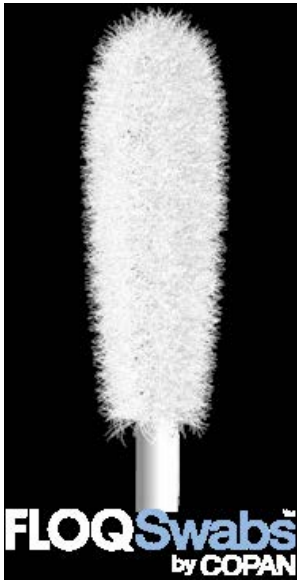
Specimen collected

- Dependent on molecule tested
  - e.g. hydrophobic vs. hydrophilic or luminal vs. intracellular
- Mucosal (cervical, vaginal) swabs / sponges, tearflo strips, and cytobrushes
  - Cervicovaginal fluid collected by InStead Cup
- Cervicovaginal lavage (typically 5 or 10 ml)
- Cervical, vaginal tissue
- Blood/PBMCs

Collection devices



Dacron Swab



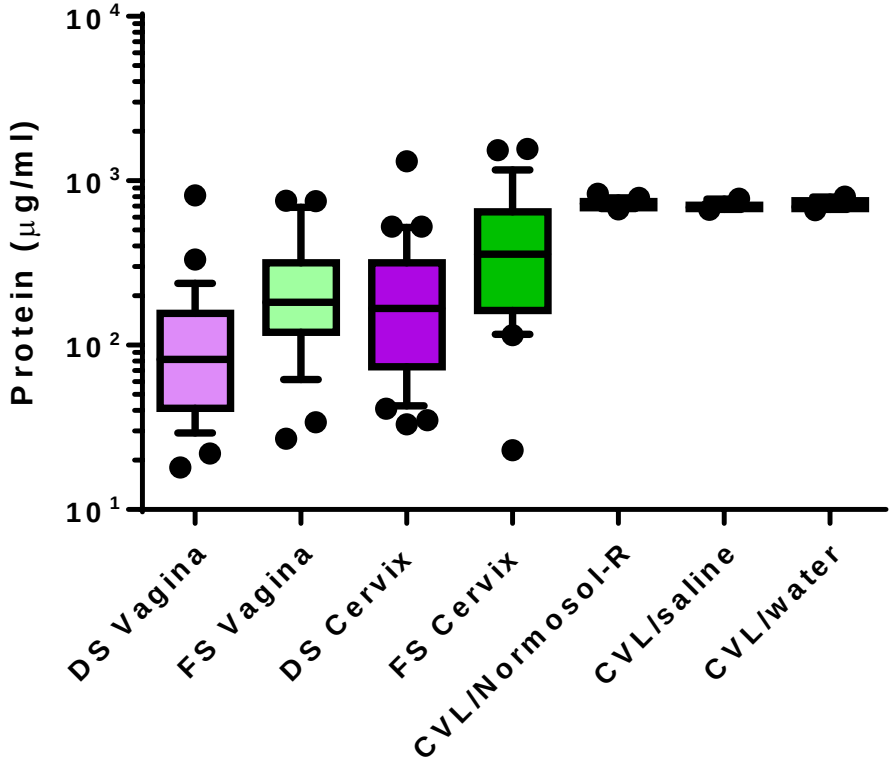
Ophthalmic sponges

Mucosal fluid processing

- Swabs/sponges
  - Low volume of PBS (or saline) added to swab, soaked for ~10 min,
  - Vortexed
  - Placed in Spin-X insert (without filter), centrifuged
  - Elute collected (can retain pellet)
  - Repeat process if desired
- CVL
  - Centrifuged to remove cellular debris (can retain pellet)
  - Whole CVL has been used for functional assays

Mucosal Fluid collection

Comparison between cervicovaginal lavage (CVL) and swabs (Dacron or flocked)

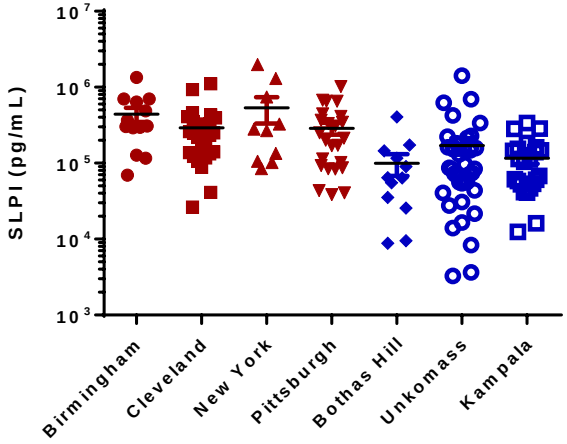


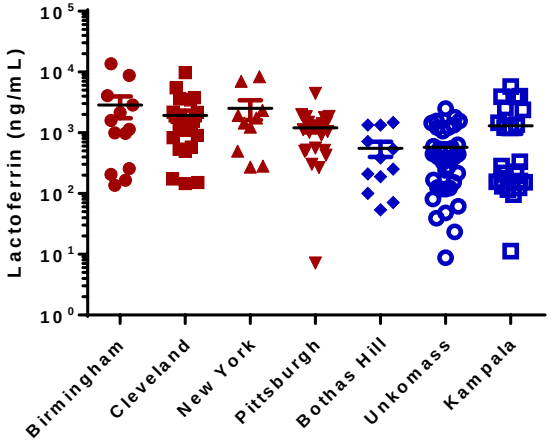
Dezzutti, C.S., et al, PLOS ONE 6(8): e23136, 2011

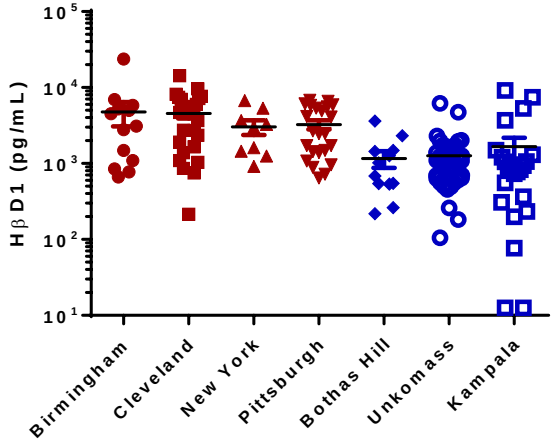
Mucosal fluid assays

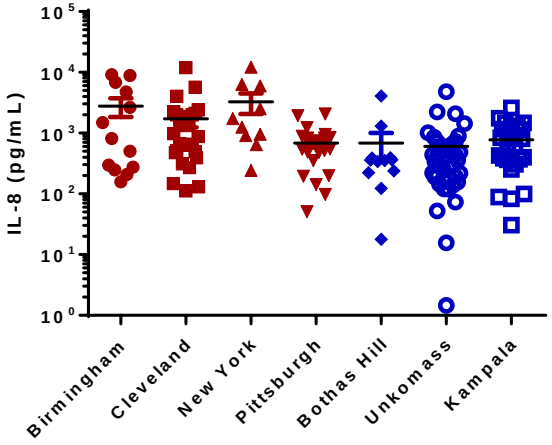
- Defining soluble cytokines/chemokines
- Functional assays:
  - Anti-HIV activity
    - Typically assayed using in vitro cell lines (e.g. TZM-bl assay or Jurkat-Tat-CCR5 assay)
  - Anti-HSV activity
    - HSV plaque reduction using Vero cell line
  - Anti-*E. coli* activity
    - Colony forming unit reduction based on plate counts

Soluble cytokines / chemokine / innate factors from baseline CVL



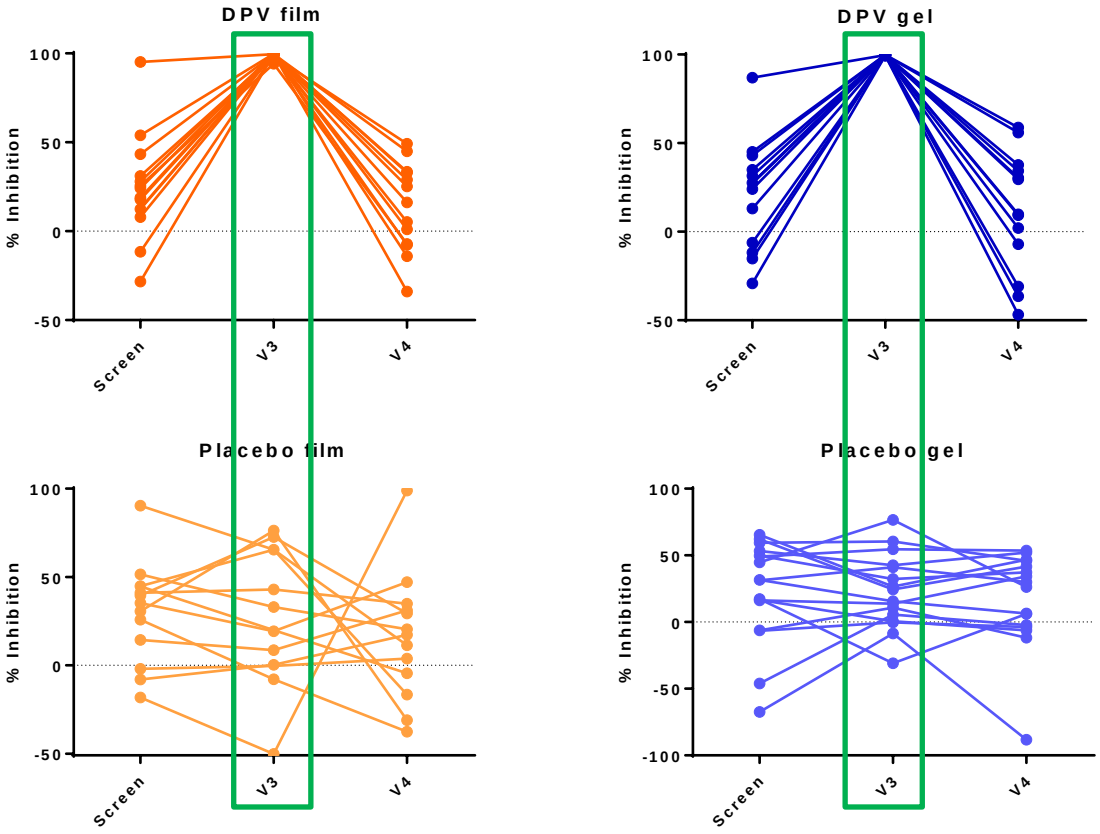






Murphy, K., et al, Am J Reprod Immunol 2015 Jun 21 Epub

Anti-HIV activity in CVL from FAME-02:
comparison between DPV film and gel users



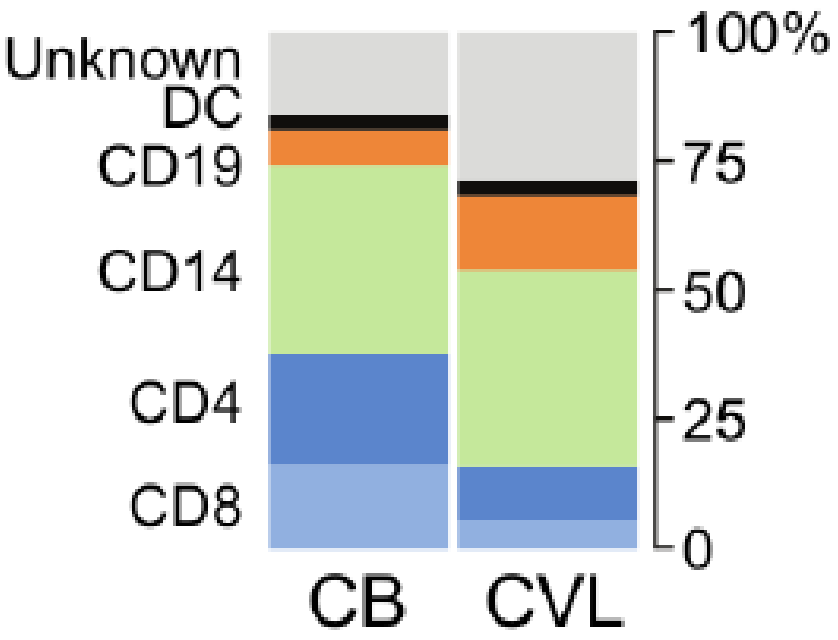
Bunge, K., et al. in submission J AIDS 2015

FGT mucosal tissue assays

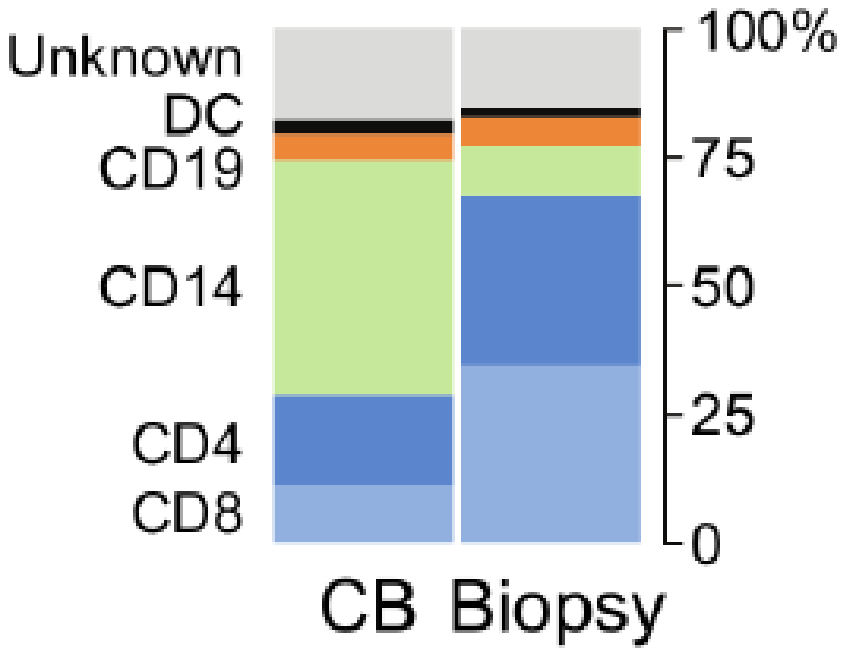
- Define cell populations (cytobrush or biopsy)
- Ex vivo challenge assay
  - Eliminated cleansing and numbing of area prior to biopsy collection
    - Consistency between FGT and GI biopsy collection
  - Limited in the number and frequency of biopsy collection
  - Requirement for fresh tissue

Cell population recovery

Cytobrush vs. CVL



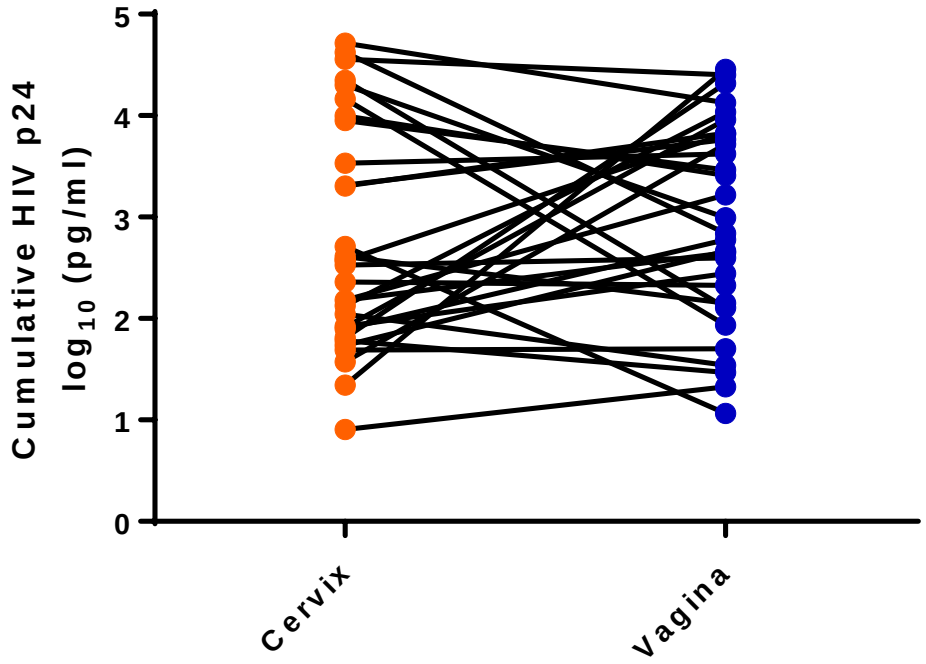
Cytobrush vs. cervical biopsy



- While possibly representative, tissue cell populations are different from luminal cell populations
- Cells have migrated out of the tissue for a reason, which should be taken into consideration

McKinnon, L.R., et al, PLOS ONE 9(1): e85675, 2014

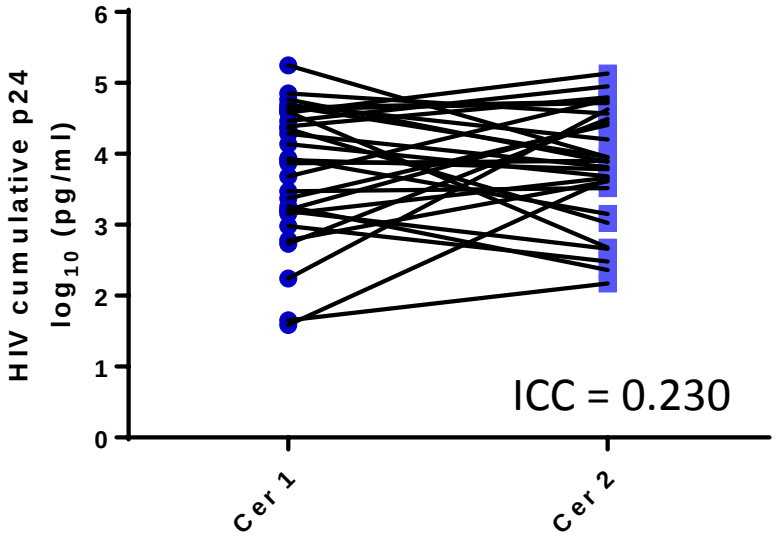
Ex vivo challenge assay: Inter-person variability in HIV replication

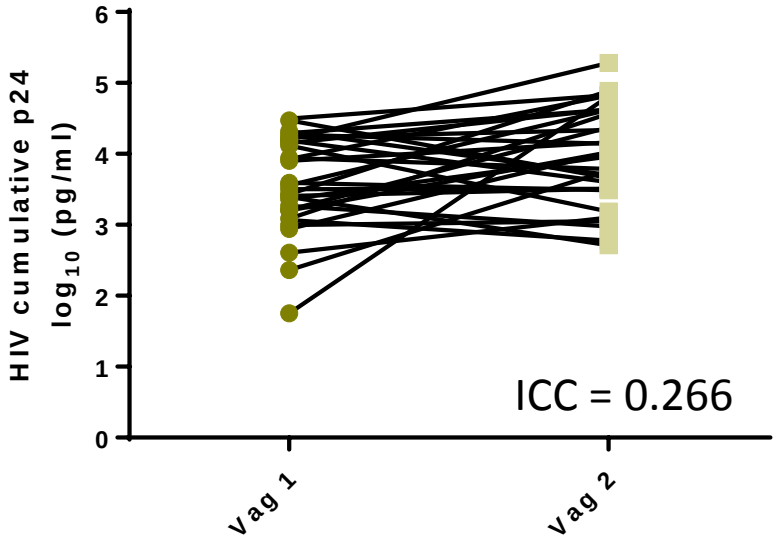


- Placebo users in FAME-02 clinical trial, n = 29
- Paired cervical and vaginal tissue

Bunge, K., et al, in submission J AIDS 2015

Ex vivo challenge assay: Intra-person variability in HIV replication

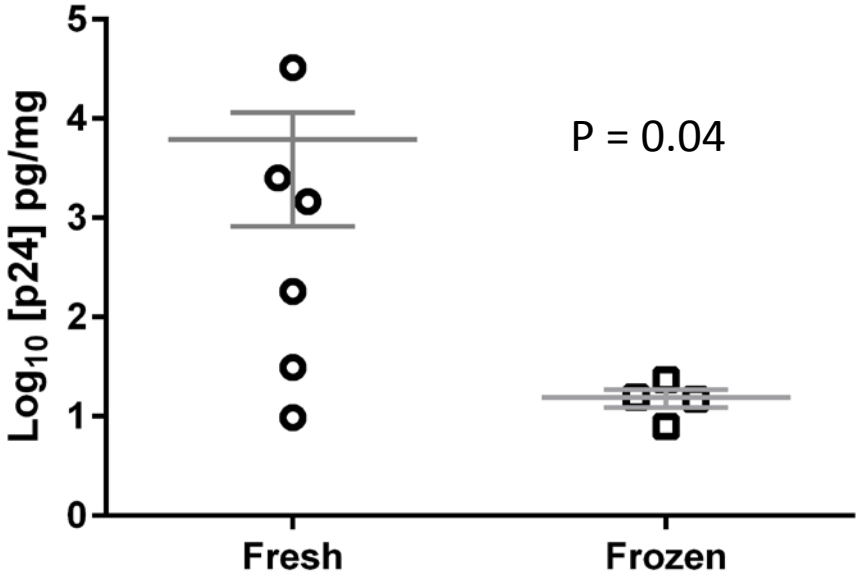




- 31 evaluable women enrolled
- 4 cervical and 4 vaginal biopsies were collected
- 2 of each were challenged with BaL or JR-CSF (data not) shown
- Significant intra-person variability for cervical and vaginal tissue

Dezzutti, C.S., et al, 2015 unpublished data

Lack of HIV replication in cryopreserved cervical tissue



- Placebo users from MTN-013
- Fresh cervical tissue was collected from local clinical site and used immediately
- Frozen cervical tissue was cryopreserved and shipped to the lab at end of study

Dezzutti, C.S., et al, 2015 in submission

Future mucosal assays

- Incorporation of omics into trials: collection of additional specimens, addition of preservatives, different processing?
- Upper genital tract sample collection (uterus / fallopian tube)?
- Biomarkers of HIV risk:
  - Inflammation (soluble and cellular)
  - Y chromosome (PSA)
  - HIV nucleic acid (exposed uninfected)
- Biomarkers of product efficacy:
  - Adherence (PK?)
  - Ex vivo challenge assay
  - Pharmacogenomics
- Development of a specimen repository – what specimens to collect?

Key points

- Establishing baseline (normative) values for the population(s) in the trial so product effects can be defined
- Inter-person is similar to intra-person variability for HIV replication in cervical and vaginal tissue; placebo groups equivalent to baseline specimen
- Close relationship between clinic and laboratory for specimen management and testing
- Focused working groups to provide best practices on specimen collection, processing, and assay development

Acknowledgements

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