9th International Conference on HIV TREATMENT AND PREVENTION ADHERENCE

DNA and Protein Biomarkers Offer Increased Accuracy for Assessing Vaginal Microbicide Gel Adherence

(Abstract 275)

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Background/Rationale

- Currently, there are no reliable, highly sensitive, objective adherence markers for microbicide trials
- Direct relationship between adherence and product effectiveness

CAPRISA 004	# HIV	N (%)	HIV incidence		Effect
			TFV	Placebo	
High adherers (>80% gel adherence)	36	336 (38)	4.2	9.3	**54%**
Intermediate adherers (50-80% adherence)	20	181 (20)	6.3	10.0	38%
Low adherers (<50% gel adherence)	41	367 (42)	6.2	8.6	28%

Karim et al, Science 2010

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Current Adherence Measures

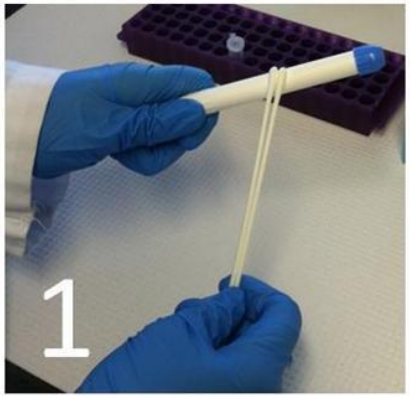
- Self Report – subjective, biased
  - Examples: FemPrep, VOICE (MTN-003) trials
- Visual Inspection of Returned Applicators (VIRA)
  - Reported sensitivity of 76% (62% - 84%)
  - Cannot determine incorrect use, wiping
- Ultraviolet Light (UVL) Assessment
  - Reported sensitivity of 84% (range 79 - 87%)
  - Cannot distinguish semen exposure independently
- Dye Stain Assay (DSA)
  - ↓ Sensitivity with HTI applicators
- Electronic Event (WiseBag)
  - Daily versus Peri-Coital dosing
- Pharmacokinetic Samples
  - Expensive, Invasive
  - Unable to detect placebo

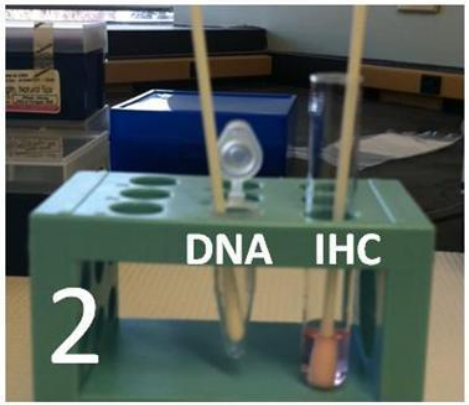
Preliminary Data

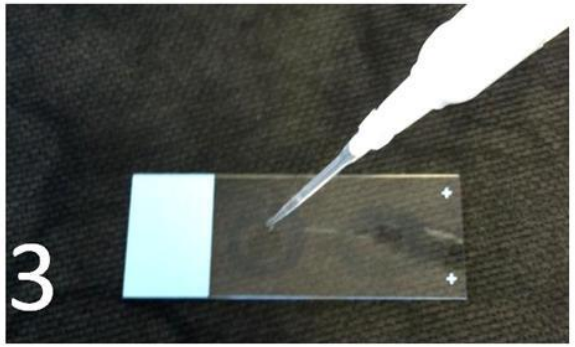
Determine objective, biological biomarkers which can be used as a composite to measure vaginal insertion of gel applicators and semen exposure

DNA
= Sensitivity

Protein
= Specificity







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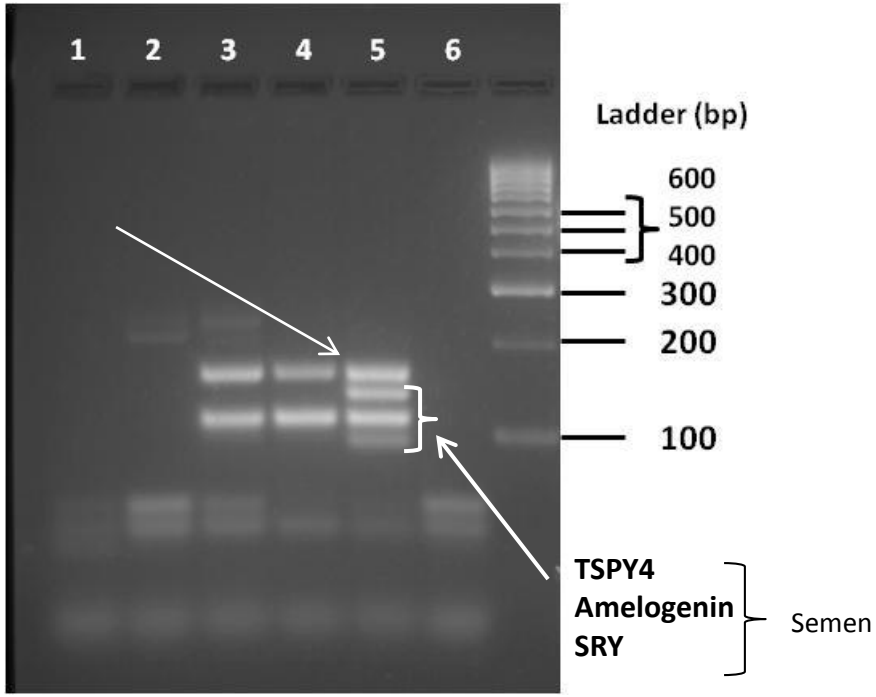
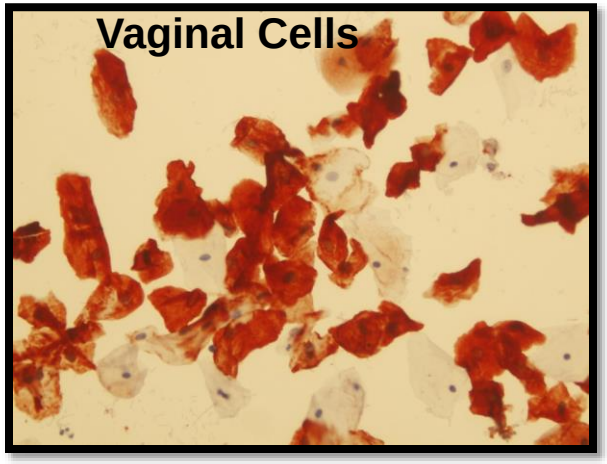
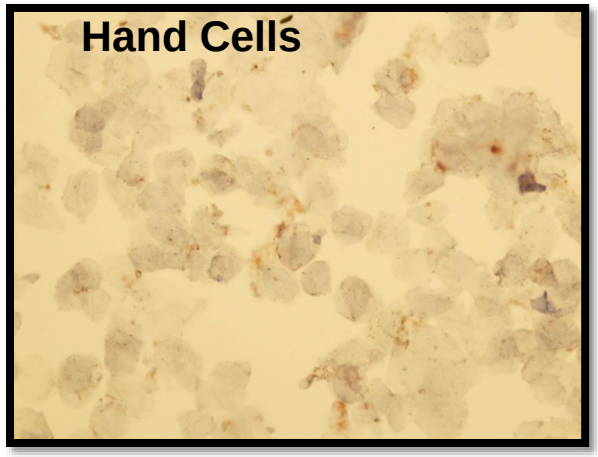
DNA and Protein Biomarkers







Cytokeratin 4

Lane 1: Un-inserted (control) applicator
Lane 2: Sham applicator (no amelogenin)
Lane 3: Inserted applicator
Lane 4: Vaginal Swab
Lane 5: Inserted applicator + semen exposure
Lane 6: No DNA negative control

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Objectives

- **CONRAD D13-125 Study**
- **Primary Objective:** Validate DNA and protein biomarkers of vaginal insertion versus sham use in a clinical study of observed applicator use
  - Study design included field conditions of wiping applicators, correct/incorrect use, sham insertion
  - Compare DNA/Protein biomarkers to VIRA, UVL
- **Secondary Objective:** Validate objective measures of semen exposure (TSPY4, SRY) versus participant report of unprotected intercourse

Methods

- Approved by the Chesapeake IRB (Pro00008154)
- Registered with ClinicalTrials.gov (NCT01804023)
- Cross-sectional study of 40 healthy, non-pregnant, HIV negative women aged 18 – 50 years-old
- At least 3 days from their last menstrual bleeding episode
- No vaginal creams or gels in the past 3 days
- Single screening/enrollment visit.
  - HIV, Pregnancy
  - Medical History

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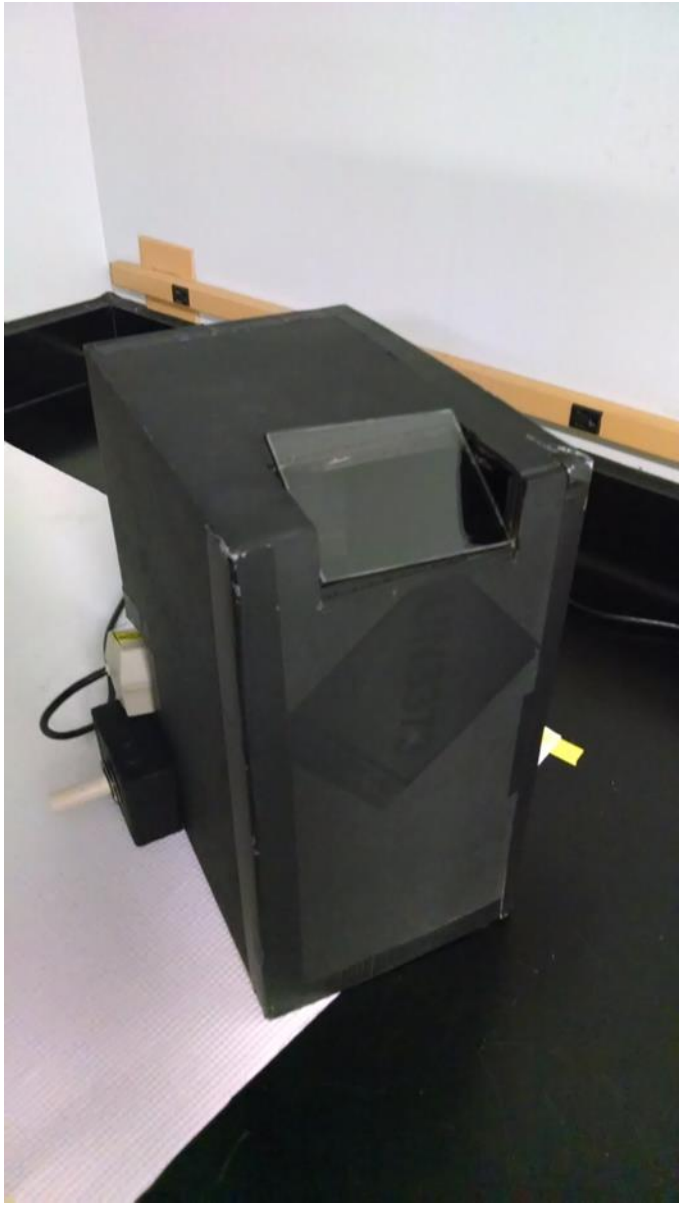
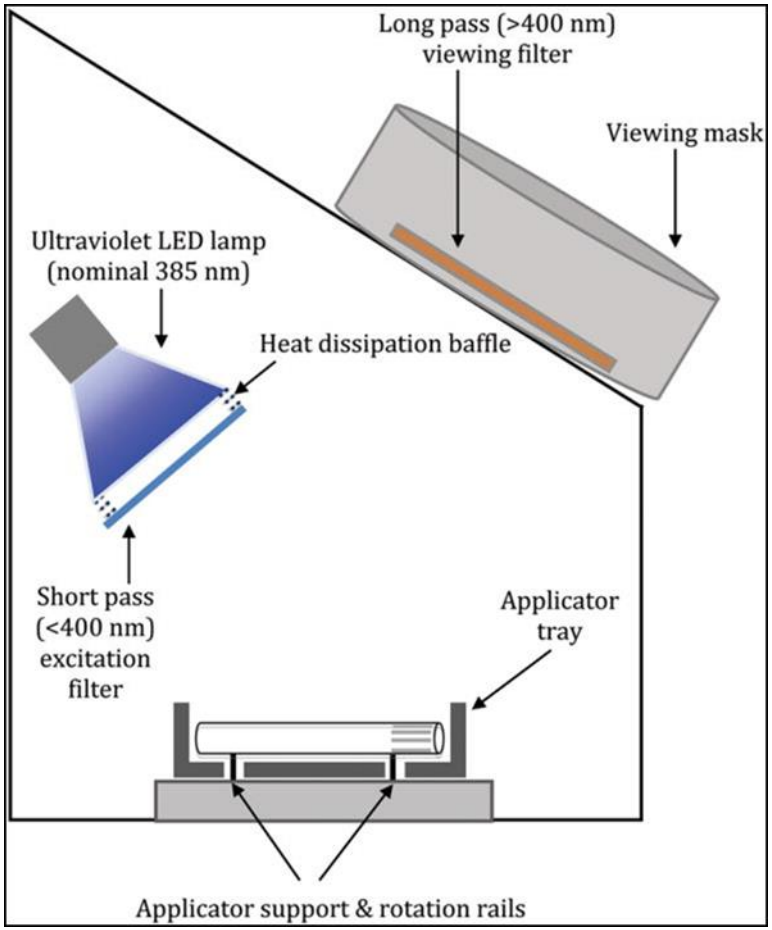
D13-125 Study Design

- Participants (n = 40) provided, under observation:
  - 1 Vaginal Swab (Positive Control)
  - 4 Sham applicators (Specificity)
  - 8 Vaginally inserted applicators (Sensitivity)
    - "Incorrect use" – vaginally inserted, gel not expelled
    - "Correct use" inserted and gel expelled
    - Wiped
- Applicators graded "Inserted" vs "Not Inserted"
  - 3 Blinded Readers → VIRA and UVL
  - Blinded Laboratory Staff → DNA/Protein Analysis
- Applicators (n = 240) evaluated within 7d of use
- Applicators (n = 240) evaluated at 30+ days of use

Applicator #	Condition	Time until processing	
		~7 days	~30 days
1	SHAM, VIRA and UVL	n = 40	
2	SHAM, VIRA and UVL		n = 40
3	SHAM, DNA/Cytokeratin	n = 40	
4	SHAM, DNA/Cytokeratin		n = 40
5	INCORRECT USE, VIRA and UVL	n = 40	
6	INCORRECT USE, VIRA and UVL		n = 40
7	INCORRECT USE, DNA/Cytokeratin	n = 40	
8	INCORRECT USE, DNA/Cytokeratin		n = 40
9	CORRECT USE VIRA, UVL, DNA/Cytokeratin	n = 20	n = 20
10	CORRECT USE VIRA, UVL, DNA/Cytokeratin	n = 20	n = 20
11	WIPED VIRA/UVL, DNA/Cytokeratin	n = 40	
12	WIPED VIRA/UVL, DNA/Cytokeratin		n = 40
Total Number Evaluated by VIRA and UVL		n = 160	n = 160
Total Number Evaluated by DNA/Cytokeratin Biomarkers		n = 160	n = 160
Total Number Evaluated by either method		n = 240	n = 240

Methods





Evaluation of Microbicide Gel Adherence Monitoring Methods.
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Sexually Transmitted Diseases. 39(5):335-340, May 2012.
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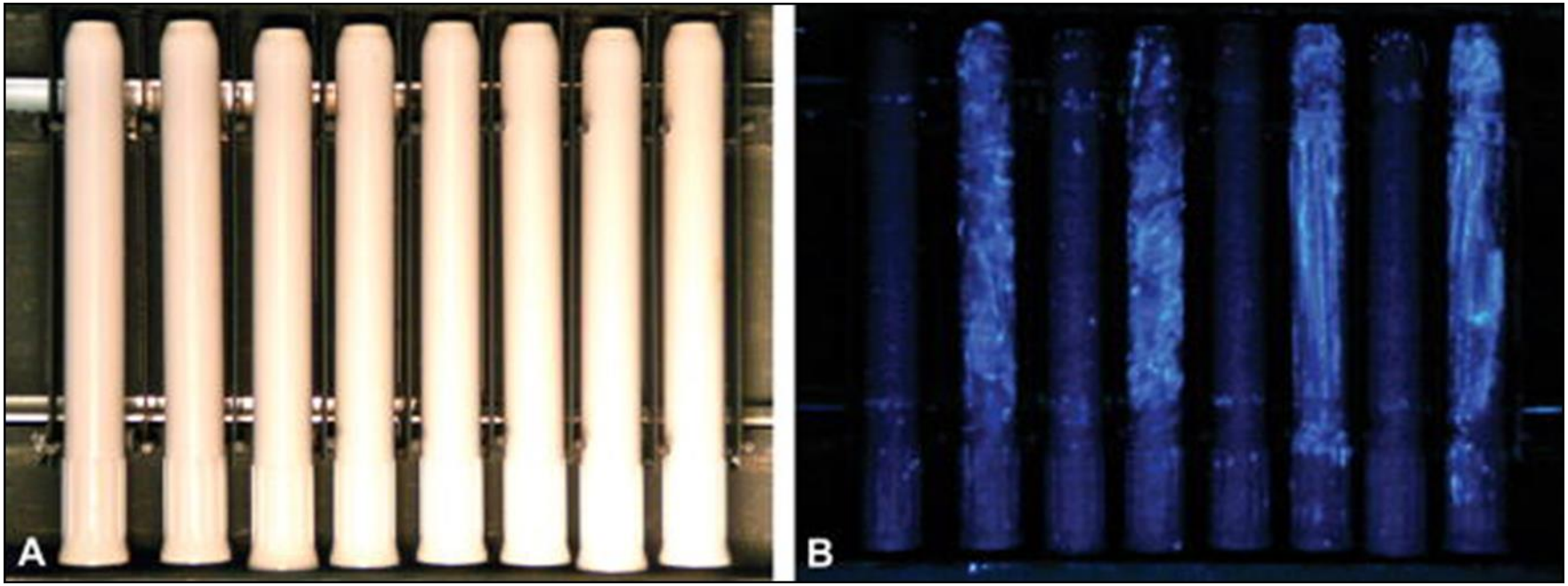
Methods



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VIRA and UVL are Subjective
Learning Curve and Significant Inter-Reader Variability

Variable	Mean %, (Inter Reader Variability p value)					
	VIRA 7d	VIRA 30d	p	UVL 7d	UVL 30d	p
Sensitivity						
All Inserted Apps	54 (IRV p = 0.36)	52 (IRV p = 0.03)	0.50	74 (IRV p = < 0.01)	92 (IRV p = 0.07)	**< 0.01**
With prior gel	70 (IRV p = 0.39)	68 (IRV p = 0.94)	0.84	93 (IRV p = 0.85)	100 (IRV p = 1.0)	0.06
Wiped	24 (IRV p = 0.84)	28 (IRV p = 0.13)	0.46	38 (IRV p = < 0.01)	84 (IRV p = 0.17)	**< 0.01**
Specificity						
All Sham	49 (IRV p = 0.65)	78 (IRV p = 0.71)	**< 0.01**	73 (IRV p = < 0.01)	66 (IRV p = < 0.01)	0.21

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DNA/Protein Biomarkers Maintain Robust, Identical Sensitivity and Specificity

Variable	7 Days	30 Days
Sensitivity (%)		
All Inserted Apps	98.3	98.3
No prior gel	100	100
With prior gel	100	100
Wiped	95	95
Specificity (%)		
All Sham	100	100

Data expressed as %

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Study Results:

DNA/Protein Biomarkers Increased Sensitivity/Specificity at 30d

Variable	VIRA or UVL	DNA/Protein	p value
VIRA - 30 DAYS			
Sensitivity All Applicators	187/360 (51.9%)	117/119 (98.3%)	**<0.0001**
Sensitivity Wiped	34/120 (28.3%)	37/39 (95%)	**<0.0001**
Specificity (Sham)	94/120 (78.3%)	40/40 (100%)	**0.0013**
UVL Light - 30 Days			
Sensitivity All Applicators	332/360 (92.2%)	117/119 (98.3%)	**0.02**
UV Specificity (Sham)	79/120 (65.8%)	40/40 (100%)	**<0.0001**

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Semen/Sperm Biomarkers

- TSPY4 and SRY
  - Jacot TA et al. *Contraception.* 2013;88(3):387-395.
- 37 Vaginal Swabs
  - 24 reported semen exposure in past 7d
  - 15 reported no condom use
  - 11/15 (73% sensitivity) with + vaginal swab.
  - No semen detected from swabs of negative reports (100% specificity)
- Vaginal Applicators
  - Feasibility demonstrated

Residual Tenofovir Detection from Applicators

Quantification of Residual Tenofovir (TFV) on Gel Applicators

	Vaginally Inserted				Controls (n=2)	
	1	**2**	**3**	**4**	**Sham[1]**	**Blank[2]**
TFV (ng)	1120	1250	659	577	BLD[3]	BLD
Storage (days)	32	55	33	13	-	-

[1] **Manually handled, gel expelled in trash**

[2] **Unopened applicator, removed from foil packet only**

[3] **BLD = Below Level of Detection**

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Conclusions

- VIRA and UVL are inexpensive, feasible
  - Ultimately subjective with significant IRV
  - Wiping applicators VIRA sensitivity 28%
- DNA and Protein Biomarkers
  - Significantly higher sensitivity, specificity
  - Reproducible w/ storage, presence of gel
- Semen Biomarkers
  - Can be assayed from vaginal swabs and returned used applicators
- Active Drug/Placebo
  - Can be assayed from returned used applicators
  - High throughput methods under development

Expected Outcomes

- A non-invasive, inexpensive, highly sensitive and specific triple adherence marker panel
  - Detect active drug or placebo use
  - Sensitive despite prolonged storage and shipping in extreme conditions
  - Inform ongoing and future HIV prevention trials
  - Correlate drug or placebo delivery with HIV or pregnancy risk exposure (semen)
- Future applications of adherence panel
  - Applicable to topical vaginal and rectal formulations; potential for other dosage forms
  - Objectively investigate acceptability and use of drug delivery systems

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Thank you