Innovations in HIV Prevention Research:
Novel adherence and diagnostic technologies
Cresta Lodge, Harare Zimbabwe
21 and 22 AUG 2019

Recent Advances in Biomarkers of Adherence

Andrea Thurman MD and Terry Jacot PhD
CONRAD

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NiX HIV PREVENTION MATTERS

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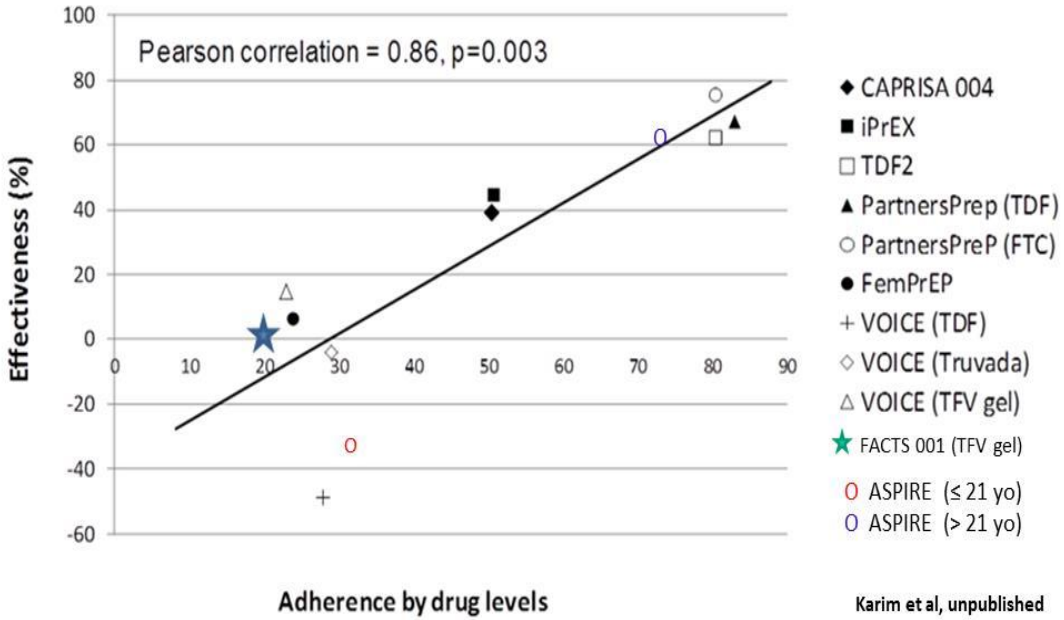
Adherence = "Achilles Heel" of PrEP Efficacy Trials



Karim et al, unpublished

- Inadequate adherence = major culprit
- Self report = overestimates adherence
- High adherence based on Rx levels = high efficacy
- Pharmacokinetic samples – invasive, expensive, not applicable to placebo
- Need to address "white coat" effect

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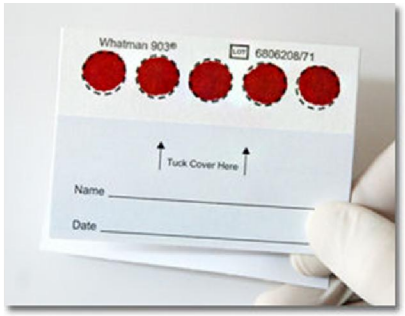
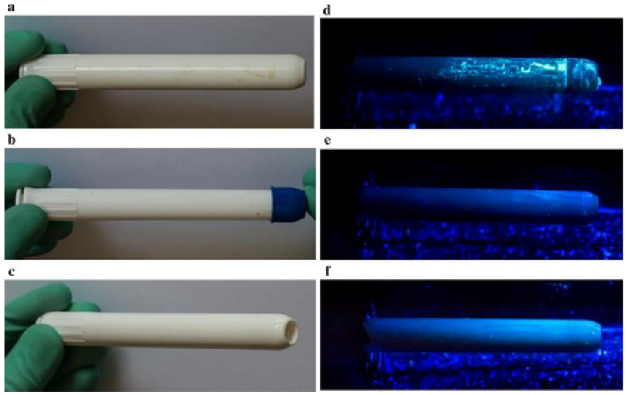
Current Objective Adherence Measures

Pill Counts
Pharmacy refills

Drug levels





VIRA/UV
Light

DPV IVR Staining and
concentration > 95 pg/mL

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Composite Measure of Product Adherence, Protocol Compliance, and Semen Exposure

Protocol Compliance
= vaginal insertion of product (applicator or swab)

DNA-Protein Biomarkers
- Vaginal Bacterial DNA
- Cytokeratin 4 (Protein)

Semen exposure
= HIV risk

Protein
PSA (interactions with TFV) 24 – 48 hrs.

Y-chromosomal DNA
- SRY 24 – 48 hrs
- TSPY4 approx 7 days

Product Adherence
= active drug or placebo exposure

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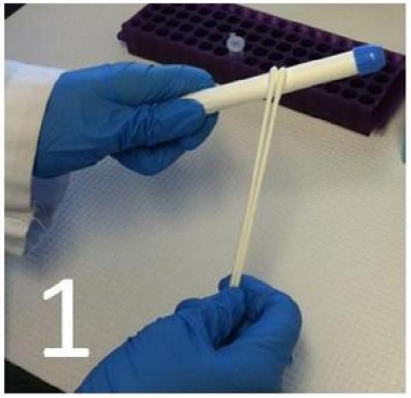
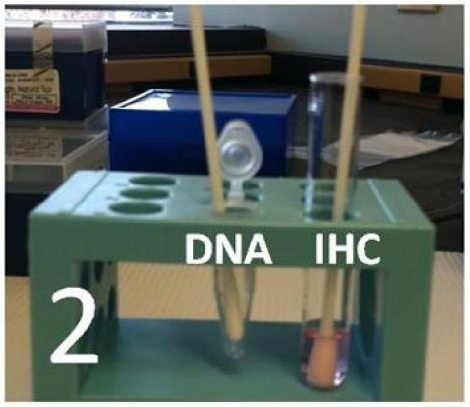
Vaginal Insertion Biomarkers

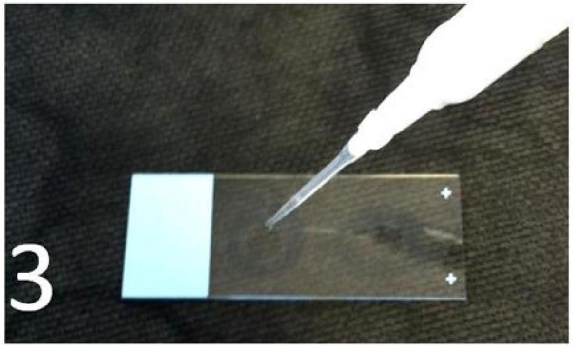
Determine objective, biological biomarkers (DNA and protein) which can be used as a composite to measure vaginal insertion of gel applicators/vaginal swabs and semen exposure

Method:

1. Obtain a double headed vaginal swab or swab a vaginally used applicator
2. Split the swab and place one into DNA extraction buffer and one into fixative (IHC) to obtain vaginal cells
3. Dry a spot of the fixative with cells onto a slide for cell staining







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Vaginal Insertion Biomarkers-DNA

Jacot TA, Nelson A, Thurman A, Kashuba AD, Archer DF, Doncel GF PLoS One. 2014 Dec 9;9(12):e114368.

Run a multiplex PCR to amplify vaginal bacterial DNA markers, amelogenin (control human gene), and semen DNA markers

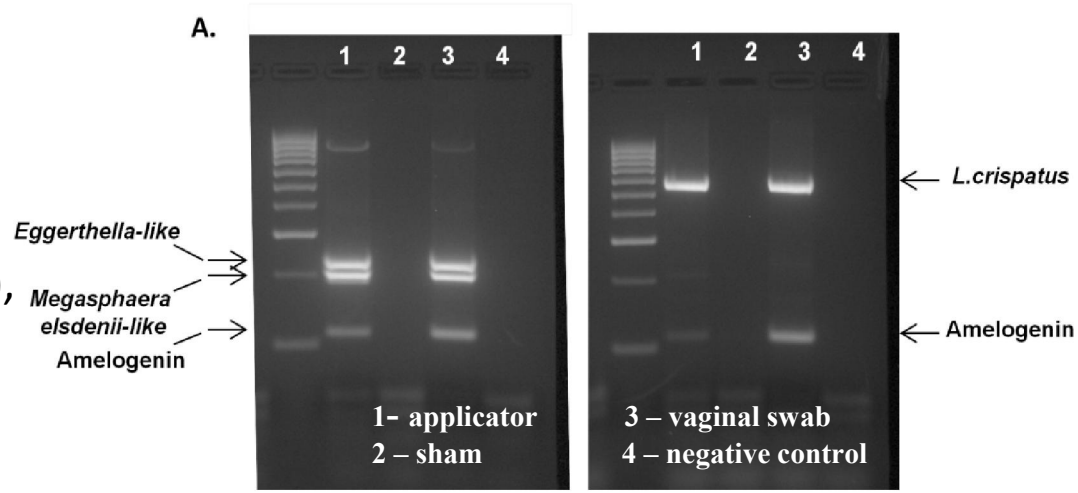
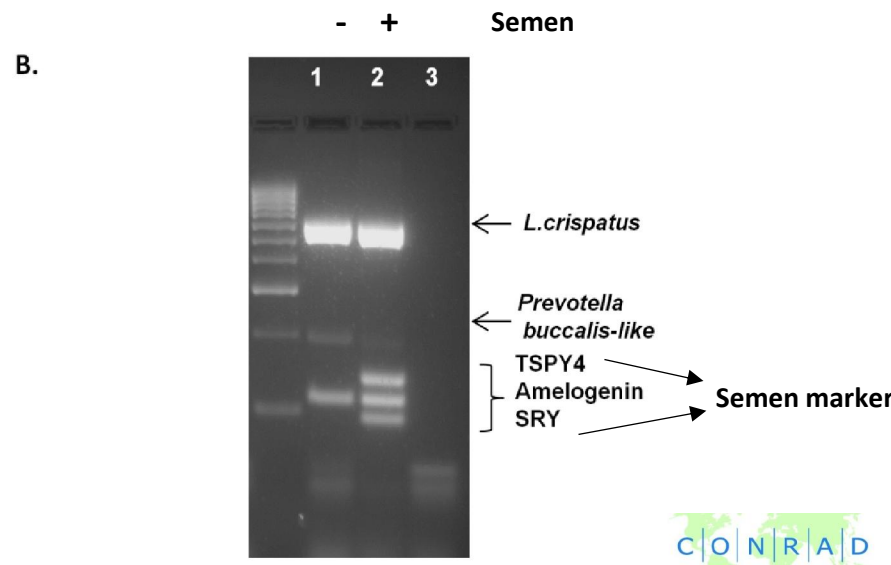


Table 1. Vaginal Bacteria Primers for Multiplex PCR.

Species
Lactobacillus gasseri
Prevotella buccalis-like
Megasphaera elsdenii-like (phylotype 1)
Eggerthella-like
BVAB1
Lactobacillus crispatus
Lactobacillus jensenii



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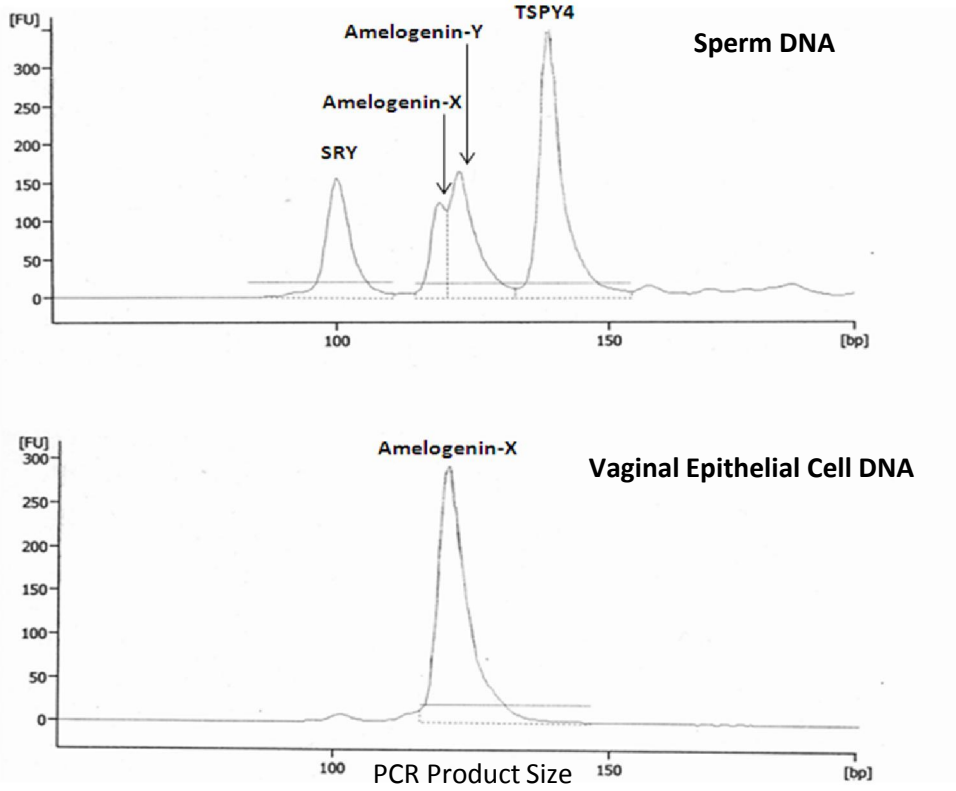
Semen Biomarkers – in vitro development

- **Prostate Specific Antigen –** Point of Care test for semen exposure in 24 hours. Interacts with some vaginal products (including HEC, UC781, TFV)
  - Snead MC et al. *Contraception.* 2014;90(2):136-141.
- **Multiplex PCR analysis platform→** Simultaneously amplify two Y-chromosomal genes, SRY (48 hrs) and TSPY4 (7 d) from one vaginal swab DNA sample
- Incorporates a human control gene, amelogenin
  - Jacot et al. *Contraception.* 2013;88(3):387-395

1. Amplified PCR reactions pipetted onto chip

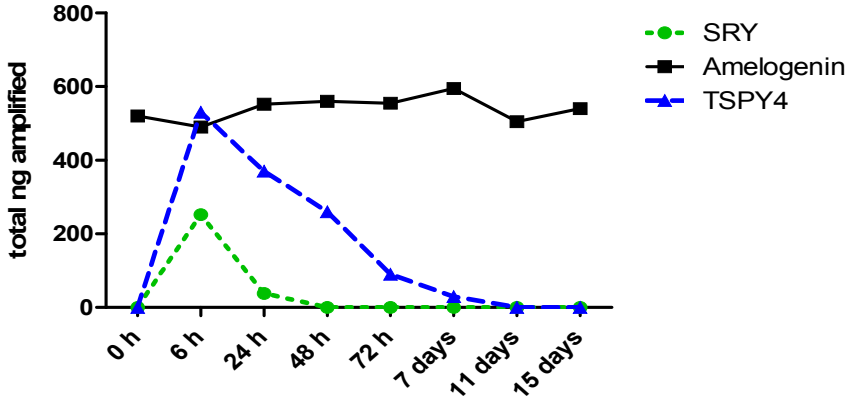
2. Chip placed in Bioanalyzer 2100 to detect amplified SRY and TSPY4

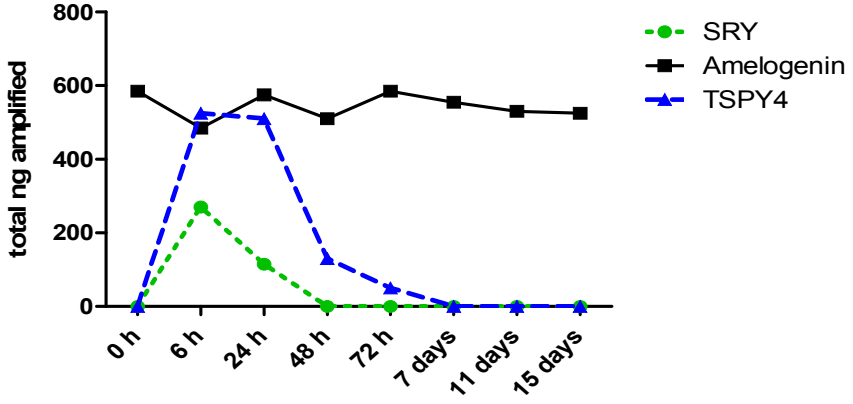


Semen Biomarkers – In vivo testing

- **Utility:** Determine condom failure or non-use, surrogate for HIV risk
- **Why DNA?:** Y-chromosomal markers are more sensitive than PSA in detecting semen exposure over 7 days.

Multiplex PCR analysis platform:
Allows incorporation of two markers:
SRY – reflects exposure up to 48 hours
TSPY4 – reflects exposure up to 1 week

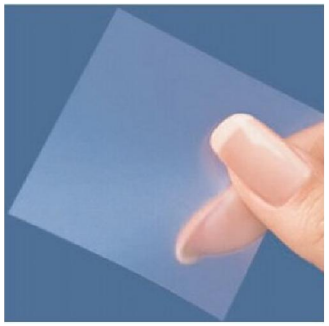




Thurman AR, Jacot TA et al. Contraception 2016;94:512-520.

Topical Product Adherence

Vaginal Gel



Vaginal Film

Vaginal Insert

Intravaginal Ring

Drug (TFV) exposure (vaginal swab & applicator)

- LC-MS/MS
- Spectroscopy(FTIR or RAMAN)

Placebo exposure (vaginal swab)

- Excipient bioassays
- Spectroscopy

Montgomery ET, Beksinska M, Mgodi N, et al. End-user preference for and choice of four vaginally delivered HIV prevention methods among young women in South Africa and Zimbabwe: the Quatro Clinical Crossover Study. *J Int AIDS Soc.* 2019;22(5):e25283.

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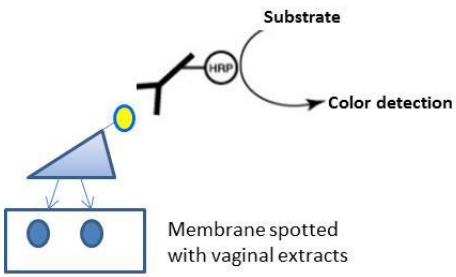
Excipient Based Approaches for Placebo Microbicide Adherence Markers (In vivo)

I. Hydroxyethylcellulose (HEC) (Gel)



Baseline

15 min post gel use

12 hrs post gel use

24 hrs post gel use

48 hrs post gel use

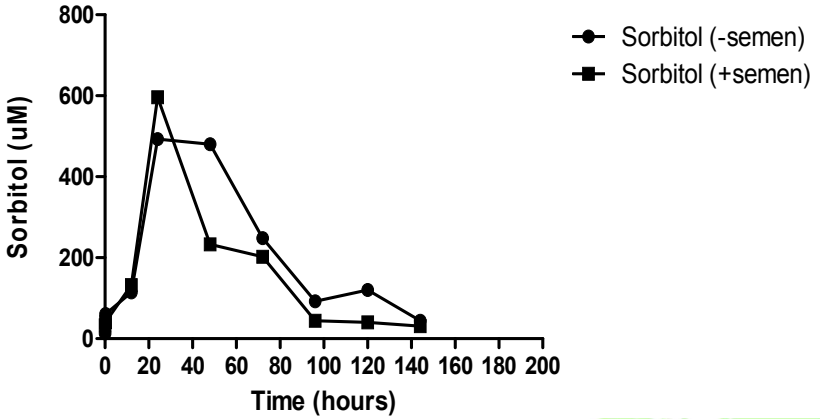
72 hrs post gel use

Colormetric Assay with Probe

II. Glycerin (Film)

Time after vaginal film use	mM Glycerin (-semen)	mM Glycerin (+semen)
0 min	0	0
15 min	3.8	1.9
12 hours	5.8	0.72
24 hours	0.91	0.25
48 hours	0.29	0.048
72 hours	0.13	0.019
96 hours	0.028	0

III. Sugar alcohols (sorbitol) (Insert)



Jacot TA et al. Clin Translational Med, 2018, 7(37).

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Product Adherence – FTIR Spectroscopy

Results:

Name	Value
IS SWAB VAGINALLY INSERTED?	YES, VAGINALLY INSERTED SWAB
IS SEMEN PRESENT?	YES, SEMEN IS PRESENT
IS HEC PRESENT	NO HEC DETECT IN SWAB
IS PLACEBO INSERT-C PRESENT	NO, PLACEBO INSERT C NOT DETECT

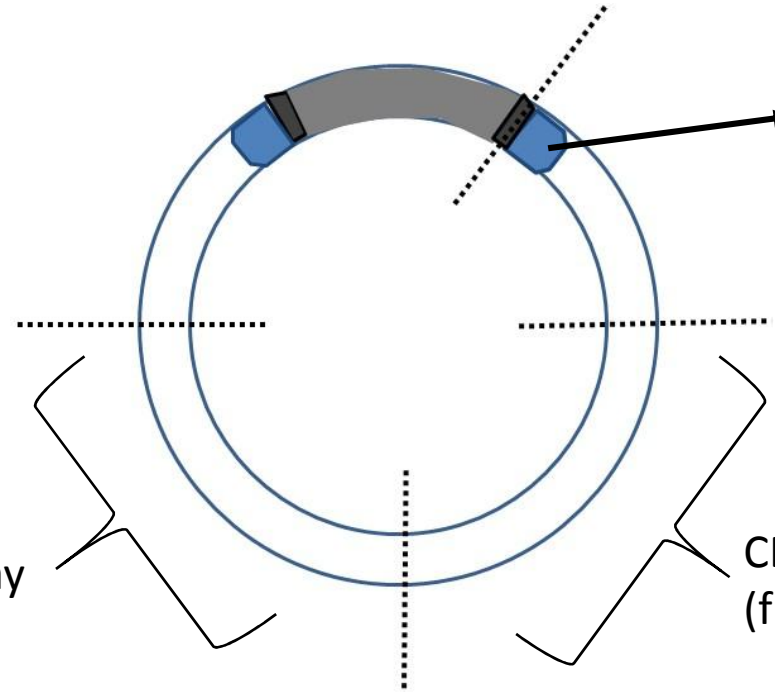
Allows ability of on-site clinic personnel to get information about active or placebo or active microbicide product use and semen exposure simultaneously

Adedipe O, Jacot TA et al. Plos One, 2018, 13(5):e0197906.

Determining Placebo Markers for Intravaginal Rings

Three general approaches:

1. Markers deposited on the ring = swabbing the surface for total carbohydrate assay (Biofilms?), ninhydrin staining, semen biomarkers
2. Excipients eluting out of the ring – glycerin
3. Markers penetrated into the ring = bioanalytes with free amine groups



Preliminary Clinical Studies

- ❑CONRAD D15-135 at EVMS (Placebo)
  - Link to USAID/BMGF Quatro Study in Zimbabwe and South Africa
- ❑FACTS 001 gel applicator analysis (TFV)
- ❑CONRAD MPT IVR Phase 1 Studies (TFV, TFV/LNG, Placebo IVRs)
  - ❑128 Study – 2 weeks of IVR use
  - ❑138 Study – 3 months of IVR use (Cyclic/Continuous)

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Results: 135 Study (Film, Insert, Gel)

Percent of swabs (n = 10 per product) positive for placebo product detection

No Semen Exp	HEC Gel		Vaginal Film		Vaginal Insert	
Time	**HEC**	**FTIR**	**Glycerin**	**FTIR**	**Sorbitol**	**FTIR**
0	0	0	0	0	0	0
15 min	100	100	100	100	100	100
6-12 hrs	100	100	90	100	90	80
24 hrs	90	50	70	90	80	20
48 hrs	40	50	40	70	70	40
72 hrs	22	50	20	40	30	40
96 hrs	20	60	10	30	60	50
120 hrs	10	50	0	20	30	50
144 hrs	10	50	0	10	10	40

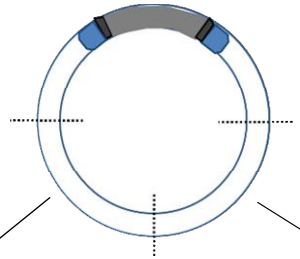
With Semen exposure:

	HEC Gel		Vaginal Film		Vaginal Insert	
Time	**HEC**	**FTIR**	**Glycerin**	**FTIR**	**Excipient**	**FTIR**
0	10	0	0	0	0	0
15 min	100	100	100	100	90	90
8-16 hrs (after sex)	100	70	70	90	90	100
24 hrs	50	70	40	100	60	50
48 hrs	20	30	30	80	40	30
72 hrs	30	40	20	50	30	30
96 hrs	20	10	0	30	10	20
120 hrs	20	10	0	40	0	30
144 hrs	0	20	0	40	0	22

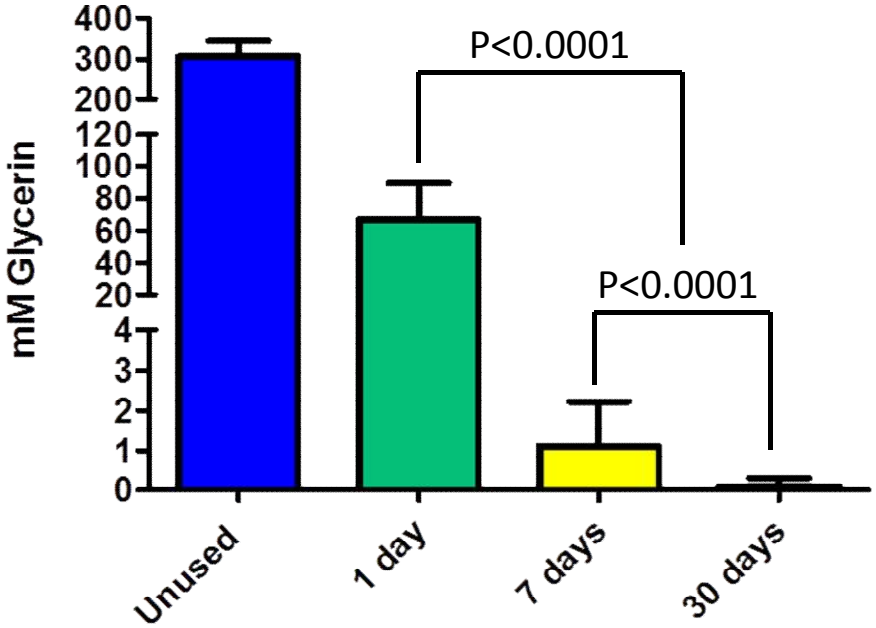
Jacot TA et al. Clin Translational Med, 2018, in press.

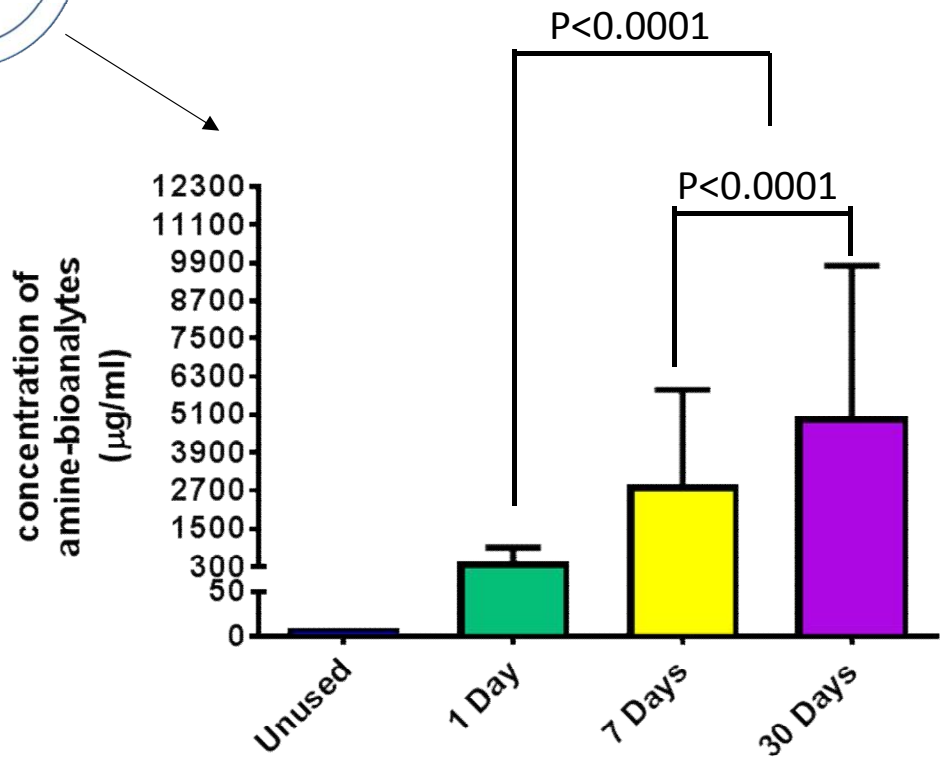
Placebo TFV/LNG IVR Adherence

Glycerin Assay
Excipient "Out"



CBQCA Assay
Biological analytes "In"





From the CONRAD 135 Study

Unused rings: N=3

Used rings: N=20

Jacot TA et al. Clin Translational Med, 2018, in press.

Objective Adherence in QUATRO Study

- Acceptability study of the four placebo delivery systems in South Africa (n = 100) and Zimbabwe (n = 100).
  - Used Placebo Vaginal Film, Vaginal Insert, Vaginal Gel, Vaginal IVR for one month each
  - After using each product for one month, 180 women chose one of the four products to use with sex for another month and to self-swab weekly for pre-coital methods.
- Double-headed vaginal swabs and used IVRs were returned to the clinic, stored frozen, and shipped to our lab.
- FTIR Analysis and Excipient Extraction for placebo product
- Vaginal Insertion and Semen Biomarkers
- IVR for residual excipient and penetrated bioanalytes

Total Swabs Received at CONRAD/EVMS

	BASELINE	GEL	FILM	INSERT	RING
South Africa	100	344	347	336	88
Zimbabwe	100	381	384	384	101
South Africa M5	N/A	84	44	112	21
Zimbabwe M5	N/A	28	160	72	26
TOTAL	200	837	935	904	236

Grand Total: 2,876

≥85% of swabs were evaluable

On Demand Product Adherence - Months 1-4

Proportion of women used product at least twice during the month?

	Individual Sites				Both sites			Returned Only Unopened Swabs	
	Number of women out of 100 total per site with evaluable swabs		Number of women with at least 2 positive swabs for product		Total number of women with evaluable swabs	Total number of women with at least 2 positive swabs for product		Total Never Used (both sites)	
	South Africa	Zim	South Africa	Zim	Count	Count	(%)	Counts	(%)
GEL	84	95	70	89	179	159	89	5	3
FILM	84	93	67	89	177	156	88	2	1
INSERTS	80	94	51	81	174	132	76	26	11

Swabs considered positive by either methodology (Excipient or FTIR)

Biomarkers indicated that the majority of women used product, thus informing their choice for month 5

On Demand Product Adherence - Month 5

How many women used product?

	South Africa			Zimbabwe			Both Sites		
	Number of women	% women with at least 2 evaluable swabs positive for product		Number of women	% women with at least 2 evaluable swabs positive for product		Number of women	% women with at least 2 evaluable swabs positive for product	
		Excipient	FTIR		Excipient	FTIR		Excipient	FTIR
Gel	21	67% (14)	62% (13)	7	86% (6)	100% (7)	28	71% (20)	71% (20)
Film	11	36% (4)	54% (6)	40	52% (21)	90% (36)	51	49% (25)	82% (42)
Insert	28	50% (14)	57% (16)	18	61% (11)	67% (12)	46	54% (25)	61% (28)

Possible reasons for imperfect adherence:

- Didn't use product, Swabbed outside the required window (decay of markers within 12 hours)
- Used product more than 12 hours before sexual activity

Intravaginal Ring Adherence

What percent of total women from both sites used the IVR for 1 month?

Biomarkers

	Months 1 - 4 (174 women) N (%)	**Month 5 (47 women) N (%)**
≤ 1 week	39 (22%)	9 (19%)
2-3 weeks	25 (14%)	1 (2%)
4 weeks	110 (63%)	37 (78%)

Self-report

During Crossover (Months 1 – 4)

- About 70% of women reported using the ring for 1 month

During Month 5 Choice

- 88% of women who chose the ring reported using it every time they had sex

What about Implants/Injectables?

- Compare to DMPA refills
  - Used to get pregnancy test, wait for spontaneous menses
  - Now use QUICK START
- Women presents and not sure when she got her last ARV injection or implant
- What do you do?
  - HIV test ✓
  - Give another dose?
- Test ARV concentration in blood?
- Point of Care Test for ARV concentration in blood

Acknowledgements