

# Pigtailed Macaques as a Model for Rectal Microbicide Use



Microbicides 2008  
New Delhi, India



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# Safety/Efficacy Requirements

## *Preclinical Evaluations for ANY Topical Microbicide*

***Desirable:*** Safe and efficacious for  
both vaginal and rectal use

***Essential:*** Acceptable safety profile  
after repeated product use



Rectal Model  
Development  
*Macaca nemestrina*

# Comparison

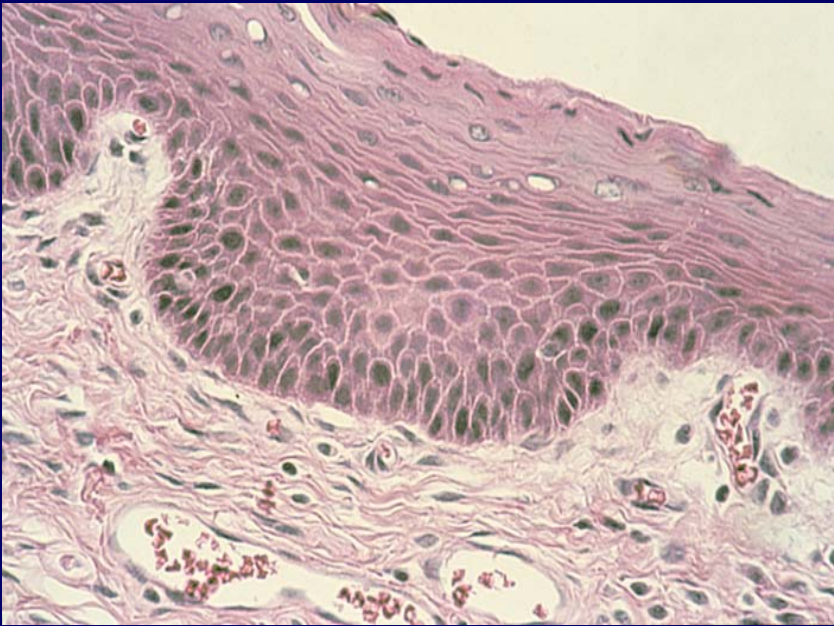
## *Rectal Microflora in Monkeys and Humans*

<u>Organism</u>	<i>M. nemestrina</i> <u>(n=132)</u>	Human* <u>(n=40)</u>
Any <i>Lactobacillus</i>	94%	68%
<i>Lactobacillus</i> H <sub>2</sub> O <sub>2</sub> <sup>+</sup>	80%	48%
<i>Lactobacillus</i> H <sub>2</sub> O <sub>2</sub> <sup>-</sup>	51%	20%
Beta hemolytic streptococci	0%	33%
<i>Enterococcus</i> spp.	17%	38%
Any viridans streptococcus	91%	60%
Viridans streptococci H <sub>2</sub> O <sub>2</sub> <sup>+</sup>	33%	25% (n=20)
Viridans streptococci H <sub>2</sub> O <sub>2</sub> <sup>-</sup>	84%	55% (n=20)
Coagulase negative staphylococcus	45%	28%
Coliforms	28%	100%
Anaerobic positive cocci	69% (n=78)	98%
Black anaerobic gram neg rods	90%	95%
Other anaerobic gram neg rods	98% (n=54)	98%

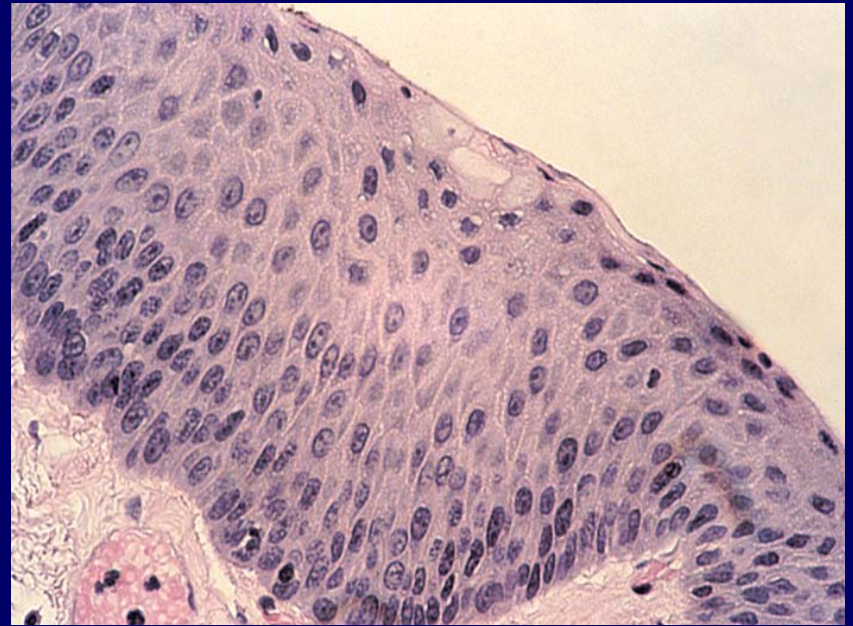
\*Women aged 18–30, nonpregnant, sexually active

# Normal Rectal Biopsies

## *Anal Canal*



Macaque



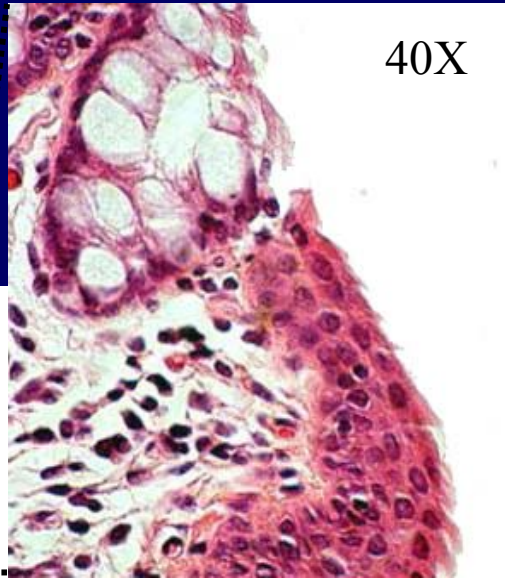
Human

# Macaque Histology

## *Transition Zone—Squamous to Columnar Epithelium*

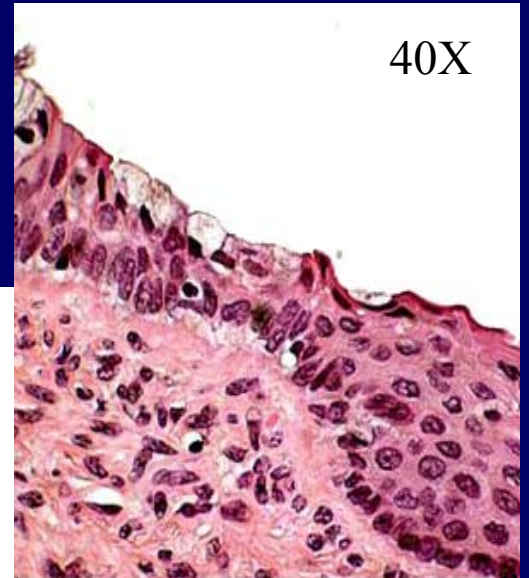
Rectal

40X

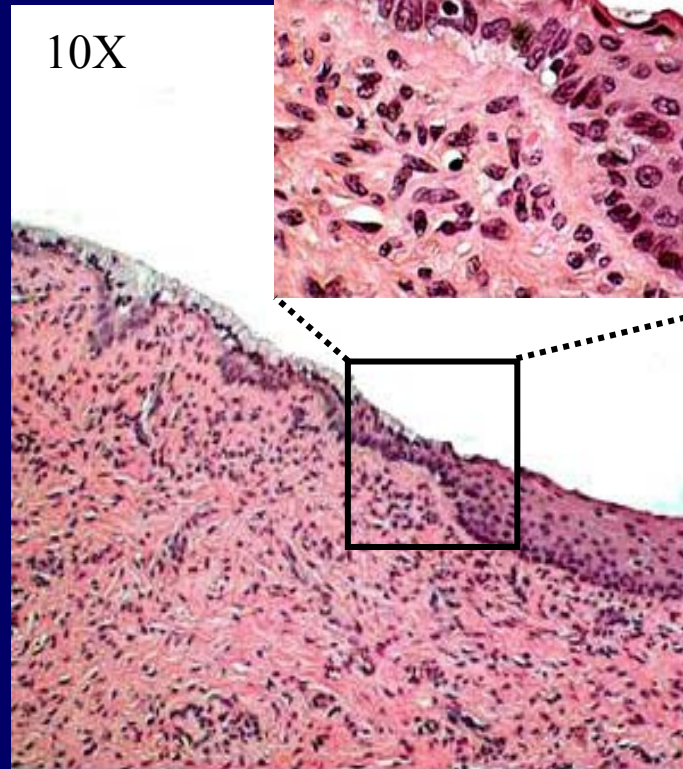


Cervical  
and  
Vaginal

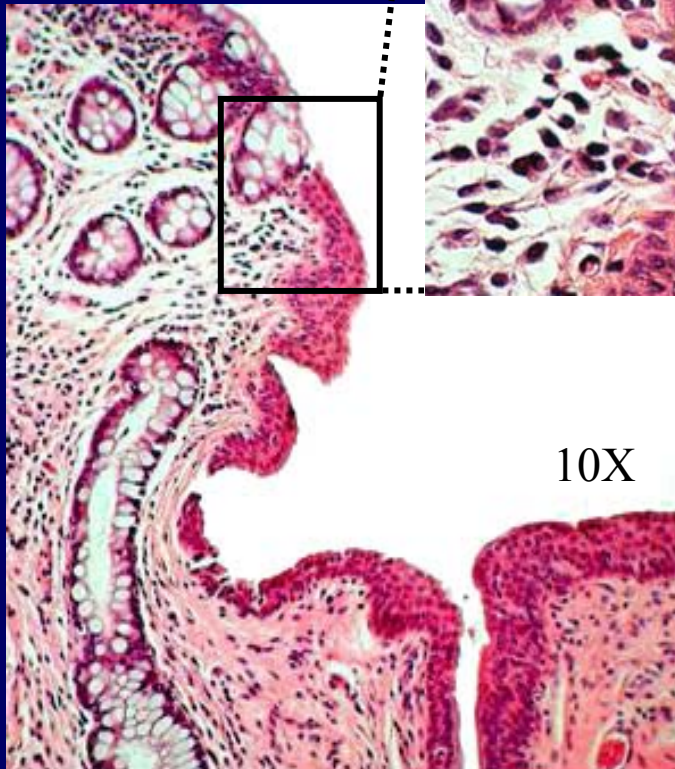
40X



10X

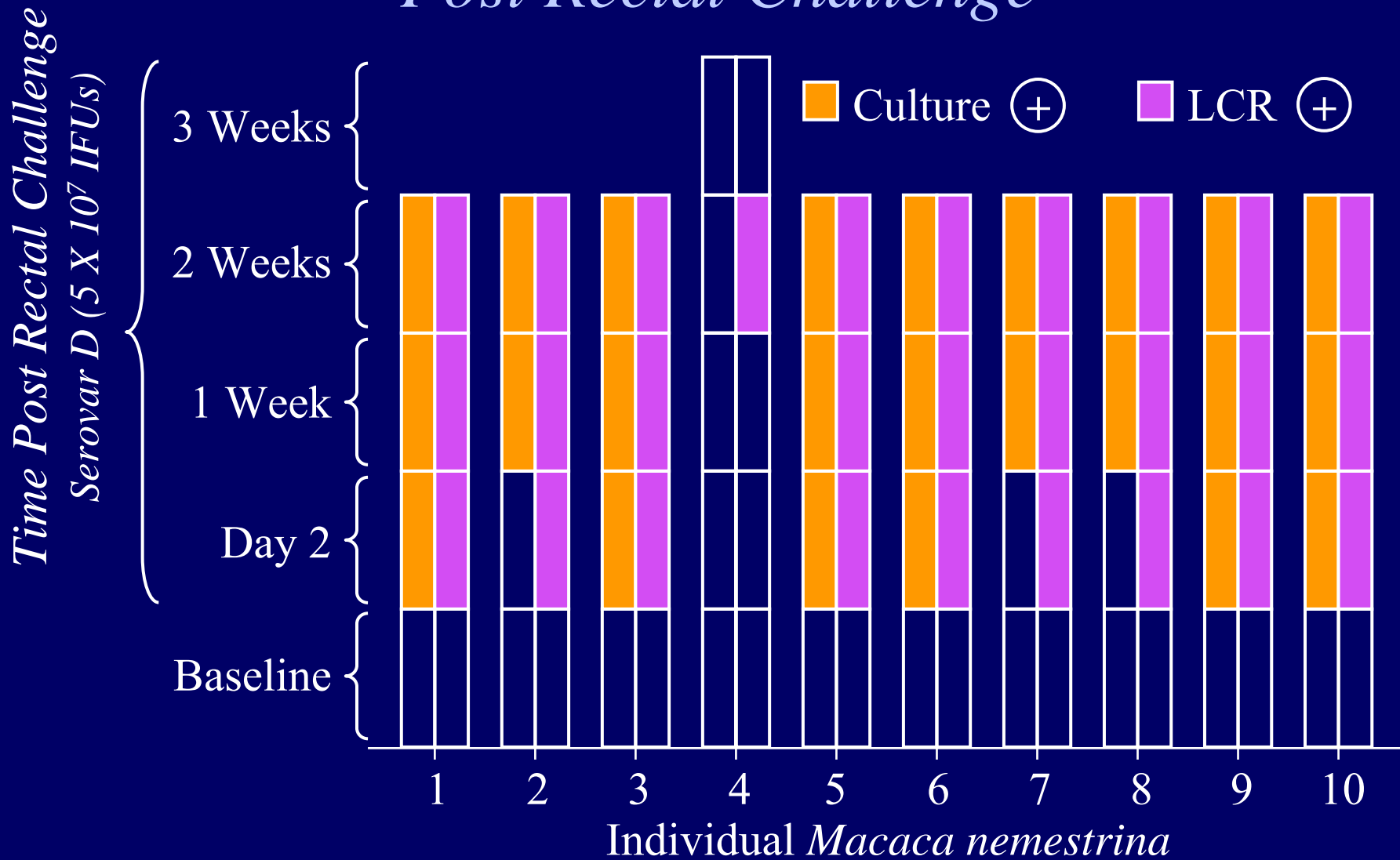


10X




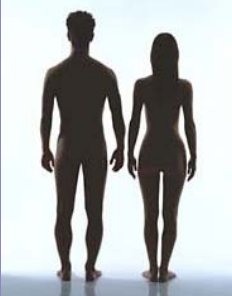
# Detection of Chlamydia

## *Post Rectal Challenge*



# Summary

## *Pigtailed Macaque Model*

	<i>Susceptibility to C.t.</i>	<i>Rectal Epithelium Thickness</i>	<i>Rectal pH</i>	<i>Rectal Microflora</i>	
				<i>Lactos</i>	<i>Prevalence</i>
	✓	≤15 cell layers	6.0–7.5*	✓	94% (n=132)
	✓	≤15 cell layers	6.0–7.5	✓	68% (n=40)

\*95% Confidence Interval for the mean normal macaque rectal pH (n=73)

# Purpose

## *Rectal Safety and Efficacy*

To perform preclinical safety and efficacy evaluations of topical microbicides for rectal use in the animal model.

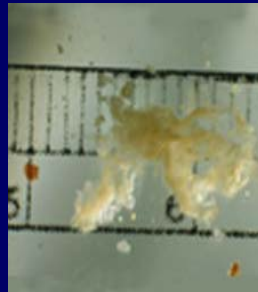


# Rectal Lavage Evaluation

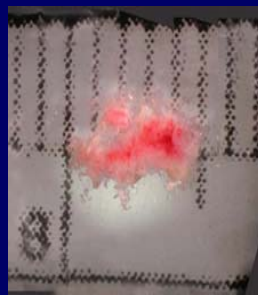
## *Primate Model*

Days 1–4, Time 0 and Time 30; Day 5 Follow-Up

Examine rectal lavage sample under dissecting microscope



Measure and count epithelial sheets  $\geq 3$  mm on one side



Presence of blood



Presence of stroma

Fix for histology (H&E)

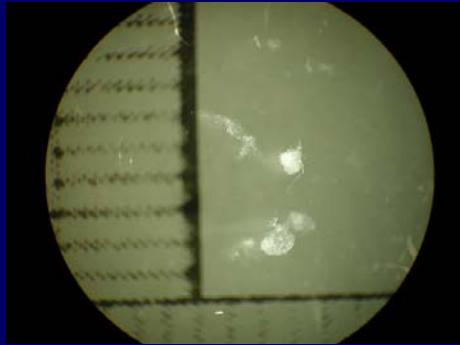
# Products Evaluated

## *Rectal Safety*

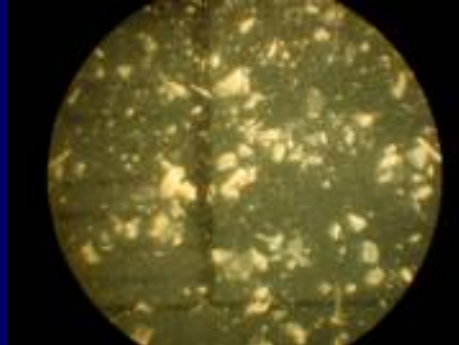
- 4.0% Nonoxynol-9
- 1.0% C31G
- 1% Octylglycerol
- 0.5% 3-Octylglycerol (3-OG)
- 10% Polystyrene Sulfonate
- 6% Cellulose Sulfate
- Carraguard
- UC781 (0.1% and 1.0%)
- BufferGel™
- 3.0% SPL7013 Dendrimer
- *Combination formulation (failed)*

# Guidelines for Rectal Lavage

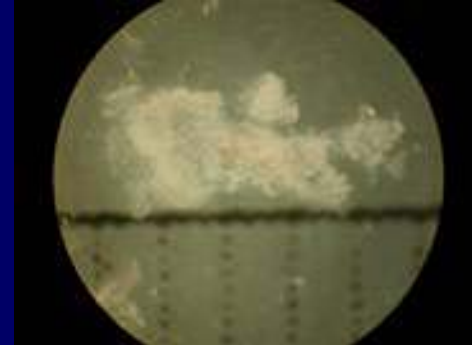
## Epithelial Sheets



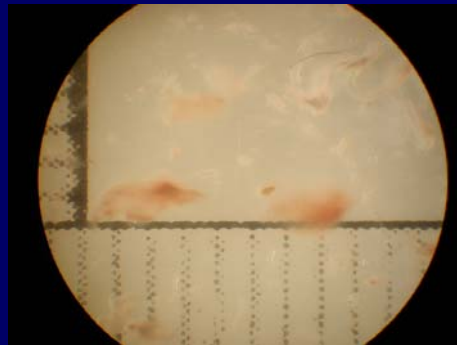
<3mm—Few



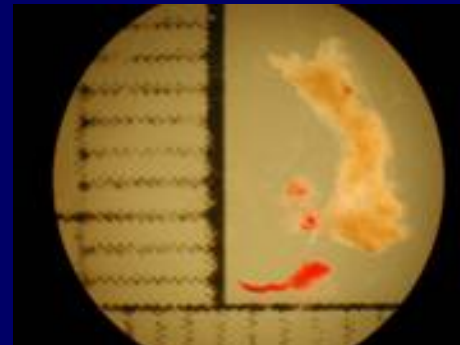
<3mm—Dense



≥3mm



Stroma with  
epithelial sheet



Blood with  
epithelial sheet

# Rectal Lavage Observations

*Epithelial Sheets >3 mm*

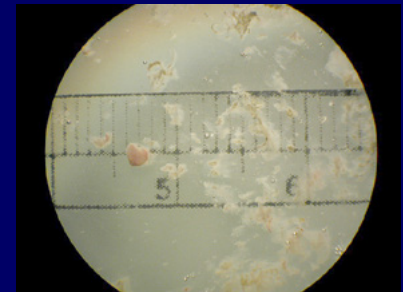
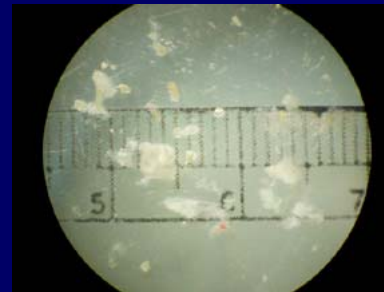
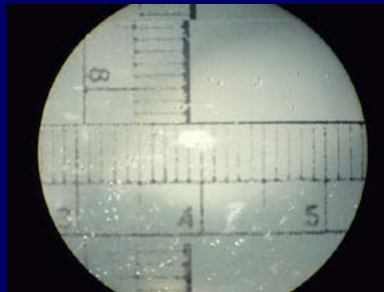
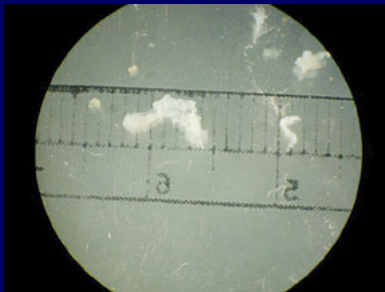
BufferGel™

Polystyrene  
Sulfonate

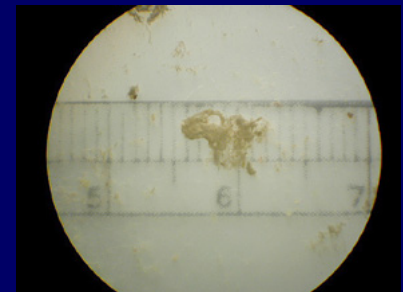
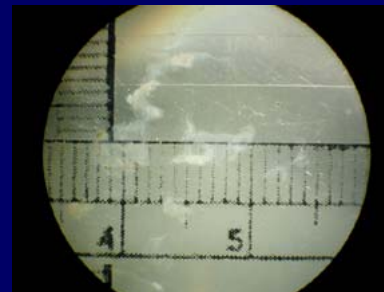
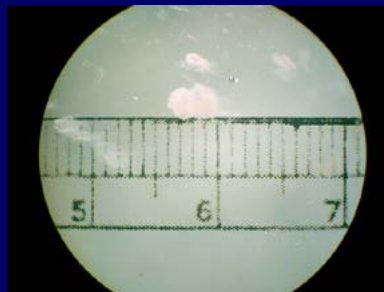
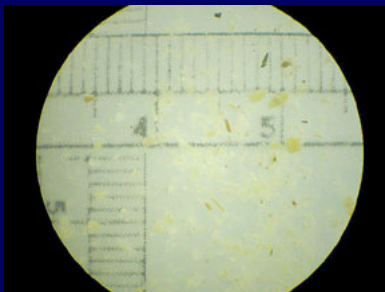
6%  
Cellulose  
Sulfate

1% Octyl-  
glycerol

*Product*



*No Product*



# Guidelines—Rectal Application

## *Topical Microbicide Safety*

### Proceed if:

### Reformulate if:

#### **Microbiology**

- Unchanged
  - Preserved H<sub>2</sub>O<sub>2</sub> production
  - Shifts of unclear significance

- ↑ Pathogenic organism(s)
- ↓ H<sub>2</sub>O<sub>2</sub> production
- Suppression of all flora

#### **pH**

- Unchanged
- Transient ↓ pH

- Permanent pH shift

#### **Rectal Lavage**

- Absence of epithelial sheets
- Individual epithelial cells

- Epithelial sheets  $\geq 3$  mm in at least 1 dimension with stroma attached to epithelium, and/or
- Blood in lavage

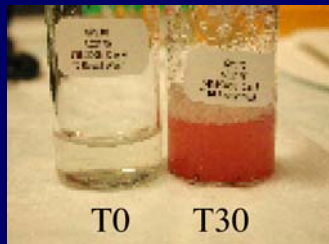
# Rectal Safety

## *Combination Formulation Failure\**

**BLOODY**  
Lavage

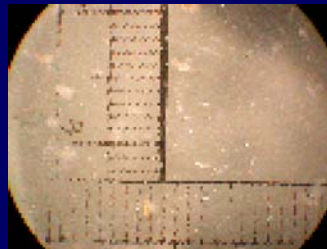


Day 4  
**Combo**  
Animal



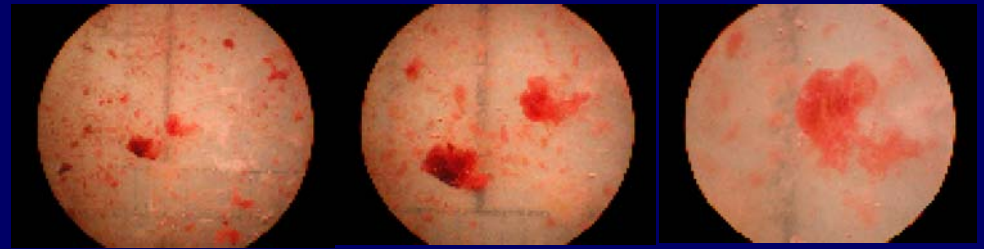
T0 T30

Day 4, T0  
24 hrs post  
3rd application



7X

Day 4, T30 post 4th application



7X

15X

30X

# Products Evaluated

## *Rectal Safety*

- 4.0% Nonoxynol-9
- 1.0% C31G
- 1% Octylglycerol
- 0.5% 3-Octylglycerol (3-OG)
- 10% Polystyrene Sulfonate
- 6% Cellulose Sulfate
- Carraguard
- **0.1% UC781\*** and 1.0% UC781
- BufferGel™
- 3.0% SPL7013 Dendrimer
- *Combination formulation (failed)*

*\*Product in clinical development for rectal use*

# Background

## UC781

- ◆ UC781, a non-nucleoside reverse transcriptase inhibitor of HIV-1, was tested in 0.1% and 1.0% gel formulations (*provided by Biosyn, Inc.*)
  - Reported antiviral activity at nanomolar concentrations



# Background

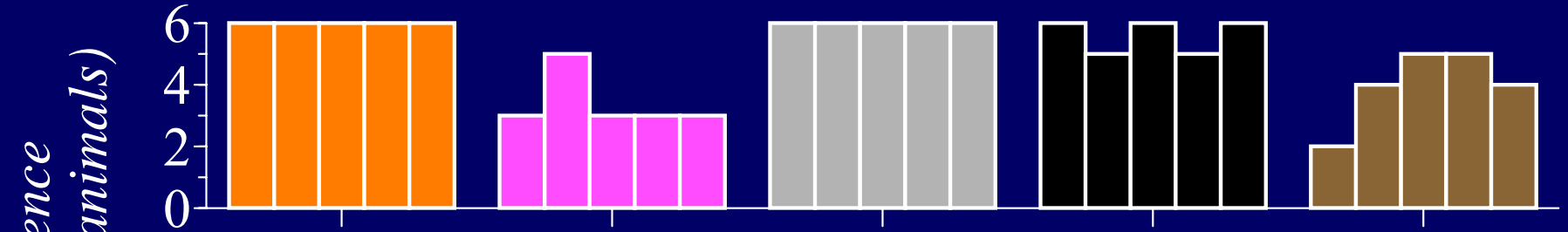
## *UC781*

Preclinical safety tests in the pigtailed macaque model:

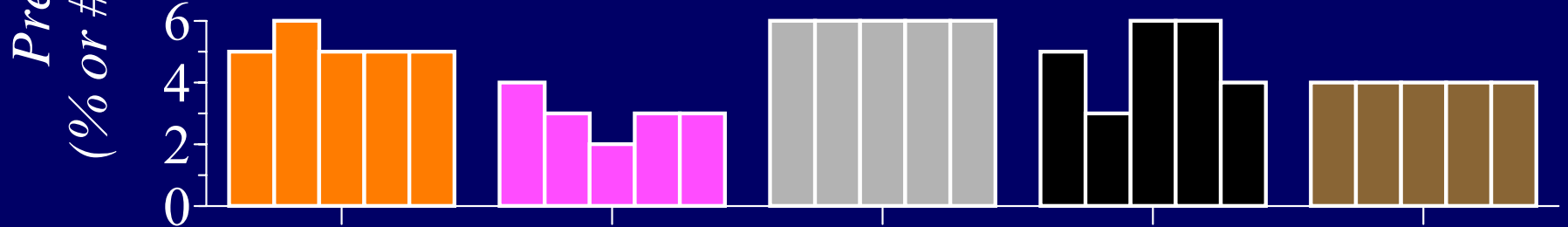
- ◆ Microbiology
- ◆ pH
- ◆ Rectal lavage
- ◆ Cytokine analyses

# Rectal Microbiology

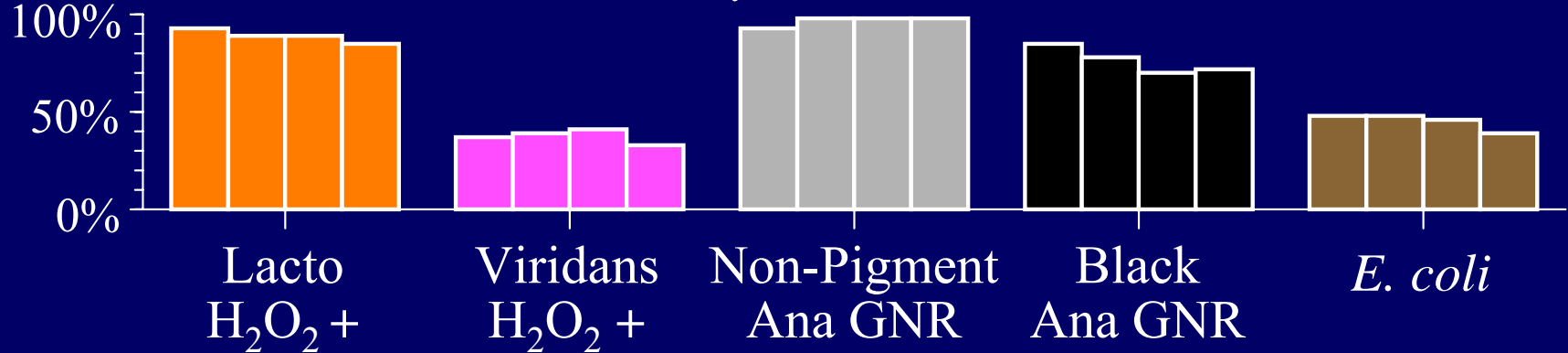
**0.1% UC781** ( $n=6$ ) (days 1–5, T0)



HEC Placebo ( $n=6$ ) (days 1–5, T0)

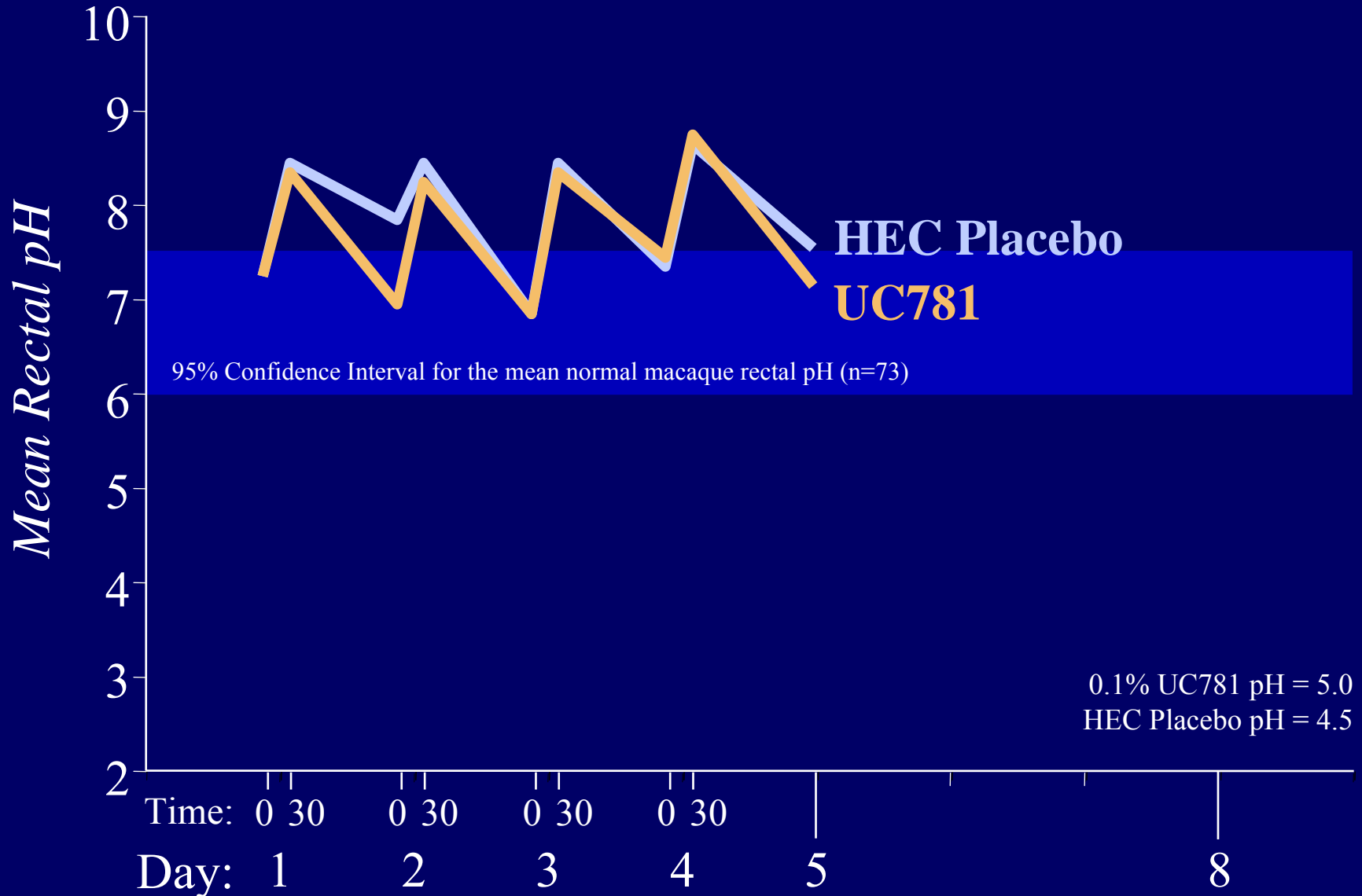


No Product ( $n=46$ ) (days 1–4, T0)



# Mean Rectal pH for **0.1% UC781** & Placebo

—*Macaca nemestrina*—



# Representative Rectal Lavage—0.1% UC781 *Macaca nemestrina*

## 0.1% UC781

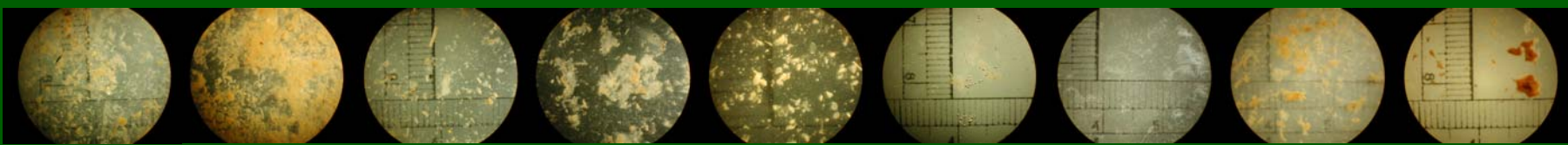
Day 1

Day 2

Day 3

Day 4

Day 5



T0

T30

T0

T30

T0

T30

T0

T30

T0



HEC Placebo

# Summary

## *Rectal Tissue Safety*

Only safety failures noted by our criteria:

◆ Bloody rectal washes — **Combination gel**

◆ ↑ Cytokine profile — **1.0% UC781**  
— Cytokines not routinely assessed  
in all studies

(IL-1 $\beta$ , IL-6, GM-CSF, TNF $\alpha$ , IFN $\gamma$ , MIP-1 $\alpha$ , IL-12<sub>p40</sub>)

# Rectal Efficacy Study

## *BufferGel*<sup>™</sup>

- ▶ Aqueous vaginal gel with acidic buffering action (*pH* 4.0) designed to prevent vaginal neutralization by semen
- ▶ Developed by ReProtect, Inc.
- ▶ Spermicidal
- ▶ Viricidal
  - HIV
  - HSV-2
- ▶ Partially protective against HSV and *Chlamydia trachomatis* in a mouse vaginal model

*Achilles SL, et al. STD 2002;29(11):655-664.*

*Zeitlin L, et al. STD 2001;18:417-423.*


*Mayer K, et al. Clin Infect Dis 2001;32:476-482.*

# Rectal Chlamydia Efficacy Protocol

## *Chlamydia trachomatis* (n=8)

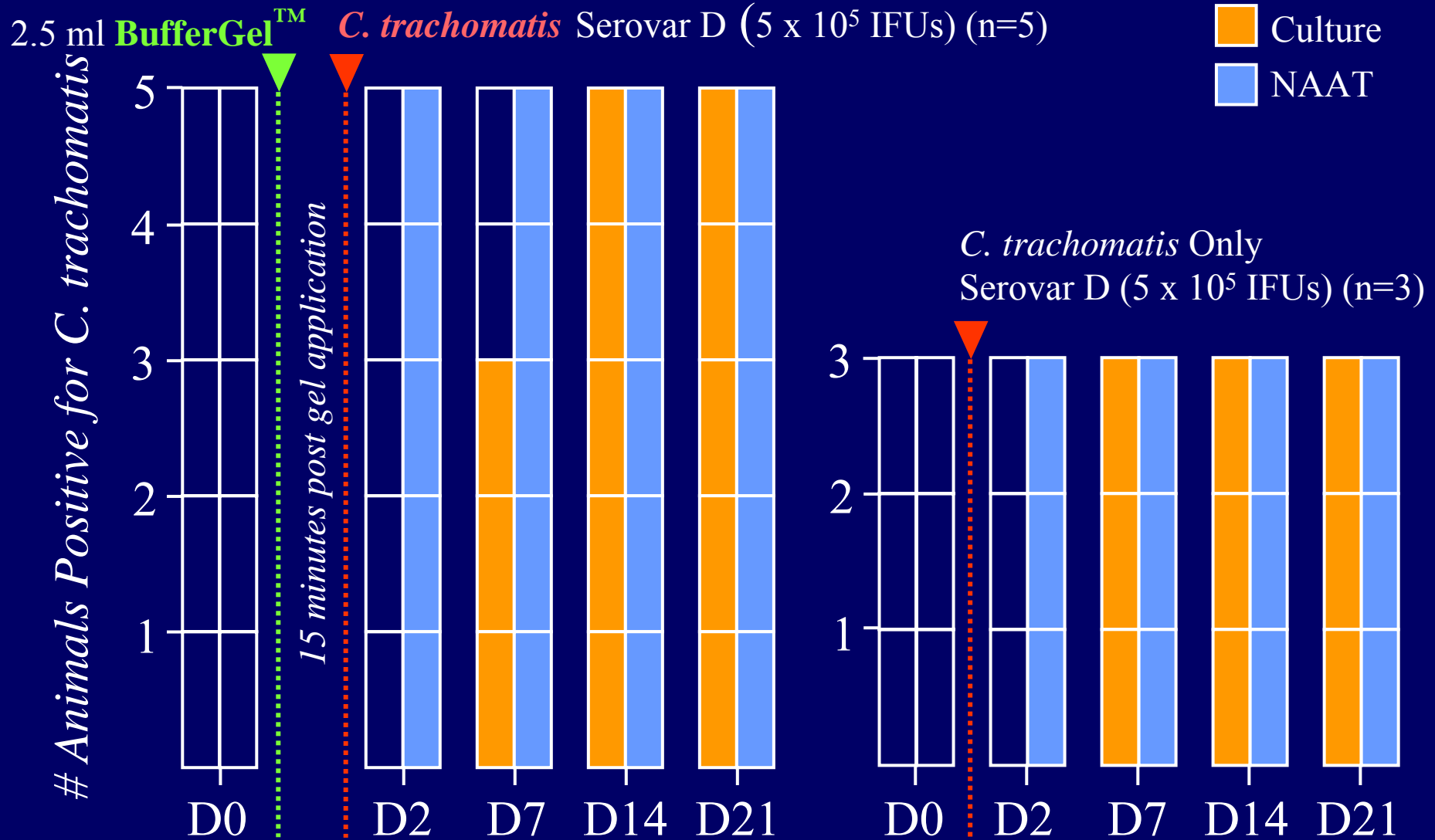
Single rectal application  
2.5 ml **BufferGel™** (n=5)

Rectal challenge  
***Chlamydia trachomatis***  
Serovar D ( $5 \times 10^5$  IFUs) (n=8)

		Baseline Day 0	D0	D2	D7	D14	D21
Rectal Swabs							
	Culture	✓	15 min post gel application	✓	✓	✓	✓
	NAAT*	✓		✓	✓	✓	✓

\*Nucleic Acid Amplification Test (Aptima Combo 2)

# Pretreatment Rectally with BufferGel™ Did Not Protect Against *Chlamydia trachomatis*



# Efficacy Summary

## *Pigtailed Macaque Rectal Model*

***BufferGel:*** A single rectal application of BufferGel™ did not protect against rectal *Chlamydia trachomatis* infection

Patton DL, Cosgrove Sweeney YT, Cummings PK, et al. Safety and efficacy evaluations for vaginal and rectal use of BufferGel in the macaque model. Sex Transm Dis 31(5):290-6, 2004

# Summary

## *Pigtailed Macaque Rectal Model*

- ◆ The pigtailed macaque is a clinically relevant model for assessing rectal safety of topical microbicides
- ◆ The rectal lavage assessments are sufficiently sensitive to identify product toxicity in our macaque model
- ◆ This model can be used to assess a product's ability to prevent rectal infections with *Chlamydia trachomatis*