

Enhanced Screening for *Trichomonas vaginalis* using Nucleic Acid Amplification Tests

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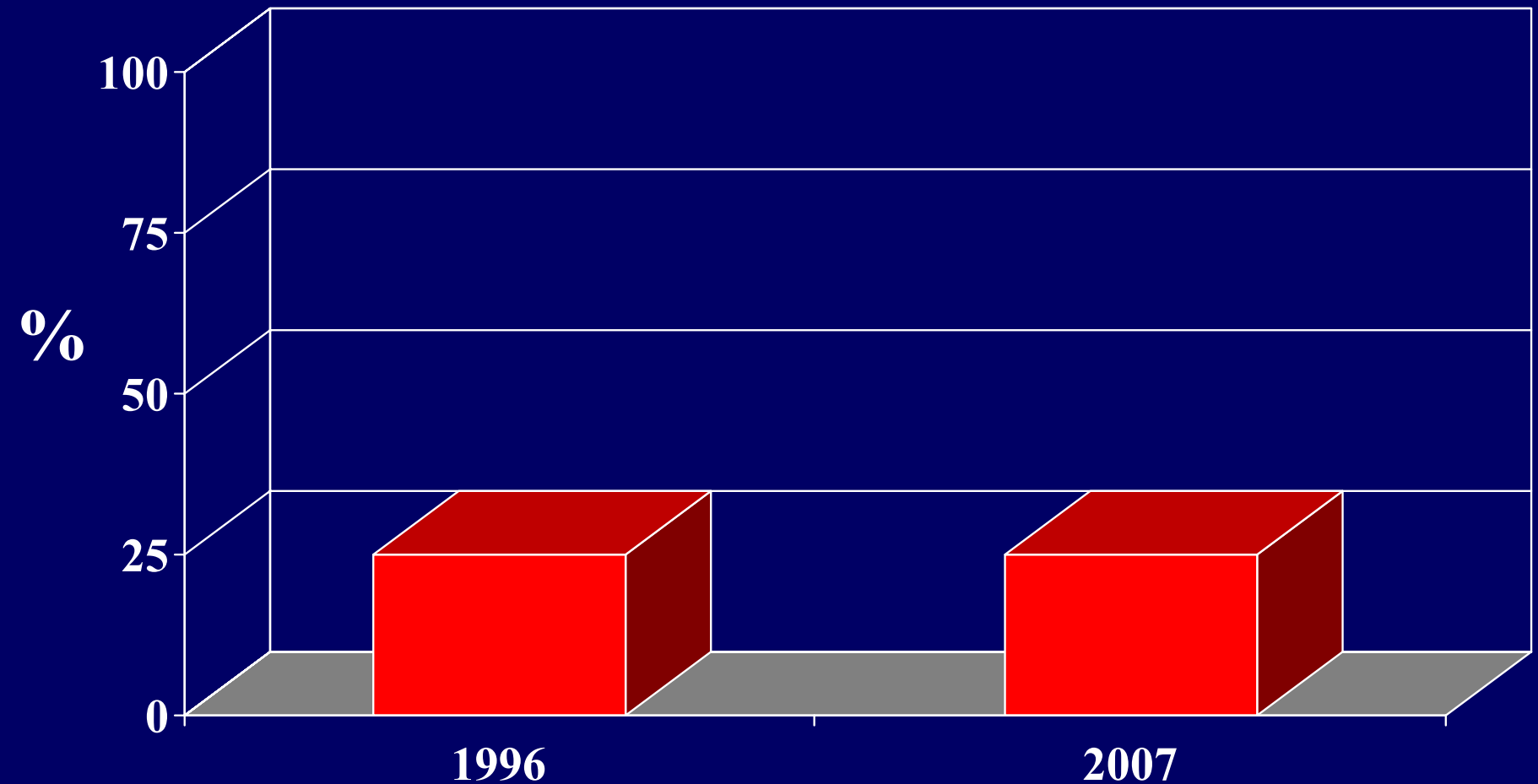
**University of Alabama at
Birmingham**

Trichomoniasis

Vaginal infections caused by *T. vaginalis* among most common conditions in women attending reproductive health care centers

- **Most prevalent in age group 20-45**
- **Based on WHO estimates, approximately 5 million new cases annually in US (Cates, 1999)**
- **Worldwide, over 180 million cases**
- ***T. vaginalis* accounts for 15-20% of all vaginitis**
- ***T. vaginalis* may be significantly underdiagnosed in the US due to reliance on wet mount for diagnosis**

Prevalence of Trichomoniasis JCDH STD Clinic 1996-2007



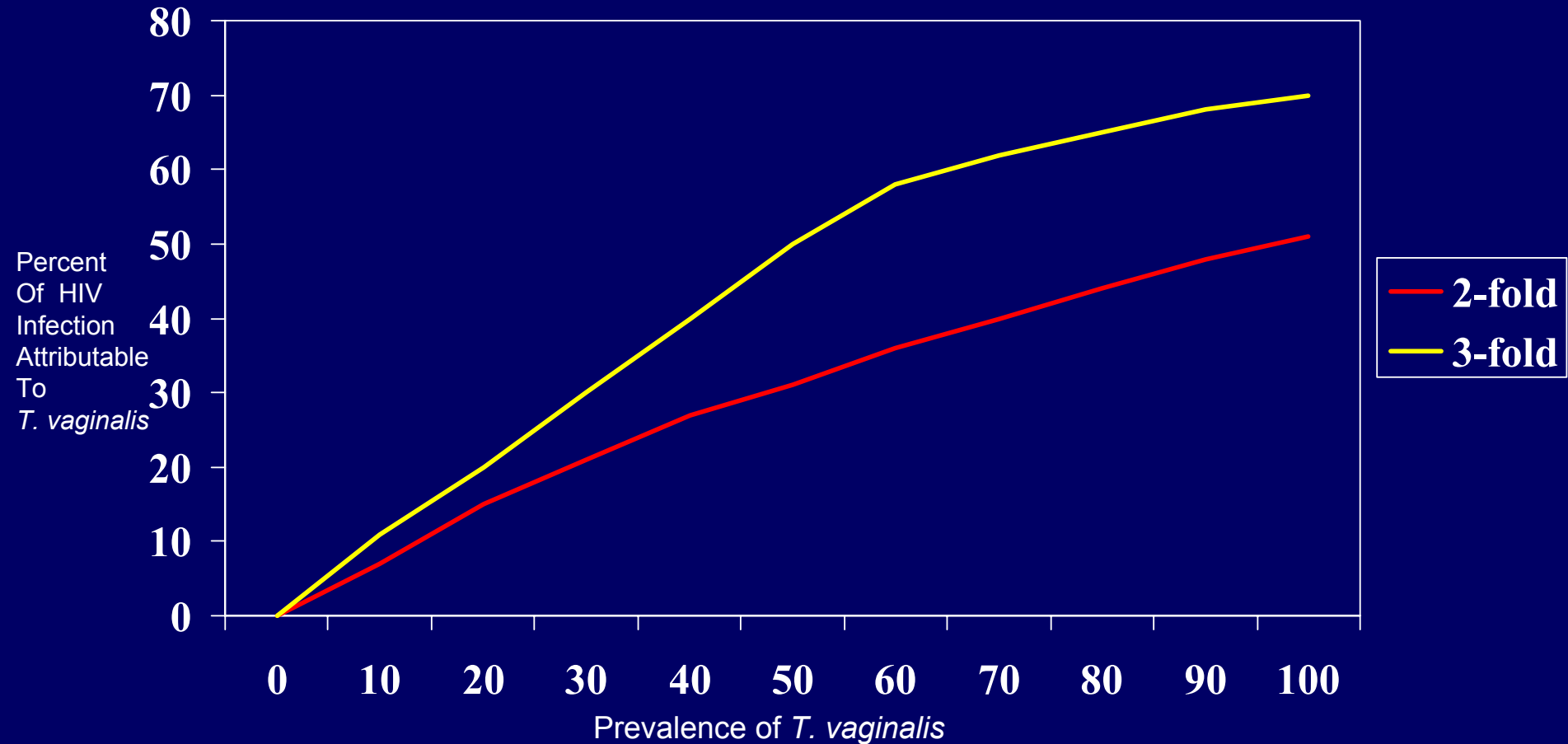
Studies Comparing the Prevalence of *Trichomonas vaginalis* infection with that of other STDs among women in the US

Year	City	Trichomonas (%)	Chlamydia (%)	Gonorrhea (%)
1996	New York	51	9	5
1994	New York	27	7	2
1994	New York	20	15	No data
1992	Baltimore	26	21	14
1990-94	New York	22	6	1
1985	San Francisco	28	25	No data

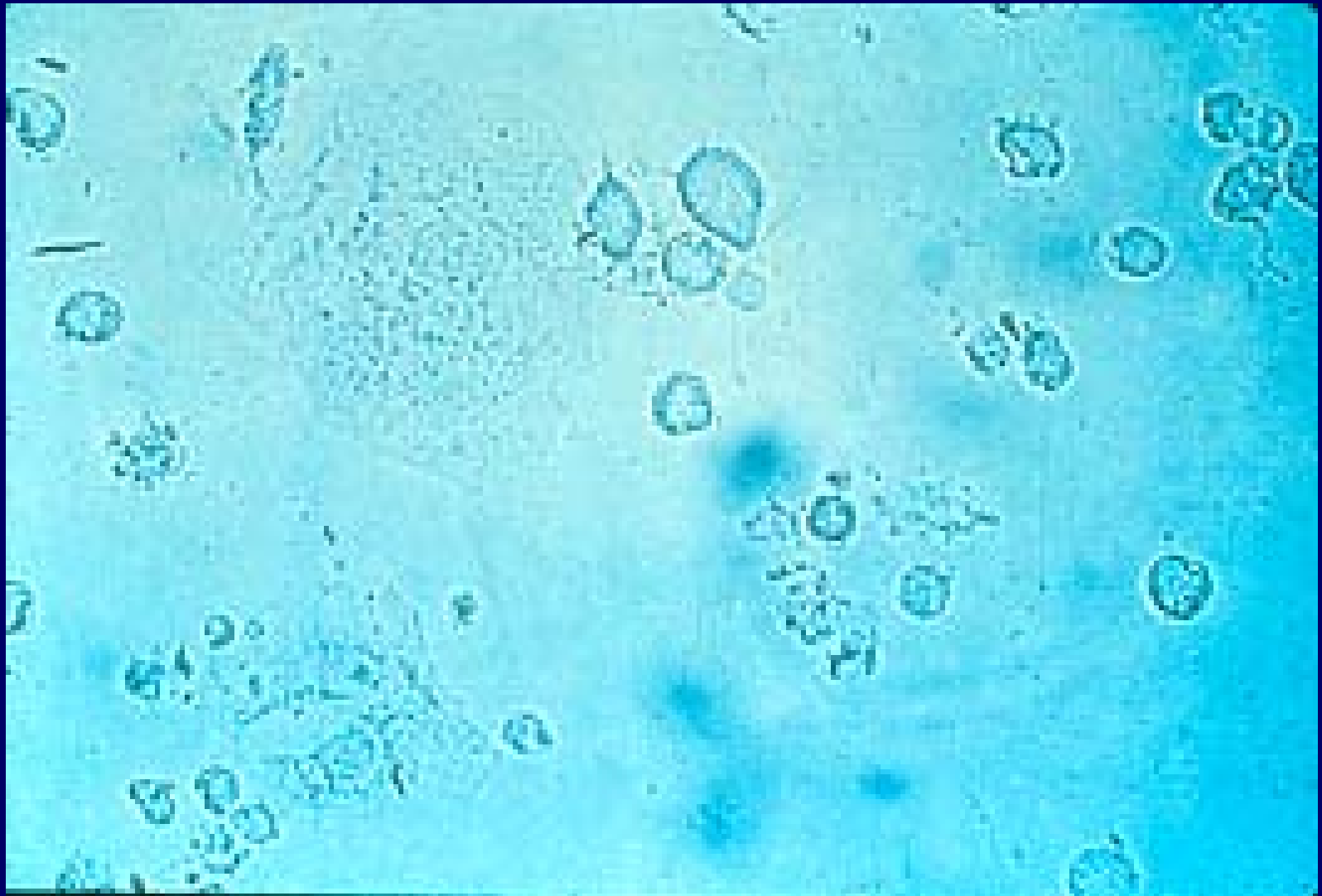
Trichomoniasis Prevalence Rates - Men

- 11% in men at risk for STDs in Seattle (Krieger, 1995) – urethral, urine, coronal sulcus cultures
- 12% of symptomatic men attending a California STD clinic (Borchardt, 1995) – urine sediment culture
- 2.9% of men at a Denver STD clinic (Joyner, 2000) – urine sediment culture
- 58% of Washington, D.C. men at high risk for STDs (Saxena, 1991) – urethral and urine culture, DFA urethral swab, urine sediment microscopy, urethral pap

Percent of HIV infection attributable to *T. vaginalis* infection



TRICHOMONAS WET PREP



InPouch™ TV

TRICHOMONAS VAGINALIS TEST

PATIENT NAME _____ DATE _____
PATIENT ID _____ DOCTOR _____
CLINIC _____

FOR IN VITRO DIAGNOSTIC USE
STORE AT 18-25°C • Patent Pending

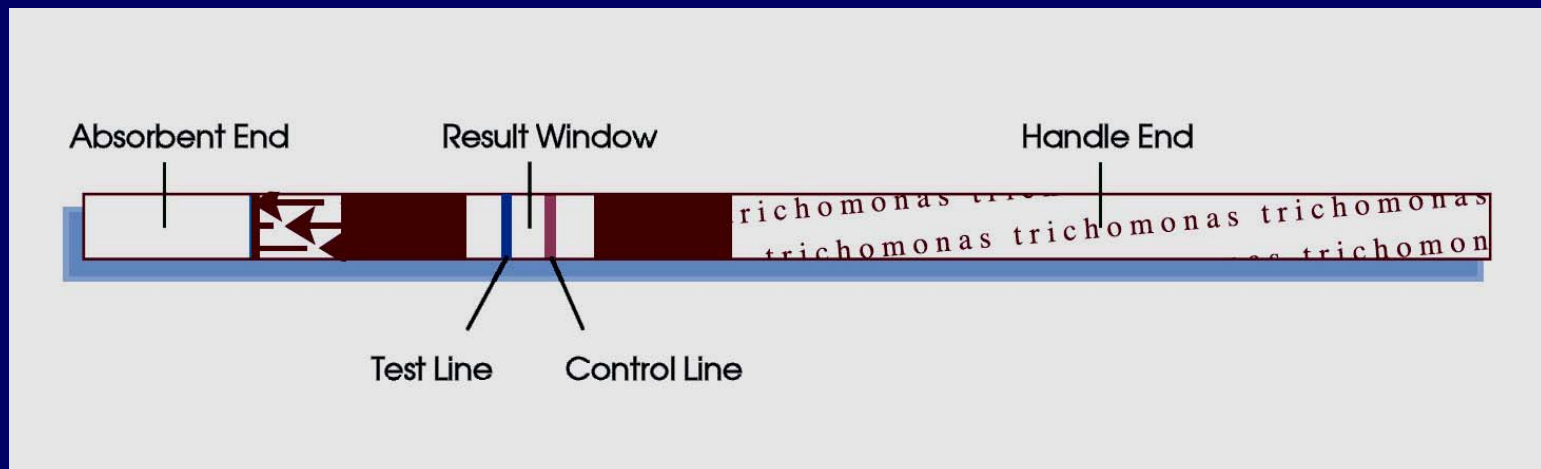
**BIOMED
DIAGNOSTICS**

1-800-954-6466
San Jose, Calif.

LOT 24K049
EXP JAN 04



OSOM[®] Test Stick



Positive test result

A blue Test Line and a red Control Line is a positive result for the detection of Trichomonas antigen.

Comparison of XenoStrip/OSOM rapid test performance to *T. vaginalis* culture, *Seattle and Birmingham 2002

Site	Prevalence	No. true pos	No. false pos	No. false neg	No. true neg	<u>Sensitivity</u>		<u>Specificity</u>	
						%	95% CI	%	95% CI
Seattle	43/497	33	1	10	453	76.7	61.4-88.2	99.8	98.8-99.9
Birmingham	92/439	73	10	19	337	79.4	69.6-87.1	97.1	94.8-98.6
TOTAL	135/936	106	11	29	790	78.5	70.6-85.1	98.6	97.6-99.3

* Gold standard is trichomonads visualized in culture. CI, confidence interval.

Summary of PCR for *T. vaginalis* in females

Authors (year)	Type of specimen	Sensitivity/specificity of PCR (%)
Jeremias et al. (1994)	Vaginal Swab	100/97.8
Heine et al. (1997)	Vaginal Swab	91.8/95.2
Shaio et al. (1997)	Vaginal Swab	100/100
Madico et al. (1998)	Vaginal Swab	96/98
Paterson et al. (1998)	Tampon specimen	92.7/92.1
Van der Schee et al (1999)	Vaginal swab	95.8/98.1
	Urine	100/97.4
Ryu et al. (1999)	Vaginal swab	100/96.8

Comparison of diagnostic tests for *T. vaginalis* in females*

Diagnostic Methods	No. True Positive	No. False Positive	No. False Negative	No. True Negative	<u>Sensitivity</u>		<u>Specificity</u>		<u>Predictive value (%)</u>	
					%	95% CI	%	95% CI	Positive	Negative
Vaginal swab culture	50	0	3	137	94.3	83.4 – 98.5	100	96.6 – 100	100	97.9
Vaginal PCR	47	4	6	133	88.7	76.3 – 95.3	97.1	92.2 – 99.1	92.2	95.7
Vaginal wet prep	31	0	22	137	58.5	44.2 – 71.6	100	96.6 – 100	100	86.2

*n=190. The gold standard is trichomonads visualized from any wet prep or culture (n=53). CI, confidence interval; TVK3 and 4

Trichomoniasis Diagnostic Methods - Men

Test Results	Sensitivity	Specificity	Cost	Ease of Use	Time to Process
Culture – urethral swab	<60%	high	medium	medium	long
Culture – urine sediment	<60%	high	medium	easy	long
Culture – swab & urine	>60%	high	medium	medium	long
Wet mount	~30%	low	low	easy	short
PCR	high	high	high	difficult	long

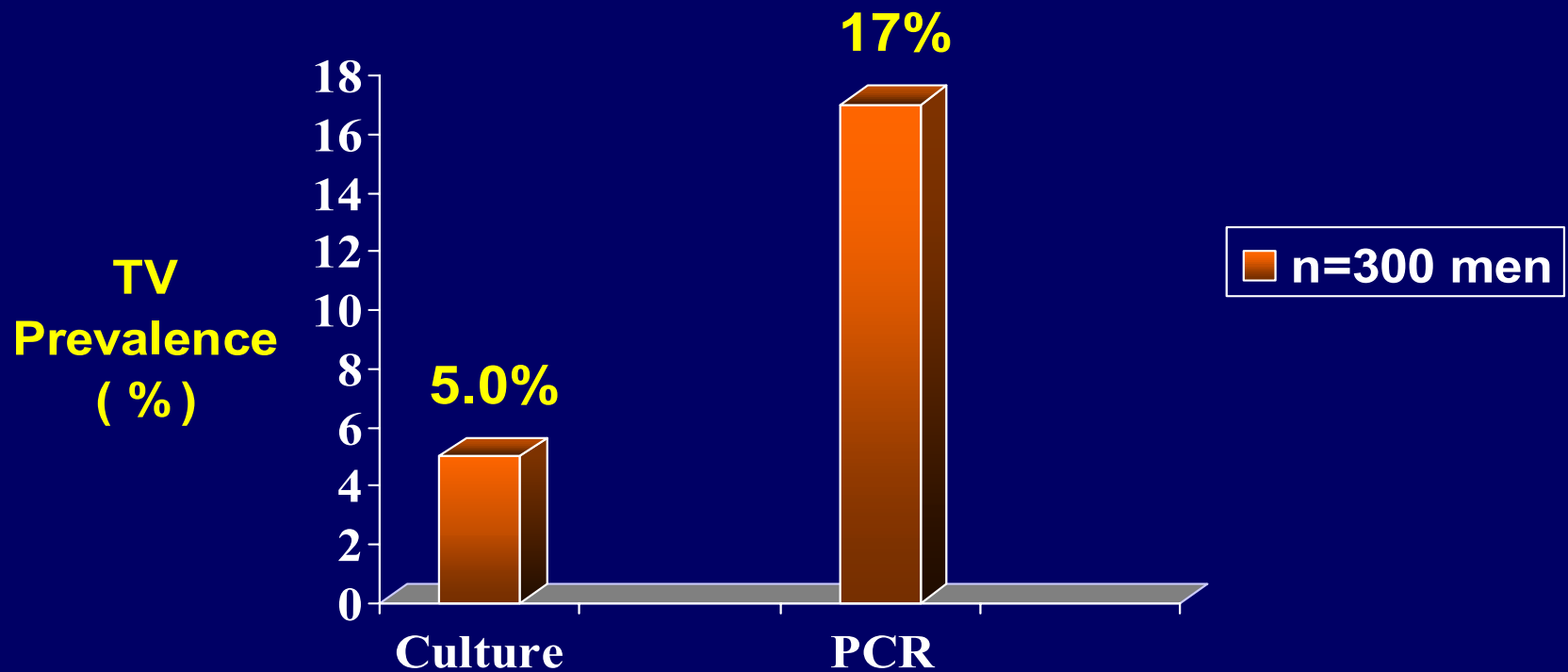
PCR for *T. vaginalis* in Males

Malawi/STD Clinic

- 13% prevalence by culture/wet-mount
- 20.8% prevalence by combined PCR/culture/wet-mount

Detection of *T. vaginalis* in Males

PCR vs. Culture



Comparison of Diagnostic Tests for *T. vaginalis* in Males

Diagnostic Method	No. true positive	No. false positive	No. false negative	No. true negative	Sensitivity	Specificity
Urethral swab culture	11	0	4	285	73	100
Urethral PCR	12	19	3	266	80	93
Urethral Pellet Culture	8	0	7	285	53	100
Urine PCR	15	35	0	250	100	88

*Gold standard is Urethral and/or Urine Culture Positive

Schwebke JR, and Lawing LF. J Clin Microbiol 2002

Objectives of Gen-Probe ASR Study

- Compare TMA (Gen-Probe APTIMA analyte specific reagents for *T. vaginalis*) to wet prep, culture, and PCR (B-tubulin, LabCorp)
- Evaluate various specimen types including vaginal endocervical, male urethral, and urine

Methods

- 300 women and 300 men who presented to the JCDH STD Clinic in Birmingham, AL
- Excluded if recent antibiotics or urination within one hour prior to exam
- Females: vaginal wet prep, vaginal culture (InPouch TV)
- Males: urethral swab and urine sediment combined culture
- Order of collection of specimens was randomized by week

Methods

- Specimens for PCR and TMA were placed into Aptima transport media (urine, urethral swab, vaginal swab, endocervical swab)
- PCR and TMA specimens shipped to Lab Corp for testing- discordant PCR and TMA results resolved with alternate Gen-Probe research use only assay

Prevalence of TV in women:

• Vaginal wet prep	16.4%	
• Vaginal culture	22.5%	
• Vaginal PCR	24.8%	ATV 28.9%
• Endocervical PCR	24.2%	ATV 26.8%
• Urine PCR	23.9%	ATV 26.2%

ATV Vaginal Swab versus InPouch Culture-Females

		In Pouch Culture		
		Pos	Neg	
ATV Vag	Pos	67	19	86
	Neg	0	212	212
		67	231	

Sensitivity 100.00%

Specificity 91.77%

TMA Alt Amp resolved: 100%

Comparison of Diagnostic Tests for TV in 298 Female Subjects

Diagnostic method and specimen	Number of Specimens			True negative	Sensitivity	Specificity
	True positive	False positive	False negative		%	%
<u>Infected patient status algorithm*</u>						
Wet prep	49	0	25	228	66.22	100
Culture	67	0	7	228	90.54	100
ATV – Vaginal swab	73	13	1	211	98.65	94.2
ATV – Endocervical swab	73	7	1	217	98.65	96.88
ATV – Urine	73	5	1	219	98.65	97.77
<u>Molecular resolved algorithm**</u>						
ATV – Vaginal swab	86	0	2	210	97.73	100
ATV – Endocervical swab	80	0	8	210	90.91	100
ATV – Urine	78	0	10	210	88.64	100

*by infected patient status algorithm (+ wet prep, vaginal culture, urine or endocervical PCR) there were 74 infected female subjects

**by molecular resolved algorithm there were 88 infected female patients

Prevalence of TV in Men

- Culture 4.0%
- Urine PCR 6.7% **ATV 11.5%**
- Urethral PCR 7.7% **ATV 16.6%**

ATV Urethral Swab versus InPouch Culture-Males

		In Pouch Culture		
		Pos	Neg	
ATV Swab	Pos	11	37	48
	Neg	1	244	245
		12	281	
Sensitivity		91.67%		
Specificity		86.3%		

TMA Alt Amp resolved: 100%

ATV Urine versus InPouch Culture-Males

		In Pouch Culture		
		Pos	Neg	
ATV Urine	Pos	11	22	33
	Neg	1	258	259
		12	280	
Sensitivity		91.67%		
Specificity		92.14%		

TMA Alt Amp resolved: 100%

Comparison of Diagnostic Tests for TV in 299 Male Subjects

Diagnostic method and specimen	True positive	Number of Specimens		True negative	Sensitivity	Specificity
		False positive	False negative		%	%
<u>Infected patient status algorithm*</u>						
Culture	12	0	13	274	48.00	100
ATV – Urethral swab	24	25	1	249	96.00	90.88
ATV – Urine	24	10	1	264	96.00	96.35
<u>Molecular resolved algorithm**</u>						
ATV – Urethral swab	40	9	2	248	95.34	96.5
ATV – Urine	31	3	11	254	73.81	98.83

*by infected patient status algorithm (+ urethral swab/urine culture, + urine or urethral swab PCR) there were 25 infected male subjects

**by molecular resolved algorithm there were 42 infected male subjects

Symptomatic Status vs. ATV Assay

Female Patients

Vag Swab	ATV Vag		
	Pos	Neg	
S	67	159	226
A	19	52	71
	86	211	

S-Pos 29.6%

A-Pos 26.7%

Symptomatic Status vs. ATV Assay

Male Patients

		ATV Swab		
		Pos	Neg	
Urethral Swab	S	25	170	195
	A	24	76	100
		49	246	

S-Pos 12.8%

A-Pos 24.0%

Summary

- ◆ TMA –based NAAT assay is more sensitive than culture and PCR for detecting *Trichomonas*
- ◆ Females: Vaginal swabs yield the highest PPV.
- ◆ Urine sample is possible as a non-invasive sample
- ◆ Males: Urethral swabs yield the highest PPV