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Diagnostic Rates Differ on the Basis of the Number of Read Days with the Use of the InPouch Culture System for *Trichomonas vaginalis* Screening

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The InPouch *Trichomonas vaginalis* test is the gold standard for clinical culture for *Trichomonas vaginalis* screening. The current package insert recommends an examination period of 3 days. After review of 2,499 InPouch tests spanning 13 years, we observed that examination up to 3 days will detect only 82.8% (95% confidence interval [CI], 79.0% to 86.2%) of positive specimens.

Trichomonas vaginalis is the most prevalent nonviral sexually transmitted infection (STI) worldwide (1, 2) and is a recognized risk factor for the acquisition and transmission of the human immunodeficiency virus (HIV), herpes simplex virus (HSV), and human papillomavirus (HPV) (3–5). Furthermore, increasing evidence implicates *T. vaginalis* infection as a risk factor in men for prostate cancer (6). Prior to the recent introduction of the highly sensitive (95.2 to 100%) *T. vaginalis* nucleic acid amplification test (NAAT) (7), the diagnosis of trichomoniasis has relied primarily on vaginal wet prep and culture using the InPouch *T. vaginalis* test (Biomed Diagnostics, White City, OR). The sensitivity of vaginal wet prep compared to *T. vaginalis* culture is approximately 50.0% to 66.0% (8), while the sensitivity of *T. vaginalis* culture compared to *T. vaginalis* NAAT is 75.0% to 95.7% in women and 28.6% to 100% in men at 100% specificity (9). Nevertheless, many clinics do not currently have access to *T. vaginalis* NAAT, and neither it nor the vaginal wet prep allows for the organism to be grown in culture for drug susceptibility testing and/or genotyping, if necessary.

The validation practices of next-generation molecular testing platforms use the InPouch as the comparison gold standard and follow the current instructional guidelines of an incubation and observational cutoff of 3 days postinoculation (Biomed Diagnostics document no. 100-001, revision K). The older revisions of the package insert (for example, Biomed Diagnostics document no. 100-001, revision D) indicate an examination range of 1 to 5 days. Our laboratory, one of the testing laboratories during the design and implementation of the InPouch system, uses a read range of five daily readings spanning 5 to 7 days, which is a realistic observation range for many laboratories not staffed on weekends. In actual laboratory practice, two of five collection days have a concurrent three-day observation period: Monday and Tuesday collections. Specimens collected on a Wednesday or Thursday have their third reads on day 5. Friday collections have their first observational read on day 3 (Table 1). The optimal timing for incubation and observation of the *T. vaginalis* InPouch culture is currently unclear. The objective of this study was to qualify the newer *T. vaginalis* InPouch package insert instructions with actual laboratory scheduling. We hypothesized that a cutoff of 3 days will fail to capture a substantial number of *T. vaginalis*-positive specimens.

T. vaginalis InPouch specimens were collected from women undergoing gynecological care at The University of Alabama at Birmingham (UAB) and the Jefferson County Department of Health (JCDH) sexually transmitted diseases (STD) clinic and from men clinically suspected to have *T. vaginalis* as a cause of nongonococcal urethritis (NGU). We subsequently reviewed all InPouch testing results at these institutions (UAB and the JCDH STD clinic) processed by our laboratory. InPouch cultures were inoculated in the clinic exam room for outpatients or in the patient's hospital room for hospitalized patients, where they were kept at room temperature for less than 8 h (in clinics or hospital areas without incubators) or incubated immediately at 35°C to 37°C (in clinics or hospital areas with incubators) until transfer to the laboratory. In the laboratory, the InPouch was incubated at 35°C to 37°C during the testing period. Day 1 was defined as the next day after specimen collection. InPouch cultures were moni-

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TABLE 1 Collection and observation matrix, five readings over seven days[a]

	No. of days postinoculation								
Collection day	Tuesday	Wednesday	Thursday	Friday	Monday	Tuesday	Wednesday	Thursday	Friday
Monday	1	2	3	4	7				
Tuesday		1	2	3	6	7			
Wednesday			1	2	5	6	7		
Thursday				1	4	5	6	7	
Friday					3	4	5	6	7

[a] Saturday and Sunday are included in the seven days.

TABLE 2 Positive *Trichomonas vaginalis* InPouch findings matrix by days postinoculation ($n = 419$)

Collection day	No. of positive cultures by day postinoculation								
	Tuesday	Wednesday	Thursday	Friday	Monday	Tuesday	Wednesday	Thursday	Friday
Monday	31	21	6	1	0				
Tuesday		41	27	9	1	0			
Wednesday			31	29	4	0	0		
Thursday				68	33	2	0	0	
Friday					84	19	11	1	0

tored daily for a total of five readings until positive, up to 7 days postinoculation, as part of our laboratory's standard protocol. InPouch cultures that did not grow at the end of the total of five daily readings were recorded as negative for live *T. vaginalis*. This study was approved by the UAB Institutional Review Board. Positive InPouch prevalence rates are presented in two formats: positive prevalence among the total population by day and positive prevalence per day of examination period. Statistics were calculated using OpenEpi version 2.3.1 (Open Source Epidemiologic Statistics for Public Health, version 2.3.1) and SPSS version 21 (IBM Corporation, Armonk, NY). Alpha values were set at 5%.

A total of 2,499 *T. vaginalis* InPouch specimens were collected from patients between May 2000 and April 2013. Of 2,499 InPouch specimens tested, 419 (16.8%; 95% confidence interval [CI], 15.3% to 18.3%) were positive. The day of positive culture determination for these 419 *T. vaginalis* InPouch specimens is stratified in Table 2. During the first 3 days, approximately 82.8% (95% CI, 79.0% to 86.2%) of positive *T. vaginalis* cultures were detected. Of the remaining positive *T. vaginalis* cultures, 17.2% (95% CI, 13.8% to 21.0%) were detected in four to seven days (Table 3). To test our hypothesis, the observed number of positive specimens (347) in the first 3 days was compared to the expected number of positive specimens (419), the actual total of positive *T. vaginalis* specimens; the difference between expected and observed was statistically significant (Fisher's exact 2-tailed $P = 0.005$). The average proportion of positives detected daily was approximately 4.5% of the total number of unresolved InPouches. By the final, fifth daily reading, all positive InPouch cultures had been observed.

A comparison of the observed distribution of positive InPouch specimens indicates that approximately 17.2% of positive InPouch cultures will be observed beyond the first three read days and that this differential is statistically significant. This finding has several important public health implications. The first has to do with the three-day cutoff affecting treatment management. By prematurely ending a test, *T. vaginalis* that is slow to grow for various reasons may not make it to visually critical levels, as the InPouch does require careful scanning of the pouch in three dimensions and not just along the edges of the viewing field. These false negatives represent a potential failure to treat an infected patient and a reservoir for ongoing infection. A second implication is the cutoff of 3 days in the development of next-generation molecular typing methods. There is no argument that the new molecular platforms, i.e., the APTIMA *T. vaginalis* assay (Hologic Gen-Probe, San Diego, CA), are much more sensitive; however, they cannot differentiate active colonization of live *T. vaginalis* from dead *T. vaginalis*. From a purist viewpoint, by using shorter cutoff *T. vaginalis* InPouch read times, and thereby potentially failing to identify 13.8% to 21.0% additional positive specimens, the validation calculations used by developers of NAAT procedures may not be correct, although one could argue that they probably would not change the outcome. By failing to capture additional positive cultures, the specificity and positive predictive values for the performance of the tests would be imprecise. In conclusion, our data support the recommendation that the InPouch be examined daily for 5 days over a seven-day period to reduce the possibility of a false negative for an appropriately handled culture.

TABLE 3 Positive *Trichomonas vaginalis* InPouch findings by day postinoculation

No. of days postinoculation	No. of positive cultures observed	% positive among total	Daily % positive rate
1	171	40.8	6.8
2	77	18.4	3.3
3	99	23.6	4.4
4–7	72	17.2	3.3
Total	419	100	

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