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THE INPOUCH™ TV TEST FOR TRICHOMONIASIS

KENNETH A. BORCHARDT, PHD

Trichomoniasis, perhaps one of the least well known of the current sexually transmitted diseases (STDs), is now being recognized as having major medical significance. The World Health Organization (WHO) has estimated that each year *Trichomonas vaginalis* infections produce approximately 180 million cases worldwide and 7 million cases in the United States[1,2].

INTRODUCTION

As many as one out of 5 sexually active women may be infected with trichomoniasis in their lifetime. Seven to 32% of women examined in sexually transmitted disease (STD) clinics and 5% others attending family planning clinics have been positive for trichomoniasis[3]. Three contemporary clinical studies on female patients in India, Costa Rica, and Uganda revealed infection rates of 14%, 19%, and 47,1%, respectively[4,5,6].

An important sequela of trichomoniasis may occur in the female during pregnancy, which is associated with a premature rupture of the membranes, resulting in a low birth weight of the neonate[7]. Vaginal discharge is another significant clinical presentation and in one study was observed in 25 of 26 (96%) culture positive patients with *T. vaginalis*[8].

Trichomoniasis in the male frequently produces symptoms similar to those of nongonococcal urethritis in addition to other genitourinary sequelae[9]. The majority of males present clinically either with a self-limiting or an asymptomatic infection, and 15 to 50% of females are asymptomatic[10]. It is important to acknowledge that trichomoniasis increases the hosts susceptibility to the human immunodeficiency virus[11].

DIAGNOSIS

Establishing a laboratory identification for *T. vaginalis* requires employing one of the following procedures: the Papanicolaou stain; enzyme-linked immunosorbent assay (ELISA), latex agglutination: direct fluorescent antibody (DFA); DNA probe; saline wet-mount; or an *in vitro* culture. These methods vary in their sensitivity, specificity, cost per test, and some may require special laboratory equipment (Table 1).

In the United States the saline wet-mount and an *in vitro* culture are the two most frequently employed laboratory tests. Although the wet-mount is an inexpensive and rapid test, its sensitivity may be 35 to 80%[12]. Three of the most critical factors effecting its sensitivity are: the experience of the microscopist in identifying *T. vaginalis* in clinical specimens, the density of Trichomonas present, and the transport time from patient to microscopy.

While the wet-mount is a more rapid test and may produce positive results within a shorter time period, *in vitro* culture remains the 'gold standard' for definitive identification of *T. vaginalis*[13].

Professor do Clinical Science do Laboratório de Ciências Biomédicas, Universidade Estadual de San Francisco – EUA

All of the commercial broth culture media differ in ther sensitivity; specificity; storage requirements; shelf-life; and technology time required to perform a test. Diamond's, Trichosel, and Hollander's are 3 of the media that have been available for culture. These tubed media have a variable shelf-life at 5°C. Before microscopy, transfer of an aliquot of the cultured specimen is required on to a glass coverslipped-slide.

INPOUCH™ TV TEST

The InPouch test introduced a new and unique concept for concomitant maintenance, transport, and detection of *T. vaginalis*. It consist, of a two chambered pouch which is fabricated of a clear plastic film for clarity in viewing that maintains a reduced Eh for increased growth, and eliminates media loss. A channel separates the chambers to facilitate media passing between them after inoculation. The InPouch™ test contains 4 ml of a selective medium inhibitory for both yeast and bacteria. The Figure 1 presents the two chambered pouch and a plastic viewer that is clamped over the bottom chamber for microscopy. Specimen evaluation may be completed within 2-3 minutes. The test has a shelf-line of one year when stored between 15-25°C.

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CLINICAL STUDIES WITH THE INPOUCH™ TV TEST

In the initial clinical study 102 wet-mount negative specimens were cultured in both the InPouch™ and Hollander's medium. The InPouch™ demonstrated 15 positive specimens, while 12 of the same patients were positive with Hollander's media[14].

An epidemiological study evaluated female patients in San Jose, Costa Rica, for trichomoniasis[5]. Their ages ranged between 18 to 70. Fifty-seven of the patients were from a STD clinic and 43 from two hospital populations. The patients were either asymptomatic, presented symptoms of a vaginal discharge, itching, or dyspareunia. All of the patients specimens were cultured with the InPouch™ and evaluated with a saline wet-mount. Only two of the 13 culture positive test were wet-mount positive. None of the InPouch™ negative tests were wet-mount positive. The prevalence rate for trichomoniasis was 19% in the STD population and 4,6% for the hospital group. Six patients who had been diagnosed by cytological smears were both wet-mount and culture negative. None of the 19% culture positive patients were wet mount positive.

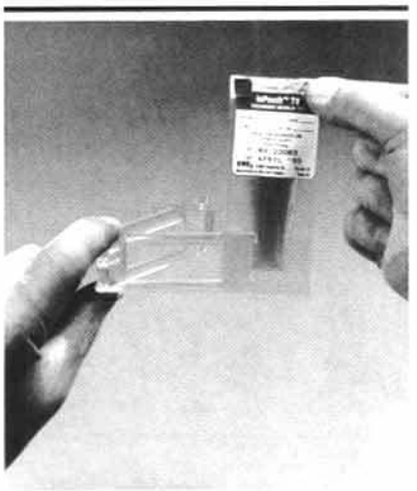

Figure 1

The prevalence of trichomoniasis was evaluated in an evening STD clinic comparing the InPouch™ to saline wet mount microscopy[15]. This study included 204 male patients who varied in their ethnic origin, general health, and ranged in age between 16 and 72 years. Appropriate diagnostic tests for other STDs were performed. Twenty-four of the 204 patients (12%) were culture positive for trichomoniasis, and only 3 of the 24 were wet mount positive. Both the youngest patient at 16 years and oldest at 72 were positive for trichomoniasis. These results confirmed the sensitivity of InPouch™ employing urine sediment.

The relative sensitivity of the InPouch™, modified Diamonds, and Trichosel was compared in supporting growth of clinical isolates at dilutions of approximately 2.0 x 10^4, 2.0 x 10^3, and 2.0 x 10^2/ml of motile *T. vaginalis*[16]. The data evaluated at 0.01 level of statistical analysis demonstrated better InPouch™ sensitivity at each dilution with a greater number of positive cultures. At 96 hours the total positives for each of the tests were: 112/129 for the InPouch™; 78/129 for modified Diamond's; and 74/129 for Trichosel.

The first self-administered vaginal swab test for trichomoniasis employed the InPouch™ and was performed in a rural population in Uganda[5]. All of the samples were

Table

Evaluation of laboratory diagnostic tests for Trichomonas vaginalis

Test	Sensitivity
Papanicolaou stain	Cytological smears are frequently inaccurate
ELISA	Only detects antibodies of the IgG and IgA class
Latex agglutination	Variability in sensitivity
DNA probe	Sensitivity of 90-99%
DFA staining	Sensitivity better than saline wet mount
Saline wet mount	Sensitivity variable from 40-70%
Culture-Hollander's	Sensitivity better than 80%
Culture-Trichosel	Sensitivity of 76.7% at 1.0 x 10^3 dilution*
Culture-Diamond's	Sensitivity of 76.7% at 1.0 x 10^3 dilution*
Culture-InPouch™	Sensitivity of 100% at 1.0 x 10^3 dilution*

*Genitourium Med. 73, *4. pp. 297-8, 1997.

collected in the homes of the participants. A total of 136 samples were available for microscopic evaluation and 47.1% of these were positive for *T. vaginalis*.

Another clinical study evaluated the validity of self-obtained vaginal specimens for trichomoniasis employing the InPouch™[7]. This study compared the self-administered vaginal swab specimen to a clinician collected specimen. A clinician-collected specimen was used for a wet-preparation examination. There was no significant difference between the self-administered positives tests (22, 84.6%) and the clinician-collected positives (23, 88.5%) specimens. Eighteen of the wet-mount tested were positive for a sensitivity of 68%.

TREATMENT FOR TRICHOMONIASIS

Treatment of trichomoniasis in the United States is restricted to metronidazole. The recommended regimen is oral metronidazole, either 2 mg in a single dose or 250 mg tid for 7 days. Treatment for the sex partner requires 250 mg oral metronidazole tid for 7 days[3]. Other derivatives of 5-nitroimidazole such as tinidazole, ornidazole, secnidazole, and nimorazole are infrequently prescribed[17]. When *T. vaginalis* resistance occurs, it is neither attributable to the failure of metronidazole absorption in the intestinal tract nor availability at the nidus of *Trichomonas* activity in the host[18]. A study determing the resistance of *T. vaginalis* employed a broth-dilution method of modified Diamond's medium. The results confirmed metronidazole resistance in some of the *T. vaginalis* isolates tested[19].

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The InPouch™ was used to evaluate *T. vaginalis* isolates from 16 symptomatic female patients[20]. Eleven of the patients responding to 250 mg tid of oral metronidazole were considered to have sensitive *Trichomonas*, whereas the 5 treatment failures were resistant. Both the minimum inhibitory concentration (MIC) and minimum lethal concetration (MLC) were determined for each isolate employing the InPouch™. The dilutions of metronidazole varied between 0.4 ug/ml and 50 ug/ml. *Trichomonas* sensitive to metronidazole demonstrated MLC's between 0.4 and 3.1 ug ml, whereas the resistant organisms (treatment failures) exhibited MLC's between 6.2 and 50 ug/ml. Statistically there was a significant difference between the clinically resistant and sensitive *Trichomonas* ($t=5.47$, $p<0.005$). This procedure using the InPouch™ offers a definitive method for defining treatment failures.

SUMMARY

Trichomoniasis is an important sexually transmitted disease worldwide. An appropriate test must be sensitive, specific, simple to use, offer an extended shelf-life, be cost-effective, and demonstrate an easy interpretation. The InPouch™ fulfills each of these criteria.

Key words: STD/Trichomoniasis, clinical significance, InPouch™ Test, diagnosis.

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