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| 13. ABSTRACT (Maximum 200)<br>Lower genital tract infections occur commonly among 17-25 year old women and pose a significant problem for military women especially on deployment. This project is to develop a rapid "self-test kit" for common, treatable cervical/vaginal and urinary tract infections. We have completed the developmental phase of the test kit and proceeded to test the performance in women with genital complaints that will collect and test their own urine and vaginal secretions. Additional modifications may be added as technology provides, however, in the present form the test kit offers sensitivity, specificity and predictive value that exceeds that available in standard clinical settings as well as resource poor environments. Specifically, this test exceeds the performance of clinical/wet mount evaluation and syndromic management schemes. A number of modifications were made and tested during the second year. We have thus far tested the kit in 234 women with genital complaints. The self-test kit results suggested appropriate treatment in the majority of cases. Specifically, 90% of women were targeted for curative therapy. We have also enrolled the first 30 women in the self-test phase and have very encouraging results with all women successfully able to obtain specimens and perform testing. The patient interpretation of dipstick results was equally successful with 100% agreement between patient and clinician interpretation of results for lactoferrin dipstick and 94% for the pH/amine test. Thus, we remain optimistic that this project will result in a self-test kit for use on deployment and/or in other resource poor environments. |                                                             |                                                                   |                                       |                |
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PRINCIPAL INVESTIGATOR: Daniel V. Lander, M.D.

CONTRACTING ORGANIZATION: Magee Women's Hospital  
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Patricia M. Brown

5/28/99

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Daniel V. Lander MD 9/28/98  
PI - Signature Date

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## Introduction

The primary goal of this proposal is to develop a "self-test kit" to be used by military women in the rapid diagnosis of the common, treatable cervical/vaginal and urinary tract infections. Testing is performed on self-collected vaginal (introital) swabs (Q tips) and a urine sample. The secondary goal is to confirm the effectiveness of treating these infections with currently available, effective, single dose, low toxicity agents that could be included in a "self-care kit" (self-test kit plus single dose treatment packs) or administered by medical personnel in the field. The specific technical objectives of this proposal are:

1. To adapt the vaginal lactoferrin test to a simple, easily readable dipstick test to identify infection with *Trichomonas vaginalis*, *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*;
2. To evaluate a vaginal amine and pH testing in a simple, easily readable, test for the diagnosis of bacterial vaginosis and *Trichomonas vaginalis*;
3. To combine the vaginal lactoferrin, amine/pH test and urine leukocyte esterase/nitrite dipstick into a simple to use and understand "self-test kit;"
4. To develop a simple and reliable algorithm for military women that combines symptomatology with rapid dipstick testing of vaginal fluid and urine which accurately predicts the presence of cervical/vaginal or urinary tract infections;
5. To test subjects' ability to select appropriate single dose treatment based on symptom/testing algorithm;
6. To demonstrate successful identification and eradication of infections predicted by "self-test kit", verified by "gold standard" diagnostic testing and treated with single dose, low toxicity antimicrobial agents;

Infections of the urogenital tract, particularly by sexually transmitted organisms, are a common and important health related problem to military women. These infections not only affect the mental and physical health of women, they may adversely affect the ability of military women to perform their duties. These conditions and symptoms may also cause embarrassment to women working and living in close quarters. Additionally, these conditions lead to decreased productivity and time off from the workplace for evaluation, diagnosis and treatment. All of these factors may significantly impact the ability and readiness of military women to perform their assigned tasks and duties. Furthermore, the adequately trained health care providers, laboratories and advanced technology required for rapid diagnosis and treatment of these conditions may not always be readily available to deployed military women especially while in remote areas or developing countries. Speculum examination requiring special tables, stirrups, directed lighting and other specialty equipment may not be easily accessible in many deployment situations.

Cervicitis, vaginitis and urinary tract infections occur in upward of 20 million women each year in the United States.<sup>1-4</sup> These infections occur most commonly in the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> decade of life. The prevalence of these infections is highest in the 17-25 year old age group particularly the STDs. Thus, these infections will commonly occur among women in the U.S. Armed Services by virtue of their age range alone. Additional considerations including socioeconomic background, increased frequency of sexual activity, numbers of partners and prevalence of STDs in their partner pool will enhance the risk. In one study, of 1,744 military men deployed aboard ship for six months to South America, West Africa and the Mediterranean, 49% reported prior sexual contact with a commercial sex-worker and 22% reported a history of a STD before deployment. During the subsequent six-month deployment, 42% reported sexual contact with a commercial sex-worker, 10% acquired a new STD and 10% reported inconsistent condom use.<sup>5</sup>

Recent preliminary reports from a survey of Army personnel indicate that 18% of women respondents report having had at least one STD over a 2 year period and this may be an underestimate especially if women with an STD history were less likely to respond to the survey.<sup>6</sup> In another study of 476 asymptomatic active duty army women presenting for routine pap smears, 39(8.2%) tested positive for chlamydia. This is a high rate of asymptomatic chlamydia infection. These statistics are further compounded by the facts that only about 50% of all unmarried military personnel report using a condom during last intercourse and women under the age of 25, the age group at highest risk for acquiring an STD, account for two-thirds of the enlisted women that are pregnant at any given time.

There is additional accumulating evidence that other, less obvious, factors may influence the high rate of STDs among military women. Statistics show that 31% of women on active duty in the U.S. Army smoke cigarettes and 17% are heavy smokers.<sup>6</sup> This is significantly higher than the number of smokers in the general population.<sup>7</sup> Several recent studies have demonstrated that smoking is a significant risk factor in the acquisition of numerous STDs including *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and pelvic inflammatory disease and its sequelae.<sup>8-10</sup>

Delayed diagnosis and treatment of STDs and urinary tract infections may well lead to significant, even life threatening long-term sequelae. Serious renal infections, permanent infertility and life-threatening ectopic pregnancies are all recognized and well documented sequelae of lower urogenital tract infections in women.<sup>1-4</sup> Recent studies also indicate that the presence of these cervical/vaginal STDs significantly increase the risk of HIV acquisition.<sup>11,12</sup>

The most common forms of lower urogenital tract infections in women are cervical and vaginal infections (cervicitis and vaginitis) and bladder or urethral infections (cystitis or urethritis). The sexually transmitted organisms *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are responsible for most cases of cervicitis while *Trichomonas vaginalis*, *Candida* species, and bacterial vaginosis account for nearly all cases of infectious vaginitis/vaginosis.<sup>2-4,13,14</sup>



Chlamydial infections are the most common bacterial STDs in the developed world. There are an estimated 4 million chlamydial infections annually in the United States alone with over 2 million occurring in women.<sup>2,15,16</sup> Over million cases of gonorrhea occur in the United States each year.<sup>2</sup> Presenting complaints include vaginal discharge, dysuria and abnormal uterine bleeding. Both gonorrhea and chlamydia can and often do present with minimal or very subtle symptoms necessitating screening and/or testing for minimal symptomatology in the "at risk" populations. Sequelae of these infections include pelvic inflammatory disease, ectopic pregnancy, permanent infertility and chronic, often debilitating pelvic pain.<sup>2,16</sup>

Infectious vaginitis and vaginosis account for some 8-10 million outpatient visits a year in the United States.<sup>17</sup> The three conditions accounting for the vast majority of these cases are trichomonas vaginitis, candida vaginitis and bacterial vaginosis.

Vaginal yeast infections commonly occur in women. It has been estimated that 75% of women will have at least one episode of yeast vulvovaginitis, with 40-45% having two or more episodes.<sup>18</sup> The predominant organism causing these infections is *Candida albicans*, and occasionally non-albicans candidiasis species (*Candida tropicalis*, *Candida (Torulopsis) glabrata* or other *Candida* species). The most common presenting complaint is vaginal and/or vulvar pruritis with or without vaginal discharge, however some 30% of women with yeast infections may present with discharge alone.<sup>19</sup>

An estimated 3 million cases of trichomoniasis occur in the United States annually. This infectious form of vaginitis is caused by *Trichomonas vaginalis*, a sexually transmitted motile protozoan.<sup>14</sup> It accounts for approximately 10-15% of all cases of clinically evident vaginal infections. Infection with this organism is most often characterized by a copious, foul smelling discharge but the clinical presentation can be quite variable including a significant number of women without specific vaginal complaints.

Bacterial vaginosis (formerly known as Gardnerella vaginitis, Haemophilis vaginitis and nonspecific vaginitis) is the most common cause of malodorous vaginal discharge in women.<sup>18</sup> It has been estimated to be the etiology in as many as 45% of women with vaginitis/vaginosis.<sup>14</sup> Bacterial vaginosis(BV) is caused by a shift in the vaginal flora from the normal high concentrations of hydrogen peroxide-producing lactobacilli to a mixed flora consisting of high concentration of anaerobic organisms, *Gardnerella vaginalis* and *Mycoplasma hominis*.<sup>20</sup> This shift in flora is associated with a homogenous, white vaginal discharge, elevated pH (>4.5), the production of amines and the presence of clue cells.

Urinary tract infections, especially bladder infections (cystitis), are the most common bacterial infection in adult women accounting for over 7 million office visits per year in the United States.<sup>2-4</sup> Lower urinary tract infections may involve the urethra or the bladder. The usual presentation is internal dysuria (not external dysuria which is more associated with vulvar or vaginal infection). Acute urethritis is most often due to

*Chlamydia trachomatis* or *Neisseria gonorrhoeae*. The vast majority of lower urinary tract infections in women are cystitis rather than urethritis. Acute, uncomplicated cystitis in young women is caused by *Escherichia coli* 80-90% of the time. The remaining 10-20% are caused by a variety of other organisms usually Gram negative bacteria including *Klebsiella*, *Proteus*, *Enterobacter* and *Pseudomonas* spp. and less commonly the Gram positive *Staphylococcus saprophyticus*, group B streptococci and enterococci. Pyelonephritis generally a sequelae of cystitis, is recognizable by fever and lower back pain in addition to dysuria. This condition can require hospitalization and even lead to sepsis.

In summary, urogenital infections are common among military women as in the civilian population, but the nature of deployment may complicate the diagnosis and treatment of these infections. Rapid diagnostic test that could be self-administered in the field without the need for special medical facilities would be logistically and economically advantageous. Single dose treatments are now available and within the standard of care. The 1998 Centers for Disease Control guidelines for the Treatment of Sexually Transmitted Diseases (STDs) were recently released and provide additional single dose treatment regimens for these infections and may further facilitate treatment success against the urogenital infections targeted by this proposal.<sup>18</sup>

## **Body**

The first year of this project was divided into two phases in the original proposal. Phase I, aimed primarily at development of the self-test kit and the data form and Phase II, aimed at collecting specimens from 100 women for evaluation and refinement of the self-test kit. Each "Statement of Work" task listed in the original proposal is printed in italics and addressed separately below.

The nature of this study required that Phase I and II be carried out simultaneously to optimize the available time in accomplishing our stated goals. The overall goal of Phase I and II was to develop our proposed self-test kit, and to compare its sensitivity and specificity to gold standard testing. The intent is to develop a self-test kit for treatable, lower genital tract infections that can direct women to treatment regimens that will result in cure. The primary goal of Phase II was to assess and modify the self-test kit to optimize diagnostic accuracy and treatment efficacy before recruiting more women to test the kit (Phase III).

As outlined in the "Statement of Work" in our original proposal and in accordance with the specific objectives of this project we are now in Phase III and recruiting women for evaluation of the self-test kit. The progress for each task in the "Statement of Work" is described below.

### Phase I Tasks:

1. *Determine optimal test format for the Lactoferrin dipstick including establishment of cutoff and appropriate threshold for sensitivity.*

Status: Completed

Lactoferrin dipsticks were provided by TechLab, Blacksburg, VA and have highly correlated with enzyme-linked immunosorbant assay (ELISA) values. We have now studied over 200 samples to determine the cutoff value (400ng/ml) that will optimize sensitivity and specificity for identification of STDs. We spent significant time this year optimizing this test by collecting specimens and performing lactoferrin testing in an additional 134 women (total 234). Studies of receiver-operator curves (ROC) indicated that the optimal cutoff value for distinguishing STDs from BV, yeast, UTI or no infection was 400ng/ml. We also determined that lactoferrin dipsticks read at 90 seconds will reflect as positive all values of 400 and greater.

Lactoferrin results of  $\geq 400$ ng/ml have been studied on 234 women and this data for analyzed sensitivity and specificity in predicting the presence of one or more of the three targeted STDs (*Trichomonas vaginalis*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*). These results are described below under Phase II, Task 2.

2. *Combine the vaginal lactoferrin, amine/pH test and urine leukocyte esterase/nitrite dipstick into a simple to use and understand self test kit.*

Status: Completed.

This work was completed during Years 1 with the exception of determining the optimal cutoff value for the dipstick (400ng/ml). The lactoferrin dipstick is able to detect levels above 400 ng/ml with a high degree of accuracy and is included in the self-test kit. As we stated in last years progress report, defensins (another soluble product of polymorphonuclear leukocytes recruited to the lower female genital tract in women infected with *Trichomonas vaginalis*, *Chlamydia trachomatis* or *Neisseria gonorrhoeae*) were studied extensively over the past year for the purpose of improving detection of the STDs. We are now developing a dipstick capable of detecting optimal cutoff level of defensins in vaginal fluid (1100 ng/ml). The defensin data is shown below in Phase II, Task 2. If the defensin dipstick is developed and improves the sensitivity and specificity of the test kit then it will be included.

The pH/Amine test (FemExam card from Litmus Concepts, Calif., USA) is now FDA approved for the diagnosis of bacterial vaginosis and as reported in last year's report is included in the test kit. New cards have been produced to rectify the quality control issue discussed in last year's progress report. The new card (with the correct barrier thickness

to avoid excess false positive results) has been tested in 184 women since the problem was discovered last year (see results in Phase II, Task 2).

We are currently collaborating with Litmus Concepts in the development of a yeast detection card able to identify the presence of Candida species which would significantly enhance the sensitivity and specificity of our test kit. Preliminary results on this new rapid test card were encouraging and a prototype card was developed for preliminary testing. Thirty patients were included in the preliminary trial and the prototype proved to be user-unfriendly, being cumbersome even for the clinician to interpret. Dr. Lawrence of Litmus Concepts is now reconfiguring the test to a more user-friendly format since the in vitro data on the Candidal proteins that serve as the basis for the test are very encouraging.

The leukocyte-esterase dipstick has been commercially available for some time now from two different companies the Bayer Multistix and Boehringer Mannheim Chemstrip. We have compared these two tests for detection of leukocytes and nitrites and found that the Chemstrip to have lower numbers of false positives for predicting urinary tract infections. Although neither test was very highly sensitive in this population of women. Since our prevalence of urinary tract infection was so low in women with genital complaints this is not a fair assessment of the test's capacity to identify urinary tract infection. The Chemstrip dipstick has been included in the test kit and with larger numbers of women with urinary tract infection it will be possible to calculate the predictive value of this test in women with genital complaints.

3. *Prepare IRB application and create patient consent forms for IRB approval and patient enrollment.*

Status: Completed.

The IRB application has been prepared and approved at our institution and has been reviewed and approved by The Surgeon General's Human Subject Review Board. This board will require, and be provided with, ongoing information to maintain our approved status.

4. *Establish data collection instruments for patient demographics and relevant specimen information.*

Status: Completed.

Detailed data collection instruments have been created and tested in the first 100 patients. These forms were included in the appendix. They have been further tested in another 132 patients (total=232) and require no additional modification except as will be needed to add new fields for new tests (i.e. defensin dipstick, yeast card etc)

5. *Develop a database for this information.*

Status: Completed.

An extensive database has been developed and is currently being used in our evaluations. The database contains 349 variables. Their variables include information of demographic and behavioral characteristics, symptoms results of physical examination, and laboratory testing. The data are written onto scannable forms, scanned, verified, labeled and coded, and imported to statistical package (SPSS for Windows) for descriptive analysis.

6. *Develop patient instruction sheets for sample collection and performance of the rapid test kit.*

Status: Completed.

Patient instruction sheets have been created for the collection of vaginal swabs. These sheets have been tested in over 300 patients collecting vaginal swabs for Chlamydia PCR testing and have assisted women in self-collecting specimens that yielded results similar to those obtained on simultaneous clinician-collected samples. These instruction sheets, provided in last years progress report, are successfully being used in Phase III of this study.

Phase II Tasks:

Status: Completed.

1. *Begin recruitment and patient sampling for the self-test kit development phase.*

Women presenting to the study sites with complaints of dysuria or vaginal discharge, itching, burning or irritation, between the ages of 18-40 were recruited as study participants. The exclusion criteria for the study were the use of antibiotics or other treatment for urogenital infections in the past two weeks and age outside the specified age range. During the clinic visit a complete medical history was taken. Upon completion, a pelvic exam was performed on each woman. The clinician collected three simultaneous vaginal (introital) swabs and performed the pH/amine test card, the lactoferrin dipstick, the leukocyte-nitrite dipstick, a wet mount form microscopic examination and recorded the results of each. A clean, unlubricated speculum was be placed into the vagina, and 6 sterile dacron swabs were used to obtain vaginal material from the posterior vaginal fornix. The following tests were performed to evaluate the self-test results and to determine the exact infectious agents present: Swab #1: Lactoferrin and defensin dipstick and ELISA, Swab #2: PCR for *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis*, Swab #3: pH/amine test, bacterial vaginosis Gram stain, Swab #4:

wet mount, *Trichomonas* culture, Swab #5: Yeast culture, and Swab #6: *N. gonorrhoeae* culture (cervical). A total of 234 women were recruited for the test development. Ongoing improvements are now tested on samples collected during Phase III recruitment.

The demographics of enrolled women are displayed in Table 1. All women recruited had at least one urogenital complaint including vaginal discharge in 77%, pruritus in 42%, abnormal vaginal odor in 41% and burning or pain in 38%. The results of gold standard testing are shown in Table 2. Among the first 234 patients, gold standard testing indicated that 96(41%) women had bacterial vaginosis (BV), of which, 23 women had a concurrent sexually transmitted disease (*N. gonorrhoeae*, *C. trachomatis*, or *T. vaginalis*), 67 women had BV alone and 6 had BV and yeast. There were an additional 24 women with one of the STDs that did not have BV. Overall, 47(20%) of women had one or more of the STDs, specifically 27 had *T. vaginalis*, 16 had *C. trachomatis*, and 6 had *N. gonorrhoeae*. Yeast was cultured from 21 women with pruritus and no other infection. In 90(39%) women studied, none of the above pathogens were identified despite symptoms. In the remaining 3 women, missing data prevented definitive classification of their infection status. These results are similar to our overall population of women with genital complaints. In addition to the organisms included in gold standard testing, visual and bimanual examinations did not reveal evidence of any other vaginal disease such as genital herpes or human papilloma virus that might account for their vaginal complaints.

**Table 1. Demographics of Women Enrolled**

| N=233             |     | Age Mean = 25.9 yr. |     | S.D. = 5.9 yr.  |          |
|-------------------|-----|---------------------|-----|-----------------|----------|
| Race              |     | Marital Status      |     | Years Education |          |
| African-American  | 57% | Single              | 73% | Mean 13.12 yr.  |          |
| European-American | 40% | Married/Cohabiting  | 17% | S.D.            | 2.06 yr. |
| Multiethnic       | 2%  | Separated/Divorce   | 10% |                 |          |
| Other             | 1%  |                     |     |                 |          |
| Employment Status |     | Tobacco Use         |     | Alcohol Use     |          |
| Employed          | 67% | Any smoke           | 61% | Any Use         | 68%      |
| Full-Time         | 39% | Heavy Smokers       |     | Heavy Use       |          |
| Part-Time         | 28% | ≥1ppd               | 17% | (Daily)         | 4%       |
| Unemployed        | 33% | Non-Smokers         | 39% | None            | 32%      |
| Douching Habits   |     |                     |     |                 |          |
| Ever              | 72% |                     |     |                 |          |
| Never             | 28% |                     |     |                 |          |

**Table 2. Gold Standard Testing**

| <u>DIAGNOSIS</u>    | <u>NUMBER</u>  |
|---------------------|----------------|
| STD +/- BV          | 47(20%)        |
| STD, No BV -----    | 24             |
| BV +/- YEAST        | 73(31%)        |
| BV Only -----       | 67             |
| YEAST Only          | 21 (9%)        |
| No Organism (neg.)  | 90(39%)        |
| <u>Missing data</u> | <u>3(1.3%)</u> |
| <b>TOTAL</b>        | <b>234</b>     |

2. *Analyze test kit performance compared with "gold standard test results and evaluate the kit's accuracy in predicting the presence of cervical/vaginal or urinary tract infections.*

Status: Completed

Lactoferrin Testing for STDs

Lactoferrin levels were determined using the Leuko-ELISA Kit (TechLab, Blacksburg, VA.). The vaginal sample is diluted 1:10 in kit diluent and a 100ul aliquot is added to an antibody coated 96 well microtiter plate. The plates are incubated at 37°C for 10 min., washed, conjugate is added and the plate is incubated at 37°C for 10 min. The wash step is repeated and 1 drop of substrate is added, the plate is incubated at room temperature for 5 minutes. Following the substrate incubation, 1 drop of color intensifier is added and the plate is read at 450 nm. A positive test result is an absorbance reading >0.400. The lactoferrin ELISA was performed in Dr. Phillip Heine's laboratory.

Lactoferrin levels were determined on clinician-collected vaginal swabs obtained from 202 women. The data on these women was analyzed using several different cutoff values to optimize sensitivity and specificity. The optimal cutoff value was found to be 400ng/ml. This value was determined using receiver-operator curves to identify optimal levels of sensitivity and specificity. The sensitivity/specificity and predictive value of the lactoferrin test using this 400ng/ml cutoff are shown in Table 3.

**Table 3. Sensitivity, specificity and predictive value of lactoferrin test.**

|                            | STD (TV, CT or GC) |     |     |
|----------------------------|--------------------|-----|-----|
|                            | (+)                | (-) |     |
| Lactoferrin<br>> 400 mg/mL | 32                 | 73  | 105 |
| Lactoferrin<br>≤ 400 mg/mL | 8                  | 89  | 97  |
|                            | 40                 | 162 | 202 |
| Sensitivity = 80%          |                    |     |     |
| Specificity = 55%          |                    |     |     |
| PPV = 31%                  |                    |     |     |
| NPV = 92%                  |                    |     |     |

The fact that 80% of women with an STD had a positive lactoferrin test (sensitivity = 80%) will identify more than three-fourths of infected patients prior to making treatment decisions. While this does not provide the sensitivity of gold standard testing such as PCR, treatment decisions for these three STDs are now made on the basis of wet mount (*T. vaginalis*) which has an estimated sensitivity of 50% and risk assessment and symptoms (*C. trachomatis*, *N. gonorrhoeae*) which have a sensitivity of 42%. (20b). The specificity of lactoferrin was 54% and the positive predictive value 28%. Thus, our data (sens=80%/spec=55%/ppv=31%/npv 92% compares favorably with recently published data on using risk assessment, symptoms and signs as predictors of STDs. In that study, Ryan and colleagues showed a sensitivity of 42%, a specificity of 74% and a positive predictive value of 34% in women with vaginal discharge for predicting the presence of *C. trachomatis* or *N. gonorrhoeae* using risk assessment and symptoms only<sup>23</sup>. The addition of speculum and bimanual examination and microscopy improved these results to a sensitivity of 52%, a specificity of 66% and a positive predictive value of 33%. Since our self test kit involves no expertise, speculum or examination and is more sensitive and only slightly less specific, there is a great motivation to continue with our test development especially if improved sensitivity and specificity is possible. In this direction, we began last year and continued this year to evaluate other soluble white blood cell (WBC) products that can be measured in vaginal fluid and are potential candidates for colorimetric card or dipstick testing. We have begun ELISA testing for defensins, WBC products that have been highly associated with infection where there is a significant local neutrophil response. The mean defensin level measured from vaginal swabs in 26 women with an STD in our Phase I/II patients was 17,682 ng/ml compared with the 8,899 ng/ml mean value among 28 women without identifiable pathogens by gold standard tests. We have continued to evaluate the remaining women to determine sensitivity/specificity of defensin in predicting the presence of TV, CT and/or NG in vaginal swabs. We have looked at various cutpoints to obtain the point at which specificity is optimal.



Table 4 shows sensitivity/specificity data on 201 women tested with both lactoferrin and defensin with a cutoff value of 400ng/ml for lactoferrin and 1,100 ng/ml for defensin.

**Table 4. Sensitivity, specificity and predictive value of combined lactoferrin and defensin tests with and without an STD.**

|                                             | STD (TV, CT or GC) |     |     |
|---------------------------------------------|--------------------|-----|-----|
|                                             | (+)                | (-) |     |
| Lactoferrin >400<br>and<br>Defensin > 1,100 | 28                 | 49  | 77  |
| One or both below<br>cutpoints              | 12                 | 112 | 124 |
|                                             | 40                 | 161 | 201 |
| Sensitivity = 70%                           |                    |     |     |
| Specificity = 70%                           |                    |     |     |
| PPV = 36%                                   |                    |     |     |
| NPV = 90%                                   |                    |     |     |

#### PH/Amine Testing for BV

The rapid diagnosis of BV is based on pH and volatile amines (trimethylamines) using the FDA-approved (as of February 1997) FemExam card (Litmus Concepts, Calif., USA). In collaboration with Dr. Paul Lawrence at Litmus Concepts we originally obtained 100 test cards from a single production lot. Our ongoing quality assurance program lead to review of the first 54 tests which indicated a sensitivity of 91% but a specificity of only 46%. This was of particular concern to us since we were involved along with 4 other centers in testing of this card in over 600 women prior to FDA approval which showed this device to be highly specific as well as sensitive in the diagnosis of BV. We changed to using cards from a separate lot for further testing and Dr. Lawrence and colleagues at Litmus Concepts began investigations into the possible cause of decreased specificity. Meanwhile we tested an additional 45 patients (one specimen was mishandled) with cards from a separate lot and although specificity improved to 65% this was well below the demonstrated capability of the card. After intense investigation at Litmus Concepts it was discovered that a whole block of cards had been produced with a barrier layer thinner than the original acceptable limits, leading to an excess of secretions being allowed to contact the test envelope. This causes an inordinate number of false positive tests that would explain our lack of specificity. The overall sensitivity and specificity in the first 99 patients was 85% and 54% respectively, despite the manufacturing defect. The quality assurance program at Litmus Concepts is

now able to detect any variations in barrier thickness and should prevent further variations in sensitivity and specificity. During the past year with further modifications to ensure maintenance of barrier thickness we studied 180 patients with the pH/amine card and found a sensitivity of 84% and a specificity of 66%. (Table 5)

**Table 5. BV diagnosis by Gram stain**

|     | BV(score >7) | Normal/intermed.(<7) |     |
|-----|--------------|----------------------|-----|
| Pos | 65           | 35                   | 100 |
| Neg | 12           | 68                   | 80  |
|     | 77           | 103                  | 180 |

Sensitivity  $65/77 = 84\%$   
 Specificity  $68/103 = 66\%$   
 PPV  $65/100 = 65\%$   
 NPV  $68/80 = 73\%$

#### Yeast Diagnosis

Our original algorithm depended on women having symptoms of pruritus or burning in the absence of positive lactoferrin and pH/amine testing. Yeast was identified by culture in 53(23%) of women in this study, however, only 29(55%) had symptoms of pruritus. Among the 29 women with yeast infection diagnosed by pruritus and positive yeast culture 22/29(76%) would have been treated appropriately because all other self test results were negative in a woman with pruritus. This represents a significant improvement in the area of yeast infection identification and appropriate treatment since last years report. In our collaboration with Dr. Paul Lawrence at Litmus Concepts we are continuing to work on developing an accurate Yeast Card to detect vaginal candida and we are continuing ongoing testing of prototype cards for this purpose. The first prototype was tested this year in 30 women but suboptimal packaging made the test somewhat confusing and not particularly user-friendly for a self-test application. The Candida protein moieties used in the test performed well in *in vitro* studies and will remain the basis for the card development for yeast identification in vaginal swabs.

**Table 6. Treatment Algorithm**

| pH/Amine | Lactoferrin | Leukocyte Esterase/Nitrite | Proposed Treatment                                        |
|----------|-------------|----------------------------|-----------------------------------------------------------|
| +        | +           | +                          | Azithromycin, Ciprofloxacin<br>Metronidazole & Fleroxacin |
| +        | +           | -                          | Azithromycin, Ciprofloxacin<br>& Metronidazole            |
| +        | -           | -                          | Metronidazole                                             |
| +        | -           | +                          | Metronidazole & Fleroxacin                                |
| -        | +           | +                          | Azithromycin, Ciprofloxacin<br>& Fleroxacin               |
| -        | -           | +                          | Fleroxacin                                                |
| -        | +           | -                          | Azithromycin, Metronidazole<br>& Ciprofloxacin            |
| -        | -           | -                          | If pruritus treat with Fluconazole                        |

Urinary Tract Infections by Leukocyte /Esterase Testing

Urinary tract infections were detected by culture in 27/220 (12%) of women.

Leukocyte/esterase/nitrites dipsticks identified 11/27(40%) of the women with positive cultures.

Since only 2 women had a positive urine culture and nothing else, the majority of positive urine cultures were in women with other infections and it was not clear whether they had symptomatic urinary tract infections or asymptomatic bacteriuria and symptoms from their cervical/vaginal infection. In many cases treatment of their cervical/vaginal infection would also provide efficacy for urinary tract infection. We will be reevaluating the data on an ongoing basis to confirm therapeutic response in all cases.

Overall Assessment of Self Test Kit

There were 58/234(25%) women presenting with urogenital tract symptoms that were found to have all negative testing by gold standard tests. This is not unexpected and is consistent with our outpatient clinics and many other populations of symptomatic women. In the typical clinical setting these women are treated empirically, based on symptoms until the results of cultures or other type of diagnostic testing become

available. Our rapid tests selected for the self test kit correctly ruled out infection in 33(57%) of these women obviating the need for unnecessary antimicrobial therapy.

Among 234 patients tested, 47 had one or more of the STDs, the most prevalent of which was TV (27/47). Concurrent BV was identified in 23 women with an STD and our treatment algorithm (see below) for BV is metronidazole 2g which is also the treatment for TV. Thus, in many cases, women with TV would be cured because of their BV treatment even when their self-test doesn't identify TV. This may further enhance the ability of self-test results to guide selection of curative single dose treatment. Overall, of the 180 women tested with self test kit 162(90%) would have been directed by the self-test kit to take an antimicrobial agent able to affect a cure in those women with a treatable infection.

3. *Analysis of patients' ability to select appropriate single dose treatment based on symptom/testing algorithm.*

Status: Studies ongoing.

We have analyzed data on the 34 women who collected their own specimens and performed the self-test kit on themselves. They were able to perform and accurately read the lactoferrin dipstick test results in all cases 34/34(100%) which was extremely encouraging in this regard. In the case of the pH/amine card test, these same patients 32/34(94%) women were able to perform the test and correctly interpret the test results. Antibiotic selection is based on correct interpretation of the test results which overall was very high and thus it is expected that antibiotic selection accuracy will be equally high. As testing continues in Phase III and antibiotic regimens finalized, patient selection of antibiotics will be assessed directly.

3. *Make any and all modifications to the test kit based on findings from the developmental phase data and make a final form of the kit.*

Status: Completed but subject to ongoing modification.

The test kit currently uses the lactoferrin dipstick, the pH/amine card and the leukocyte esterase/nitrite dipstick and clinical symptoms to determine treatment selection. Defensin dipstick will be developed if defensin testing improves overall sensitivity and specificity testing as an increased number of women's data are analyzed. Yeast card will be added when test development reaches an acceptable level of accuracy.

4. *Refine and finalize instruction sheets as needed to improve the efficiency and scope of the data collection process.*

Status: Completed.

Interview forms and instructions are reviewed on an ongoing basis and modifications will be made as is deemed appropriate. As patients begin to use self-test kit and interpret results this will become a very important task. As noted above this tool has been developed and used very successfully in the first 34 women tested.

5. *Revise and finalize data collection sheets as needed to improve the efficiency and scope of the data collection process.*

Status: Completed.

The data collection sheets are also reviewed on an ongoing basis to insure the validity and accuracy of collected and entered data. We have to date entered complete data on 234 women enrolled during Phase I, II and III of this project.

### Phase III Tasks

1. *Recruitment and enrollment of patients into the study.*

Status: Ongoing.

We have begun recruitment of women to self-collect specimens, perform and interpret the self test kit results and to choose therapy. Thus, far we enrolled 34 women to begin evaluating this phase of the study and the women have shown a remarkable ability to collect specimens, perform the test and interpret results as described above in Phase II, Task 3. We are actively enrolling women at a rate of 8-10 per week in order to meet goal of 900 women by month 43 of the study.

2. *Sample collection and patient use of the self-test kit.*

Status: Ongoing.

As described above in Task 1, patients are being enrolled and patients are successfully able to collect the samples and perform and interpret the test. In this group of women performing their own self-test we noted an improved sensitivity to 86%, specificity of 76%, ppv of 33% and npv 93% by reading dipstick at 30 seconds rather than 90 seconds.

This is likely due to the fact that most true positive values of lactoferrin are well above 400ng/ml and thus turn positive in the first 30 seconds.

3. *Continuous monitoring of patient treatment selection based on symptom/testing algorithm.*

Status: Ongoing.

Patient monitoring of treatment selection is an ongoing part of patient enrollment.

4. *Data collection and entry into the patient database.*

Status: Ongoing.

All data is collected and entered into the database on an ongoing basis.

5. *Laboratory testing and reporting of all patient samples.*

Status: Ongoing.

As with the other tasks in Phase III, this is being done in an ongoing fashion as the patient are enrolled and tested according to protocol.

6. *Respond to all progress inquiries.*

Status: Ongoing.

Responses to all progress inquiries are prepared expeditiously as requested.

## **Conclusions**

The first two years of this project are completed with significant progress being made in developing a rapid self-test kit for symptomatic, treatable lower genital tract infections in women. A number of problems were encountered as described above, including suboptimal specificity of the lactoferrin and pH/amine tests. Troubleshooting and modifications have been or are being made to address each of these problems. We have begun Phase III studies with the kit although minor modifications or additions will be included as they become available (i.e. Yeast Card). We have had no problem recruiting patients for this study and therefore anticipate meeting our recruitment goals by Month 43 as indicated in the original statement of work despite additional time being spent optimizing our self-test kits.

It is notable that despite the difficulties outlined above; the self-test kit results would have directed women to appropriate treatment in the vast majority of cases. Specifically, 90% of women with BV alone, 84% of women with an STD and 87% of women with BV and/or an STD would have been directed to appropriate therapy based on lactoferrin and pH/amine testing. Planned improvements in the sensitivity/specificity of these tests will significantly enhance these results. Overall, including all 99 women with self test results, 63% of women would have received the appropriate treatment decision. If 90% of women with yeast had been identifiable using a yeast card test, then 87% of women with disease by gold standard testing would have been directed to take appropriate therapy. This number may well exceed the number treated appropriately in fully equipped clinical settings. Thus, we remain optimistic that a successful self-test kit can be developed for women with symptomatic urogenital infections.

We are currently preparing 3 separate manuscripts for publication, the first is on the first 200 women tested for lactoferrin and defensin as a diagnostic test for STDs. The second manuscript is the initial evaluation of our test kit in 234 women with genital complaints. The third manuscript is a comparison of 3 methods of determining treatment choice in women with genital complaints. The three methods are: 1) Clinical symptom/risk factor assessment; 2) Clinical symptom/sign/wet mount findings; and 3) Our test kit; upon completion these manuscripts will be submitted for DOD approval prior to submission for publication.

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Office of Research Administration  
Institutional Review Board

W. Allen Hogge, MD  
Chair

September 22, 1998

Erin Ragan, MS  
Coordinator

412-641-1120  
Fax 412-641-1385

Daniel Landers, M.D.  
Magee-Womens Hospital  
300 Halket Street  
Pittsburgh, PA 15213

RE: Self-Test Kit: Rapid Diagnosis of Urogenital Infections in Military Women.  
[MWH-95-129]

Dear Dr. Landers:

On September 21, 1998, the Institutional Review Board of Magee-Womens Hospital approved your progress report for the above-listed protocol.

Current approval date: 9/21/98  
Next renewal date: 9/21/99

Please be advised that this protocol has a one year approval period. This approval interval may have changed from your original approval due to modifications in the IRB guidelines concerning risk level definitions and corresponding renewal requirements. In addition, IRB policy has changed so that a new approval date is given at each renewal, versus having the date revert to the month and date of the original approval.

By the above renewal date, you must submit a progress report to the committee detailing the number of patients studied, any results and side effects occurring during the study, and a request for continuation, or for closure. You must report any proposed changes in your protocol to the committee prior to their implementation. Any serious or life-threatening complications of the study must be reported to me or my designate by telephone within twenty-four hours of their occurrence. All adverse consequences must be reported in writing to the committee within 14 days of their occurrence. If your study utilizes a consent document, please furnish a copy of the consent to each patient, place one copy in your files, and in Medical Records, if appropriate. The consent document must have the current approval date in the right upper corner. Copies of your research records should be maintained for a minimum of three years after your study is completed.

Sincerely,

  
W. Allen Hogge, M.D.  
Chair, Institutional Review Board  
(DHHS Assurance # M-1399, 01)

WAH/tmm



DEPARTMENT OF THE ARMY  
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND  
504 SCOTT STREET  
FORT DETRICK, MARYLAND 21702-5012

REPLY TO  
ATTENTION OF:

MCMR-RMI-S (70-1y)

23 Aug 01

MEMORANDUM FOR Administrator, Defense Technical Information  
Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir,  
VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to the technical reports listed at enclosure. Request the limited distribution statement for these reports be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

  
PHYLLIS M. RINEHART  
Deputy Chief of Staff for  
Information Management

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