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in Military Women

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13. ABSTRACT (Maximum 200 Words) 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 tested the performance in 486 women with genital complaints. We have also evaluated women's ability to self test and evaluate their own results in 289 subjects. Additional modifications have been made to correct suboptimal sensitivity, specificity and predictive value. This test exceeded the performance of clinical/wet mount evaluation and syndromic management schemes. A number of additional modifications were made and tested during this year based on sensitivity/specificity and quality assurance testing. Among the 289 women in the self-test phase, all women were successfully able to obtain specimens and perform testing. The patient interpretation of dipstick results was equally successful with 94% agreement between patient and clinician interpretation of results for lactoferrin dipstick and 80% for the pH/amine test. The self-test kit results suggested appropriate treatment in 80% of the cases. 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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Table of Contents

Cover.....	
SF 298.....	Page 2
Table of Contents.....	Page 3
Introduction.....	Page 4
Body.....	Page 7
Key Research Accomplishments.....	Page 21
Reportable Outcomes.....	Page 21
Conclusions.....	Page 22
References.....	Page 23
Appendices.....	Page 25

Introduction

The primary goal of this proposal was 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 was performed on self-collected vaginal (introital) swabs (Q tips) and a urine sample. The secondary goal was 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 were:

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.¹⁻⁴ These infections occur most commonly in the 2nd, 3rd and 4th 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.⁵

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.⁶ 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.⁶ This is significantly higher than the number of smokers in the general population.⁷ 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.⁸⁻¹⁰

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.¹⁻⁴ Recent studies also indicate that the presence of these cervical/vaginal STDs significantly increase the risk of HIV acquisition.^{11,12}

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.^{2-4,13,14}

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.^{2,15,16} Over a million cases of gonorrhea occur in the United States each year.² 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.^{2,16}

Infectious vaginitis and vaginosis account for some 8-10 million outpatient visits a year in the United States.¹⁷ 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.¹⁸ 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.¹⁹

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.¹⁴ 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.¹⁸ It has been estimated to be the etiology in as many as 45% of women with vaginitis/vaginosis.¹⁴ 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*.²⁰ 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.²⁻⁴ 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.¹⁸

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 was to develop a self-test kit for treatable, lower genital tract infections that could direct women to treatment regimens that would 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 this past year we completed recruitment of women into Phase III and performed the data analysis. The final report 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 (Blacksburgh, VA) and have highly correlated with enzyme-linked immunosorbant assay (ELISA) values. We studied over 200 samples to determine the cutoff value (400ng/ml) to optimize sensitivity and specificity for identification of STDs. We spent significant time optimizing this test by collecting specimens and performing lactoferrin testing in an additional 134 women (total 334). 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 would reflect as positive all values of 400 and greater.

Lactoferrin dipstick testing was studied in 703 women and this data analyzed for 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 was included in the self-test kit. As we stated in previous progress reports, 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*) was studied extensively for the purpose of improving detection of the STDs. We did not develop a dipstick capable of detecting optimal cutoff level of defensins in vaginal fluid because the values of defensins were so widely distributed that not enough correlation with STDs could be established to warrant development of a rapid test at this time.

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 previous progress reports was included in the test kit. New cards were produced to rectify a quality control issue discussed in year 3's progress report. The new card including a foil-wrapped swab was employed during the last two years and that data as well as the combined data is presented in Phase II, Task 2 results.

Our collaboration with Litmus Concepts included 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. Thus far this card has not been developed beyond a prototype because of difficulties with sensitivity and specificity. In addition, thirty patients were included in a preliminary trial and the prototype proved to be user-unfriendly, being cumbersome even for the clinician to interpret. Dr. Lawrence of Litmus Concepts is still 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 were 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 compared these two tests for detection of leukocytes and nitrites and found that the Chemstrip had lower numbers of false positives for predicting urinary tract infections. 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 was included in the test kit and the predictive value of this test in women with genital complaints is reported in Phase II, task 2.

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

Status: Completed.

The IRB application was prepared and approved at our institution and was reviewed and approved by The Surgeon General's Human Subject Review Board.

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

Status: Completed.

Detailed data collection instruments were created and tested in 232 patients. These forms were included in the appendix.

5. *Develop a database for this information.*

Status: Completed.

An extensive database was developed and was used in our evaluations. The database contains 349 variables. The variables include information of demographic and behavioral characteristics, symptoms, results of physical examination, and laboratory testing. The data was 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 were 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 previous years progress report, were successfully used in Phase III of this study. Analysis of Phase III patients provided additional data on self-collection on 505 patients with 95% correlation with clinician-collected samples.

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 was 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 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). Recruitment was completed on June 1, 2000. A total of 717 women were recruited.

The demographics of enrolled women are displayed in Table 1. All women recruited had at least one urogenital complaint including vaginal discharge in 48%, pruritus in 29%, abnormal vaginal odor in 37% and burning or pain in 18%. The results of gold standard testing are shown in Table 2. Among the 717 patients, gold standard testing indicated that 351(49%) women had bacterial vaginosis (BV), of which, 85 women had a concurrent sexually transmitted disease (*N. gonorrhoeae*, *C. trachomatis*, or *T. vaginalis*), 207 women had BV alone. 80 had BV and yeast. There were an additional 71 women with one of the STDs that did not have BV. Overall, 156 of women had one or more of the STDs, specifically 91 had *T. vaginalis*, 55 had *C. trachomatis*, and 38 had *N. gonorrhoeae*. Yeast was cultured from 130 women with no other infection. Pruritus was a presenting complaint in only 32% of these women. In 162(25%) women studied, none of the above pathogens were identified despite symptoms. 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=717		Age Mean = 24.9 yr.		S.D. = 5.8 yr.	
Race		Marital Status			
African-American	61%	Single	75%		
European-American	37%	Married/Cohabiting	18%		
Multiethnic	1%	Separated/Divorce	7%		
Other	1%				
Employment Status		Tobacco Use		Alcohol Use	
Employed	66%	Any smoke	60%	Any Use	48%
Full-Time	40%	Heavy Smokers		Heavy Use	
Part-Time	26%	≥1ppd	15%	(Daily)	1%
Unemployed	34%	Non-Smokers	40%	None	52%
Douching Habits					
Ever	75%				
Never	25%				

Table 2. Gold Standard Testing

<u>DIAGNOSIS</u>	<u>NUMBER</u>
STD +/- BV	156(22%)
STD, No BV -----	71
BV +/- YEAST	266(37%)
BV Only -----	207
YEAST Only	130(18%)
<u>No Organism (neg.)</u>	<u>162(23%)</u>
TOTAL	717

2. *Analyze test kit performance compared with "gold standard test results and evaluate the kit's accuracy in predicting the presence of cervical/vaginal 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 have now been determined on clinician-collected vaginal swabs obtained from 703 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 > 400 mg/mL	112	276	388
Lactoferrin ≤ 400 mg/mL	40	275	315
	152	551	703
Sensitivity = 74%			
Specificity = 50%			
PPV = 29%			
NPV = 87%			

The fact that 74% of women with an STD had a positive lactoferrin test (sensitivity = 74%) will identify nearly 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%. The specificity of lactoferrin was 50% and the positive predictive value 29%. Thus, our data (sens=74%/spec=50%/ppv=29%/npv 87% 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 only²³. 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%. Thus, without the need for expertise, speculum or examination our test kit was more sensitive and only slightly less specific. We further began 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 691 women tested with both lactoferrin and defensin with a

cutoff value of 400ng/ml for lactoferrin and 1,100 ng/ml for defensin. Although the addition of defensin measurements enhanced our specificity from 50% to 68%, it did so at the expense of sensitivity (74% to 54%). For this reason we did not develop a defensin dipstick.

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 and Defensin > 1,100		81	174	255
One or both below cutpoints		68	368	436
		149	542	691
Sensitivity = 54%				
Specificity = 68%				
PPV = 32%				
NPV = 84%				

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). As indicated in last years progress report, improvements in the barrier thickness of the pH/amine card improved sensitivity and specificity among the 180 patients tested with appropriate thickness cards. Among the 651 women tested with the pH/amine card the sensitivity remained greater than 90% and the specificity 64%. (Table 5) There seemed to us to be an inordinate number of false positive tests and further quality assurance investigations were undertaken. The card performed extremely well under laboratory conditions indicating that our sensitivity should be well over 95%, however, it was performing at 62% in the clinical setting. We then investigated each clinical site, evaluating the swabs obtained from these sites. Two significant findings resulted from these on site investigations. The first was that these clinical exam rooms were cleaned (as are most hospital and clinical sites) with an ammonia based cleaning solution. The second finding was that if the swabs in the drawers and open cabinets of these sites were not replaced after room cleaning with new swabs, the swabs themselves, even though sterile and sealed in paper, could absorb ammonia and turn the amine test card positive when wet. Fresh swabs not exposed to cleaning reagents consistently tested negative. After discussion with Litmus Concepts representatives, it was agreed that all cards would now come with foil, sealed swabs used for testing. We instituted

this change over the last six months of specimen collection (138 specimens). The overall sensitivity/specificity data is shown in Table 5a and the last years data in Table 5b. No significant improvement was noted in sensitivity and specificity.

Table 5a. BV diagnosis by Gram stain

		BV(score ≥ 7)	Normal/intermed.(<7)	
pH/Amine TESTCARD	Pos	291	117	408
	Neg	32	211	243
		323	328	651

Sensitivity $291/323 = 90\%$
 Specificity $211/328 = 64\%$
 PPV $291/408 = 71\%$
 NPV $211/243 = 87\%$

Table 5b. BV diagnosis by Gram stain

		BV(score ≥ 7)	Normal/intermed.(<7)	
pH/Amine TESTCARD	Pos	69	19	88
	Neg	7	43	50
		76	62	138

Sensitivity $69/76 = 91\%$
 Specificity $43/62 = 69\%$
 PPV $69/88 = 78\%$
 NPV $43/50 = 86\%$

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 231(32%) of women in this study, however, only 42(32%) had symptoms of pruritus. 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 Candida protein moieties used in the test perform well in *in vitro* studies and will remain the basis for the card development for yeast identification in vaginal swabs.

Urinary Tract Infections by Leukocyte /Esterase Testing

As we previously reported, 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. Because so few women had urinary complaints we have subsequently focused our study toward vaginal complaints.

Table 6. Rapid Detection of Urinary Tract Infection

		Urine culture +	Urine culture +	
Leukocyte esterase (Chemstrip)	Pos	16	103	119
	Neg	17	345	362
		33	448	481

Sensitivity 16/33 = 48%
 Specificity 345/448 = 77%
 PPV 16/119 = 13%
 NPV 345/362 = 95%

Chlamydia trachomatis and *Neisseria gonorrhoeae* Detection

We received a new instrument in our laboratory to detect the presence of *C. trachomatis* and *N. gonorrhoeae*. We compared the Becton Dickinson ProbeTec ET system to our gold standard tests. Secretions from vaginal and endocervical swabs were tested in 455 patients. The ProbeTec System was compared to Roche Amplicor PCR for the detection of chlamydia and culture for the detection of gonorrhea. Discrepant results were then tested using the Abbott LCx assay. Of the 455 patients tested, 37 (7.5%) were positive for chlamydia and 39 (8.6%) were positive for gonorrhea. The diagnostic sensitivity and specificity of chlamydia was 89% and 99.5% for PCR and 91.9 and 99.7% for ProbeTec, respectively. The diagnostic sensitivity and specificity of gonorrhea was 77% and 100% for culture and 100% and 100% for ProbeTec, respectively. We determined that secretions from vaginal swabs work just as well as those from endocervical swabs for the detection of chlamydia or gonorrhea. There was no statistically significant difference between ProbeTec and Roche for chlamydia detection. However, ProbeTec was more sensitive than culture for the detection of gonorrhea.

Overall Assessment of Self Test Kit

There were 162/717(23%) women presenting with vaginal 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 64(40%) of these women obviating the need for unnecessary antimicrobial therapy.

Among 717 patients tested, 156 had one or more of the STDs, the most prevalent of which was TV (91/717). Concurrent BV was identified in 85 women with an STD and our treatment algorithm (see Table 7) 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 591 women tested with self-test kit that had an infection, 550(93%) would have been directed by the self-test kit to take an antimicrobial agent able to affect a cure.

Table 7. Treatment Algorithm

pH/Amine	Lactoferrin	Leukocyte Esterase/Nitrite	Proposed Treatment
+	+	+	Azithromycin, Ciprofloxacin, Metronidazole & Fleroxacin
+	+	-	Azithromycin, Ciprofloxacin & Metronidazole
+	-	-	Metronidazole
+	-	+	Metronidazole & Fleroxacin
-	+	+	Azithromycin, Ciprofloxacin & Fleroxacin
-	-	+	Fleroxacin
-	-	-	If pruritis is present treat with Fluconazole

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

Status: Completed.

We have analyzed data now on 507 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 443/469 (95%) cases, which was extremely encouraging in this regard. In the case of the pH/amine card test, there was 478/507 (94%) agreement between clinician reading and patient reading. Moreover, compared to gold standard testing clinicians rather than patients tended to over read or over interpret the pH/amine card resulting in decreased sensitivity.

4. *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.

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 not be developed, since this test did not significantly improve sensitivity /specificity over that achieved by lactoferrin alone. Yeast card will be added when test development reaches an acceptable level of accuracy.

5. *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 269 women tested.

6. *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 were also reviewed on an ongoing basis to insure the validity and accuracy of collected and entered data. We have entered complete data on 717 women enrolled during Phase I, II and III of this project.

Phase III Tasks

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

Status: Completed.

We recruited women to self-collect specimens, perform and interpret the self-test kit results and to choose therapy. We enrolled 717 women and the women showed a remarkable ability to collect specimens, perform the test and interpret results. We have completed enrollment prior to collecting 900 patients due to time constraints. Enrollment was terminated June 1, 2000 in order to complete our data analysis.

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

Status: Completed.

Patients were enrolled and successfully able to collect the samples and perform and interpret the test. They were able to perform and accurately read the lactoferrin dipstick test results in 443/469 (95%) cases, which was extremely encouraging in this regard. In the case of the pH/amine card test, there was 478/507 (94%) agreement between clinician reading and patient reading. Moreover, compared to gold standard testing clinicians rather than patients tended to over read or over interpret the pH/amine card resulting in decreased sensitivity.

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

Status: Completed.

Results of treatment selection based on symptoms are reported. Decision analysis was performed to determine better diagnostic criteria. Patient monitoring of treatment selection was an ongoing part of patient enrollment.

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

Status: Completed.

All data collected has been entered and included in the final analysis.

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

Status: Completed.

All patients enrolled have had lab testing completed and recorded and are included in the analysis.

6. *Respond to all progress inquiries.*

Status: Completed.

All inquiries have been addressed.

Key Research Accomplishments

- We were able to develop a rapid self-test kit for women with genital tract complaints, which with further refinement in sensitivity would be a valuable tool for STD diagnosis in a resource-poor setting.
- We were able to collect data to assess the diagnostic capabilities using
 - 1) signs and symptoms only (syndromic approach);
 - 2) currently available clinical tools such as wet mount, pH and KOH preparations; and
 - 3) rapid tests such as pH/amine card and lactoferrin dipstick.
- We demonstrated women's ability to self-collect vaginal samples and to read rapid diagnostic tests.
- We demonstrated the equivalency of self-collected samples compared with clinician-collected samples for the detection of lower genital tract infections.
- We developed an extensive data collection instrument and collected data on over 700 women with lower genital tract complaints that will enable us to further evaluate risk factors, demographics and predictors of the various causes of lower genital tract complaints.
- We have collected sufficient data to study in a decision analysis tree in order to better define clinical diagnostic criteria based on signs, symptoms and risk factors.

Reportable Outcomes

Abstracts:

Enhanced STD Diagnosis Combining Syndromic Approach With A Self-Collected Test Kit When Resources Are Limited

Landers DV, Krohn MA, Heine RP, Wiesenfeld HC, Duarte G, Sweet RL, Hillier SL
Presented at ISSTD, February 1999

Poor Predictive Value of Symptoms for Diagnosis of Lower Genital Tract Infections

Daniel V. Landers, MD, Sharon L. Hillier, PhD, Marijane A. Krohn, PhD, and R. Phillips Heine, MD

Presented at IDSOG, August 2000

Publications:

Vaginal SLPI Levels Are Significantly Decreased In Women With Symptomatic Lower Reproductive Tract Infections

Deborah L. Draper, PhD, Daniel V. Landers, MD, Marijane A. Krohn, PhD, Sharon L. Hillier, PhD, Harold C. Wiesenfeld, MD, and R. Phillips Heine, MD
Am J OB-GYN (In Press)

Manuscripts in progress:

Poor Predictive Value of Symptoms for Diagnosis of Lower Genital Tract Infections
Daniel V. Landers, MD, Sharon L. Hillier, PhD, Marijane A. Krohn, PhD, R. Phillips Heine, MD and Harold C. Wiesenfeld (in preparation)

Decision Analysis in the Diagnosis of Female Lower Genital Tract Infection
Simhan H, Krohn MA, Meyn L, Wiesenfeld HC, Heine RP, Hillier SL and Landers DV (in preparation)

Comparison of Amplicor PCR, Culture and Strand Displacement Amplification for the Detection of Chlamydia trachomatis and Neisseria gonorrhoeae using Vaginal and Cervical Swabs
Lisa A. Cosentino, Daniel V. Landers, and Sharon L. Hillier (in preparation)

Conclusions

The main goal of this project was to develop a rapid self-test kit for the diagnosis of symptomatic, treatable, lower genital tract infections in women.

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, 89% of women with BV or TV, and 96% of women with BV, TV, GC or CT would have been directed to appropriate therapy based on lactoferrin and pH/amine testing. Overall, including all women with self-test results, 93% of women would have received the appropriate treatment decision. This number may well exceed the number treated appropriately in fully equipped clinical settings. Thus, although the self-test kit did not produce the ideal sensitivity and specificity, it exceeded the predictive value of currently available clinical algorithms for treating lower genital tract infections.

References

1. U.S. Department of Health and Human Services. Summary of notifiable diseases. United States, 1994. MMWR 1994; 431-68.
2. Sweet RL, Gibbs RS. Sexually transmitted diseases. In: Infectious Diseases of the Female Genital Tract, 3rd Edition, Williams and Wilkins, Baltimore 1995; p133-181.
3. Stamm WE, Hooten TM. Management of urinary tract infection in adults. N Engl J Med 1993; 329:1328-1334.
4. Johnson JR, Stamm WE. Urinary tract infections in women: Diagnosis and treatment. Ann Inter Med 1989; 111:906-917.
5. Cates W, Wasserheit JN. Genital chlamydial infections: Epidemiology and reproductive sequelae. Am J Obstet Gynecol 1991; 164:1771-1781.
6. Committee on Defense Women's Health Research, Institute of Medicine: Recommendations for Research on the Health of Military Women. National Academy Press, Washington, D.C. 1995; p6-18.
7. Malone JD, Hyams KC, Hawkins RE, et al. Risk factors for sexually transmitted diseases among deployed U.S. military personnel. Sex Transm Dis 1993; 20:294-8.
8. Catterson ML, Zadoo V. Prevalence of asymptomatic chlamydial cervical infection in active duty army females. Military Med 1993; 158:618-9.
9. Holly EA, Cress RD, Ahn DK, et al. Characteristics of women by smoking status in the San Francisco Bay Area. Cancer Epidemiology, Biomarkers and Prevention, 1992; 1:491-7.
10. Scholes D, Daling JR, Stergachis AS. Current cigarette smoking and the risk of acute pelvic inflammatory disease. Am J Public Health 1992; 82:1352-5.
11. Shafer MA, Pessione F, Scieux C, et al. Chlamydia trachomatis: Risk factors in women in the Parisian region. Importance of smoking and cervical ectropion. J Gynecol Obstet Biol Reprod 1993; 22:163-8.
12. Laga A, Manoka A, Kivuvu M, et al. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: Results from a cohort study. AIDS 1993; 7:95-102.
13. Plummer FA, Simonsen JN, Cameron, et al. Cofactors in male-female sexual transmission of human immunodeficiency virus. J Infect Dis 1991; 163:233-9.
14. Cates W. Epidemiology and control of sexually transmitted diseases: Strategic evolution. Infect Dis Clin North Am 1987; 1:1-23.
15. Sweet RL, Gibbs RS. Infectious vulvovaginitis. In: Infectious Diseases of the Female Genital Tract., 3rd Edition, Williams and Wilkins, Baltimore 1995; p341-362.
16. Washington AE, Johnson RE, Sanders LL, et al. Incidence of Chlamydia trachomatis infections in the United States: Using reported Neisseria gonorrhoeae as a surrogate. In: Oriel D, Ridgeway G, Schachter J, et al, Chlamydial Infections. Cambridge University Press 1986; p487-490.
17. Sobel JD. Vaginal infections in adult women. Med Clin North Am 1990; 74:1573-602.

18. Centers for Disease Control and Prevention. 1998 Sexually transmitted diseases guidelines. MMWR 1998; 47(Nº. RR-1):49-75.
19. Oriel JD, Partidge BM, Denny MJ, Coleman JC. Genital yeast infections. Br Med J 1972; 4:761-4.
20. Hillier S, Holmes KK. Bacterial vaginosis. In: Holmes KK, Mardh PA, Sparling PF, Weisner PJ, Sexually Transmitted Disease, 2nd edition. Mc Graw-Hill Inc. 1990; p547-59.
21. Lehrer RI, Lichtenstein AK, Ganz T. Defensins: Antimicrobial and cytotoxic peptides of mammalian cells. Ann Rev Immunol 1993; 11:105-28.
22. Panyutich AV, Panyutich EA, Krapivin VA, et al. Plasma defensin concentrations are elevated in patients with septicemia or bacterial meningitis. J Lab Clin Med 1993; 122:202-207.
23. Ryan CA, Courtois BN, Hawes SE, Stevens CE, Eschembach DA, Holmes KK. Risk assessment, symptoms, and signs as predictor of vulvovaginal and cervical infections in an urban US STD clinic: implications for use of STD algorithms. Sex Transm Inf 1998; 74(Suppl. 1):S59-S76.

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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

23 Aug 01

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Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir,
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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


PHYLIS M. RINEHART
Deputy Chief of Staff for
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