

# ***Trichomonas vaginalis* repeat infections among HIV-negative women**

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This trial will be conducted in compliance with the protocol and the Tulane University biomedical IRB. The Administrators of The Tulane Educational Fund through the Tulane University Biomedical IRB (FWA #00002055) is duly constituted, organized and operates in compliance with DHHS regulations as described in 45 CFR part 46, i.e. The Common Rule, FDA regulations as described in 21 CFR Parts 50 and 56. Tulane's Biomedical IRB also complies with the guidelines resulting from the International Conference on Harmonization (ICH) E-6 Good Clinical Practice guidelines as required to the extent required by third-party sponsors. In addition, the Tulane University IRB operates in compliance with portions of the Health Insurance of Portability Act of 1996 (HIPAA Privacy Rule) that apply to research, as described in 45 CFR Parts 160 and 164 as appropriate. All investigators and staff will have completed the protection of Human Subjects Training as required by the participating institutions and will adhere to all NIAID Clinical Terms of Award.

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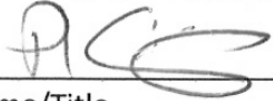
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## Signature Page 1

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## List of Abbreviations

AE	Adverse Event/Adverse Experience
ACASI	Audio/Computer Assisted Survey Instrument
BV	Bacterial vaginosis
CC	CrescentCare Health and Wellness Center
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CIB	Clinical Investigator's Brochure
CRF	Case Report Form
DCC	Data Coordinating Center
DOT	Directly observed therapy
DSMB	Data and Safety Monitoring Board
DSMC	Data and Safety Monitoring Committee
FDA	Food and Drug Administration
FMS	Future microbiota study
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JCHD	Jefferson County Department of Health
LSUHSC	Louisiana State University Health Sciences Center
MSDH	Mississippi Department of Health
MTZ	metronidazole
NAAT	Nucleic acid amplification test
NYU	New York University
N	Number (typically refers to participants)
NDA	New Drug Application
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research

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### **List of Abbreviations**

PCR	Polymerase Chain Reaction
PHI	Protected Health Information
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
TOC	Test of Cure
TV	Trichomonas vaginalis
UAB	University of Alabama at Birmingham
UMMC	University of Mississippi Medical Center

## Protocol Summary

<b>Full Title</b>	<i>Trichomonas vaginalis</i> repeat infections among HIV-negative women.
<b>Short Title</b>	TV repeat infections among HIV- women
<b>Clinical Trial Phase</b>	III
<b>IND Sponsor (if applicable)</b>	118276
<b>Conducted By</b>	Tulane University School of Public Health and Tropical Medicine
<b>Principal Investigator</b>	Patricia Kissinger, Ph.D.
<b>Sample Size</b>	1664
<b>Study Population</b>	HIV negative women who are TV positive and are the age of majority, English speaking, not pregnant or breast feeding, and are patients at participating clinics; 95% of participants are anticipated to be African American.
<b>Accrual Period</b>	30 months
<b>Study Design</b>	This study is a phase III randomized clinical trial examining two different doses of metronidazole (MTZ) for the treatment of <i>Trichomonas vaginalis</i> (TV). HIV negative women who test positive for TV at their routine exam at participating clinics will be referred to the nurse/study coordinator who will screen for eligibility, provide a description of the study, and obtain written, informed consent (N=1664). Subjects will undergo an audio computer assisted self-interview (ACASI), and will self-collect up to 4 vaginal swabs for InPouch culture and nucleic acid amplification test (NAAT) for TV, Gram stain for bacterial vaginosis (BV) and storage for future microbiota studies (FMS). Subjects will then be randomized into one of two arms: (1) metronidazole (MTZ) 2 g single oral dose (CDC recommended treatment regimen) or (2) MTZ 500 mg oral dose BID for 7 days (CDC alternative treatment regimen) Participants will be scheduled for a Test of Cure (TOC) follow up visit 4 weeks after treatment completion with a window of 3-12 weeks post-baseline for the 2 gm MTZ dose and 4-13 weeks post-baseline for the 7 day MTZ dose. If a woman is NAAT TV+/InPouch TV- at TOC, she may be asked to return for an additional follow up at 4 weeks post TOC visit (window of 3-8 weeks post TOC visit). During follow up visits

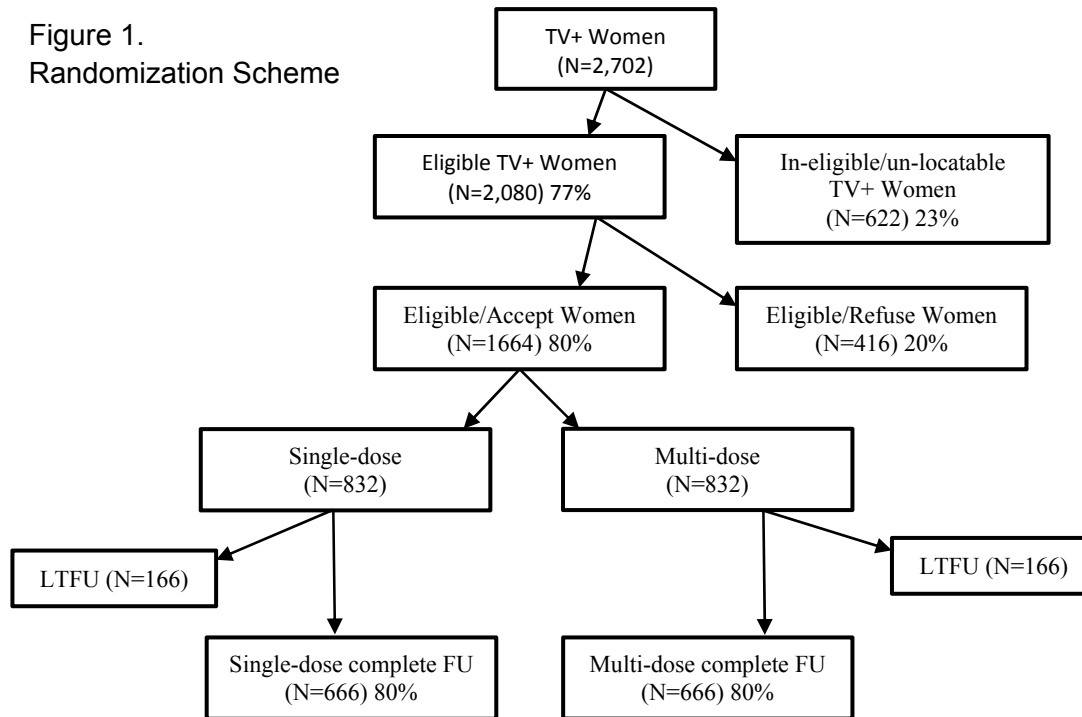
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	subjects will undergo ACASI and self-collect up to 5 vaginal swabs for TV and BV testing and FMS.
<b>Study Duration</b>	Participants will be enrolled in the study for the time it takes to complete their follow up visits (no more than 21 weeks). Accrual for the study will be 30 months in duration. Participants will be asked for permission to store their samples for future research and for investigators to use their data for secondary data analyses.
<b>Study Agent/Intervention Description</b>	Metronidazole 2g single oral dose under direct observation and Metronidazole 500 mg oral dose BID for 7 days with the first dose given under direct observation
<b>Primary Objective</b>	<p>To examine the influence of two factors on repeat infections with TV among HIV negative women: dosing and the presence of bacterial vaginosis (BV):</p> <p>We will test two hypotheses:  H1: Women receiving multi-day MTZ will be less likely to test TV positive at TOC compared to women who receive the single dose MTZ.  H2: This effect (H1) will only be observed among women who have BV (Nugent score <math>\geq 7</math>).</p>
<b>Secondary Objectives</b>	To determine the proportion of infections at follow up that are attributable to treatment failure or sexual re-exposure.
<b>Exploratory Objectives</b>	Examination of the rate and factors associated with adherence to medication, knowledge/belief/factors associated with partner treatment, sexual exposure and the nature of the sexual act and sexual partner, and condom use will be possible using the ACASI survey data. These factors will be assessed by arm to determine if any confounding occurred and if so, these analyses will be adjusted for these factors. These data will also help inform future translational research.
<b>Endpoints</b>	Repeat infection with <i>Trichomonas vaginalis</i> (TV) at 4 weeks (TOC) as measured by InPouch culture or NAAT.

## Schematic of Study Design Showing Projected Enrollment:

Figure 1.  
Randomization Scheme



# 1. KEY ROLES

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## 2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

### 2.1 Background Information

*Trichomonas vaginalis* (TV), the most common curable sexually transmitted infection (STI) in the world<sup>1</sup>, is associated with vaginitis, cervicitis, urethritis, low birth weight, preterm delivery<sup>2</sup>, pelvic inflammatory disease<sup>3</sup> and may increase the risk of HSV and HIV acquisition<sup>4-7</sup> and transmission<sup>8-10</sup>. Repeat infections are common, ranging from 5%-31%<sup>11-20</sup>, and share similar sequelae to primary infections. Determining the origin of these repeat infections and examining better treatment options is the focus of this study.

Potential causes of early repeat TV infections include: drug resistance, non-adherence to treatment, clinical treatment failure, or re-infection. Single dose therapy has removed adherence as an issue and *in vitro* resistance testing has consistently demonstrated low rates of insusceptibility. The most likely sources of repeat infections, therefore, are clinical treatment failure (dosing or host factors) or re-infection from an untreated partner.

In our study of TV-infected women who were given directly observed single dose metronidazole (MTZ) and provided with medication to deliver to their sex partner(s), repeat infections rates were high (8%) and nearly all (92%) were attributed to clinical treatment failure<sup>11</sup>. Clinical treatment failure could be due to insufficient dosing or biological factors that interfere with antibiotic treatment. The Centers for Disease Control and Prevention (CDC) guidelines for treatment of TV include: MTZ or tinidazole 2 g single dose as the recommended regimens, and MTZ 500 mg BID 7 day dose as the alternative treatment regimen<sup>21</sup>. These recommendations, however, were based on studies conducted nearly 30 years ago and while single dose was found to be equivalent to multiple dose therapies, repeat TV infection rates after single dose treatment were high (i.e. 5-20%). We recently completed a randomized controlled trial (RCT) among HIV-infected women with TV and found multi-dose MTZ to be superior to single dose treatment<sup>22</sup>. We also found that the presence of bacterial vaginosis (BV) was likely a major factor in the early failure of the single dose treatment. Whether these findings can be extrapolated to HIV-negative women will be examined in aims 1 and 2.

Re-infection from an untreated partner is another potential source of repeat infections. Since TV testing for men is difficult and many TV infected men are asymptomatic<sup>23</sup>, partner treatment is paramount to reducing repeat infections. Patient-delivered partner treatment (PDPT), or the provision of antibiotics to infected index persons to deliver to their sex partners, is a possible alternative to the standard of partner referral (PR) or telling the index woman to refer her partner for care. While PDPT has been found to be superior to standard partner referral methods for reducing repeat Chlamydia and gonorrhea infections<sup>24</sup>, RCTs of PDPT for TV have found conflicting results<sup>13-25</sup> and, to date, the CDC has not made recommendations to offer PDPT for TV infected women. However, these studies were single centered and likely underpowered, thus larger multi-centered trials are needed.

Given the high prevalence of TV, the absence of a national screening program, the deleterious reproductive outcomes associated with TV and the potential for a TV infection to

increase HIV transmission, reducing repeat TV infections is an important targeted public health approach. The overall goal of this project is to determine the influence of index treatment and host factors on repeat TV infections among HIV-negative women in two specific aims:

**Aim 1: Index treatment- Multi-dose (7 day 500 mg BID - alternative regimen) vs. single dose (2 g – recommended regimen) of MTZ for the treatment of TV.**

**Aim 2: Host factors - To determine if altered vaginal flora, as measured by Nugent score, interferes with TV treatment.**

These aims will be accomplished by conducting a phase III randomized clinical trial among TV infected HIV negative women (N=1664) attending clinics in New Orleans, LA, Birmingham, AL or Jackson, MS. These public clinics serve mostly African American, low income women, the demographic group most highly affected by TV<sup>26</sup>. Women will be randomized to a single dose or multi-dose arm. Test of cure will be conducted at 4 weeks post treatment completion. . Viable vaginal specimens from women who are TV+ at TOC will undergo MTZ susceptibility testing and TV genotyping (to compare baseline and follow-up types). Self-collected specimens from women who provide specific consent will be stored for future microbiome studies of the vaginal flora and other related studies. The information obtained from this study will be used to refine treatment recommendations and to better understand the origins of repeat TV infections. Given the strong multi-centered, RCT design, and the large sample size, results should rapidly inform treatment guidelines thus having high impact.

***The ultimate goal is improved reproductive health for women, particularly minority women, and the reduction of the potential for HIV transmission, fitting well with NIAID's mission.***

### **2.1.1 Description of the Study Agent(s)/Intervention(s)**

This is an RCT to examine two doses of MTZ:

MTZ 2 g single dose - (CDC recommended treatment regimen) Following randomization, women assigned to the single dose arm will be given the 2 g oral dose of MTZ under direct observation.

MTZ 500 mg BID 7-day dose - (CDC alternative treatment regimen) Following randomization, women assigned to the 7 day arm will be given the 500 mg oral dose BID for 7 days with the first dose given under direct observation.

### **2.1.2 Summary of Previous Pre-clinical Studies**

N/A

### 2.1.3 Summary of Relevant Clinical Studies

**Single versus multi-dose MTZ** - In the 1970's and 80's, studies were published which support switching from the standard treatment of MTZ 250 mg TID 7 day dose to a single, high-dose treatment regimen for TV. In 1972, Woodcock et al, using microscopy to diagnose TV, conducted a non-randomized comparison of women treated with MTZ 2 g single dose to women treated with MTZ 200 mg TID for 7 days. After 4 weeks, results showed roughly twice as many repeat infections in the trial group as in the control group with respective repeat infection rates of 12.3% (9/73) and 6.7% (4/60) p-value not reported<sup>16</sup>. The authors concluded that the single dose was "acceptable treatment" for TV. Another early study by Csonka in 1971, which also used microscopy to diagnose TV, but used a randomized design compared patients who were randomly given the MTZ 2 g single dose to patients given the MTZ 200 mg TID 7 day dose. Women were followed for 3 months, and possible cases of re-infection were excluded. The repeat infection rates due to probable treatment failure were 18% (7/36) for the single dose group and 6% (3/49) for the 7 day group<sup>27</sup>. The authors report that the cure rates were not statistically different ( $p=0.06$ ) and thus the two doses were equivalent, though they were not powered for an equivalency trial. The authors concluded that the MTZ single dose treatment "is a practical and acceptable alternative to the longer conventional course"<sup>27</sup>. In 1982, Aubert and Sesta reported results from their study comparing the MTZ 2 g single dose to the 250 mg TID 7 day dose, where women from a family planning clinic were diagnosed with TV by wet mount preparation and offered the option of single vs. 7 day dose treatment. At the follow-up visit, which occurred 7 to 21 days post-treatment, women were tested for TV using wet mount preparation. The repeat infection rates were similar (6.2% (6/96) for the single dose vs. 2.7% (2/74) for the 7 day dose,  $p=0.47$ )<sup>28</sup> but there are potential major biases due to the fact that women were allowed to self-select their treatment option.

In the above mentioned comparison studies, the repeat TV infection rates were nearly double for the MTZ single dose groups versus the 7 day dose groups. However, given the small sample sizes, the differences were not found to be statistically significant. The authors propose a move towards the MTZ single dose treatment because of the advantage of observing the patient take the single dose treatment<sup>16,28</sup>, the practicality and simplicity of the single dose treatment<sup>27</sup>, the classic therapeutic dictum to treat using the lowest effective dose<sup>28</sup>, and cost savings<sup>28</sup>.

There were other early randomized, double-blind evaluations comparing single dose MTZ to multiple-dose regimens that found closer results. In 1979, Thin et al. reported results from their randomized, double blind comparison of the MTZ 2 g single dose versus the MTZ 400 mg BID 5 day dose. Women were diagnosed with TV by wet preparation and culture, and were asked to return 7 and 14 days after the start of treatment. The repeat TV infection rates were similar for both groups after 14 days of follow-up (7.7% (4/52) for single dose vs. 7.6% (5/66) for 5 day dose)<sup>17</sup>, however, there was significant loss to follow-up (39%). In 1980, Hager et al. reported their results from a randomized, double-blind evaluation of the MTZ 2 g single dose versus the MTZ 250 mg TID 7 day dose. Women were also diagnosed with TV by wet mount preparation and culture, and asked to return in 14 days (range of 7-21 days after treatment). This study was

plagued by a low follow-up rate with only 37.6% of the women returning for a study visit (176/468) and the repeat TV infection rates were as follows: 14.0% (13/93) in the single dose group and 8.4% in the 7 day group,  $p > 0.10$ )<sup>18</sup>. In sum, the data to support the use of single dose MTZ is not strong and prior studies have several methodological concerns indicating that the proper dose of MTZ merits further investigation.

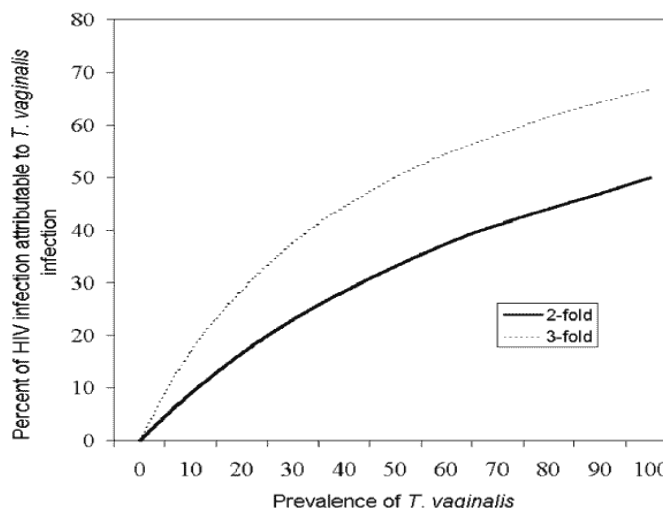
## 2.1.4 Summary of Epidemiological Data

**Epidemiology** - TV is the most common curable STI among women both worldwide and in the US<sup>29</sup>. In the U.S. there are approximately 7 million new cases of TV each year<sup>30</sup> and prevalence rates range from 3% in a nationally representative sample of women<sup>26</sup>, to 14% in adolescents<sup>31</sup>, 13-36% in pregnant women<sup>32-33</sup>, 11-26% in women attending STD clinics<sup>34-37</sup>, 27% among an urban, inner-city population<sup>38</sup>, 38% among drug users<sup>39</sup>, and up to 47% in newly incarcerated pregnant women<sup>40</sup>. The highest prevalence of TV infection in US women is seen among African-Americans with rates ranging from 13-51%<sup>41</sup>. Despite the high rate of TV in both the general and selected sub-populations, there is no screening program in the US for TV. And since over 80% of cases can be asymptomatic<sup>42</sup> most TV infections likely go undetected.

**Sequelae of TV** - Studies show an association between TV and vaginitis, cervicitis, urethritis, bacterial vaginosis, candidiasis, herpes simplex virus type-1 and type-2, Chlamydia, gonorrhea, and syphilis<sup>7,40,42-45</sup>. TV has also been associated with poor birth outcomes such as low birth weight, preterm delivery, pelvic inflammatory disease<sup>3,46-48</sup>, and premature rupture of membranes<sup>2,49-50</sup>. A recent study also showed an association between maternal TV infection and intellectual disability in children<sup>51</sup>. Although rare, TV infection can be transmitted perinatally<sup>52</sup> and cause vaginal and respiratory infections in neonates<sup>53-54</sup>. Treatment during the prenatal period is controversial given found deleterious effects<sup>55-56</sup>. Therefore, effective and successful treatment of TV is imperative during the preconception phase and is important for reducing TV-associated morbidities and improving the general health status of women.

But the most compelling reason to study and control TV: it may amplify the risk of HIV acquisition<sup>4-6</sup> and transmission<sup>8-9</sup>. Cross-sectional<sup>42,57-59</sup> and prospective studies<sup>4-6</sup> have shown an association between TV infection in women and HIV acquisition. This greater susceptibility is biologically plausible for three reasons: inflammatory response to TV infection results in the

**Figure 2. Hypothetical level of HIV transmission attributable to *T. vaginalis* at varying prevalences of TV infection<sup>79</sup>**



appearance of HIV target cells<sup>60-61</sup>; TV infection can cause punctate mucosal hemorrhages resulting in a compromised mechanical barrier to HIV<sup>62</sup>; and TV infection may change the normal vaginal flora and therefore increase susceptibility to bacterial vaginosis<sup>63</sup>, which would increase the risk of HIV acquisition<sup>64</sup>. These consequences combine to enlarge the portal of entry for HIV in TV-infected women. A study by Sorvillo et al. estimates that in a community with a TV prevalence of 25%, as much as 20% of HIV could be attributed to TV infection<sup>65</sup>. Also, a recent study estimates that 6.2% of all HIV infections among US women may be attributable to TV infection<sup>66</sup>. Control of TV, therefore, may provide a cost-effective strategy for reducing HIV transmission especially in settings where TV is common<sup>67-68</sup> or among subgroups who are at higher risk for TV such as African Americans<sup>69</sup>. In the absence of a national screening program to detect primary infections, reducing repeat TV infections can be a targeted approach for reducing TV transmission and TV-related morbidity.

**Repeat TV infections** – Repeat TV infections are common, ranging from 5%-31%<sup>11-20</sup>, and share similar sequelae to primary infections. While it is clear that the TV repeat infection rate is unacceptably high, the source of these repeat infections is less clear. Possible sources are drug resistance, host resistance, or sexual exposure (either by an untreated original partner or a newly acquired sex partner). In our preliminary work, we found evidence that a large proportion of the repeat infections could be attributed to treatment failure (i.e. no sexual exposure and no drug resistance). In the data described in Table 1, of 301 HIV-negative women who were treated for TV with the MTZ 2 g single dose, 8.0% (n=24) retested positive for TV at one month post-treatment. Of the 24 repeat infections, 92% (n=22) were categorized as probable treatment failures using sexual histories<sup>11</sup>. From the 22 women with probable treatment failure, only 2 (9.0%) had strains with moderate resistance to MTZ and 1 (4.5%) had a strain with mild resistance. All three responded to higher doses of MTZ.

Reported rates of MTZ resistance among mostly non-HIV infected women range from 2.2-9.6%<sup>31,70-72</sup> and were usually resolved with repeat MTZ treatment at the same or higher dosage<sup>72</sup>. Therefore, resistance appears to play only a minor role in explaining probable treatment failure

**Table 1. No. (%) of patients with a second test result positive for *Trichomonas vaginalis* after treatment with metronidazole.**

Variable	No. (%) of patients		P
	Family planning clinic (n = 301)	HIV clinic (n = 60)	
Second test result positive for <i>T. vaginalis</i>	24 (8.0)	11 (18.3)	.01
Probable reinfection	2 (0.7)	3 (5.0)	.03
Probable treatment failure	22 (7.3)	6 (10.0)	.44
Probable infection from new sexual partner	0 (0.0)	2 (3.3)	.03
Isolate resistant to metronidazole	3 (1.0)	2 (3.3)	.19

**NOTE.** P values were determined by Fisher's exact test.

## 2.2 Rationale

### RATIONAL AIM 1:

**Single versus multi-dose MTZ** - In the 1970's and 80's, studies were published which supported switching from the standard treatment of MTZ 250 mg TID 7 day dose to a single, high-dose treatment regimen for TV. In 1972, Woodcock et al, using microscopy to diagnose

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TV, conducted a non-randomized comparison of women treated with MTZ 2 g single dose to women treated with MTZ 200 mg TID for 7 days. After 4 weeks, results showed roughly twice as many repeat infections in the trial group as in the control group with respective repeat infection rates of 12.3% (9/73) and 6.7% (4/60) p-value not reported<sup>16</sup>. The authors concluded that the single dose was “acceptable treatment” for TV. Another early study by Csonka in 1971, which also used microscopy to diagnose TV, but used a randomized design compared patients who were randomly given the MTZ 2 g single dose to patients given the MTZ 200 mg TID 7 day dose. Women were followed for 3 months, and possible cases of re-infection were excluded. The repeat infection rates due to probable treatment failure were 18% (7/36) for the single dose group and 6% (3/49) for the 7 day group<sup>27</sup>. The authors report that the cure rates were not statistically different ( $p=0.06$ ) and thus the two doses were equivalent, though they were not powered for an equivalency trial. The authors also concluded that the MTZ single dose treatment “is a practical and acceptable alternative to the longer conventional course”<sup>27</sup>. In 1982, Aubert and Sesta reported results from their study comparing the MTZ 2 g single dose to the 250 mg TID 7 day dose, where women from a family planning clinic were diagnosed with TV by wet mount preparation and offered the option of single vs. 7 day dose treatment. At the follow-up visit which occurred 7 to 21 days post-treatment, women were tested for TV using wet mount preparation. The repeat infection rates were similar (6.2% (6/96) for the single dose vs. 2.7% (2/74) for the 7 day dose,  $p=0.47$ )<sup>28</sup> but there are potential major biases due to the fact that women were allowed to self-select their treatment option.

In the above mentioned comparison studies, the repeat TV infection rates were nearly double for the MTZ single dose groups versus the 7 day dose groups. However, given the small sample sizes, the differences were not found to be statistically significant. From the authors’ comments, there seemed to be a desirability to move towards the MTZ single dose treatment because of the advantage of observing the patient take the single dose treatment<sup>16,28</sup>, the practicality and simplicity of the single dose treatment<sup>27</sup>, the classic therapeutic dictum to treat using the lowest effective dose<sup>28</sup>, and cost savings<sup>28</sup>.

There were other early randomized, double-blind evaluations comparing single dose MTZ to multiple-dose regimens that found closer results. In 1979, Thin et al. reported results from their randomized, double blind comparison of the MTZ 2 g single dose versus the MTZ 400 mg BID 5 day dose. Women were diagnosed with TV by wet preparation and culture, and were asked to return 7 and 14 days after the start of treatment. The repeat TV infection rates were similar for both groups after 14 days of follow-up (7.7% (4/52) for single dose vs. 7.6% (5/66) for 5 day dose)<sup>17</sup>, however, there was significant loss to follow-up (39%). In 1980, Hager et al. reported their results from a randomized, double-blind evaluation of the MTZ 2 g single dose versus the MTZ 250 mg TID 7 day dose. Women were also diagnosed with TV by wet mount preparation and culture, and asked to return in 14 days (range of 7-21 days after treatment). This study was plagued by a low follow-up rate with only 37.6% of the women returning for a study visit (176/468) and the repeat TV infection rates were as follows: 14.0% (13/93) in the single dose group and 8.4% in the 7 day group,  $p>0.10$ )<sup>18</sup>. In sum, the data to support the use of single dose MTZ is not strong and prior studies have several methodological concerns indicating that the proper dose of MTZ merits further investigation.

**Other nitroimidazoles – It should be noted that tinidazole (TDZ), which is from the same class of drugs as MTZ,** is also considered first line therapy by CDC as a 2 gram single dose. A meta-analysis of treatment for TV found that MTZ had significantly higher rates of treatment failure, clinical failure, and side effects compared to TDZ, though the only blinded study included in this analysis did not show any advantages for TDZ<sup>73</sup>. This drug has not shown superiority over MTZ for the treatment of bacterial vaginosis<sup>74</sup>. It can be argued that since TDZ is at least as good for TV infection as MTZ and may be better, that we should use this drug rather than MTZ for this trial. While TDZ has become generic, the price difference (generic TDZ is 3 times more costly) is likely to make TDZ cost prohibitive as a front line medication. Thus, public health clinics around the world will likely continue to use MTZ for TV infections for many years into the future. Future trials may also wish to include TDZ.

## RATIONALE AIM 2

**TV treatment among HIV+ women.** Prompted by our prior work and others who found that post-treatment repeat TV infection rates among HIV-positive ranged from 18-36%<sup>11,76-77</sup>, we examined potential causes of this high repeat infection rate. The purpose of the study was to determine if the single dose MTZ

(recommended) is as effective as the multi-dose (alternative) for treatment of *Trichomonas vaginalis* (TV) among HIV+ women. Using a Phase IV randomized clinical trial, HIV-positive women with culture confirmed TV were randomized to treatment arm: MTZ 2 g single dose or MTZ 500 mg BID 7 day dose. All women were given 2 g MTZ doses to deliver to their sex partners. Women were re-cultured for TV at a test-of-cure (TOC) visit occurring 6-12 days after treatment completion. TV-negative women at TOC were re-cultured at a 3 month visit. Repeat TV infection rates were compared between arms. Of the 270 HIV+/TV+ women who were enrolled, the mean age was 40 years,  $\pm$  9.4 and 92.2% African-American. Treatment arms were similar with respect to age, race, CD4 count, viral load, ART status, site, and loss-to-follow up. Women in the 7 day arm had: lower repeat TV infection rates at TOC [8.5% (11/130) versus 16.8% (21/125) (R.R. 0.50, 95% CI=0.25, 1.00;  $p < 0.05$ )], compared to the single dose arm. Because of the known potential for false negatives with TOC culture, we also examined the effect at 3 months and found that the superiority of the approach persisted [3 months [11.0% (8/73) versus 24.1% (19/79) (R.R. 0.46, 95% CI=0.21, 0.98;  $p = 0.03$ )]. We concluded that the 7 day MTZ dose was more effective than the single dose for the treatment of TV among HIV+ women. (Table 2).

**Table 2. T. vaginalis results by Metronidazole Treatment Arm at Test-of-Cure (n=255) and 3 month (n=152)**

	TV+ Rate Overall % (n)	7 day dose % (n)	Single dose % (n)	RR (95% CI)	P-value
TOC	12.5% (32/255)	8.5% (11/130)	16.8% (21/125)	0.50 (0.25, 1.00)	0.045
3 month	17.8% (27/152)	11.0% (8/73)	24.1% (19/79)	0.46 (0.21, 0.98)	0.030

RR=Relative Risk; CI=Confidence Interval;

**Altered flora, bacterial vaginosis and TV** - One possible factor in the treatment failure of TV is vaginal flora disturbances. Bacterial vaginosis (BV) is the most common vaginal condition in women of childbearing age<sup>78</sup>. The prevalence of BV in the US ranges from 29% in a nationally

representative sample (where the prevalence was 3.1 times greater for African-American women compared to whites)<sup>79</sup>, 44% in a group of women at high-risk for HIV<sup>78</sup>, and as high as 56% among injection drug users<sup>79</sup>. Like TV, BV can also increase a woman's susceptibility to HIV infection<sup>64,82-83</sup>. Several studies have shown a strong association between TV and BV<sup>33,84-86</sup>, meaning that the two frequently occur as co-infections among women. While these two vaginal infections have similar

symptomatology and are treated with similar medication, the dosing is not the same. The Centers for Disease Control and Prevention (CDC) guidelines for treatment of TV include: MTZ or TDZ 2 g single dose as the recommended regimens, and MTZ 500 mg BID 7 day dose as the alternative treatment regimen<sup>21</sup>. For BV, the first line of therapy is 500 mg MTZ BID for 7 days. Thus, the first line of therapy for TV would not adequately treat concomitant BV.

In a screening study of HIV-positive women, we found that the prevalence of TV was higher among women who had altered vaginal flora (Table 3) and that the majority (61.0%) of HIV+/TV+ women also had BV<sup>87</sup>. This high rate of BV that accompanies TV infection among HIV+ women has implications for treatment decisions.

These findings prompted us to re-examine the data from our RCT where we found multi-dose MTZ to be superior to single dose to see if BV had any influence on treatment outcomes. In analyses, stratified by treatment arm, we found that among women given single-dose treatment, those with TV/BV co-infection were 4.2 times more likely to retest TV-positive at TOC than those with baseline TV infection only. This difference was not among women given the multi-dose and was not observed for either dose at 3 months (Table 4)<sup>88</sup>.

**Table 3. Distribution of *T. vaginalis* according to Nugent Score categories and association between vaginal flora and *T. vaginalis* infections among HIV-positive women (n=356)**

Vaginal Flora (Nugent score)	Number of Women	Percentage infected with <i>T. vaginalis</i>	Odds ratio for <i>T. vaginalis</i> infection	95% confidence interval	P-value
Normal (0-3)	128	16.4%	Ref.	--	--
Intermediate (4-6)	50	40.0%	3.40	1.63, 7.08	0.0008
Bacterial vaginosis (7-10)	187	34.2%	2.65	1.52, 4.63	0.0005
Abnormal (4-10)	237	35.4%	2.80	1.63, 4.79	0.0001

**Table 4. Repeat infection rates at TOC by arm and BV status**

	Percentage overall repeat infection rate TV+ (n)	Percentage baseline coinfection TV/bacterial vaginosis	Percentage baseline single infection TV	RR (95% CI)	p Value
Test-of-cure (N=230)	13.0 (30/230)	16.1 (25/155)	6.7 (5/75)	2.42 (0.96 to 6.07)	0.05
Single-dose	18.3 (21/115)	23.8 (19/80)	5.7 (2/35)	4.16 (1.02 to 16.89)	0.02
7-day dose	7.8 (9/115)	8.0 (6/75)	7.5 (3/40)	1.07 (0.28 to 4.04)	0.92
3 month (N=138)	16.7 (23/138)	19.1 (16/84)	13.0 (7/54)	1.47 (0.65 to 3.34)	0.35
Single-dose	22.2 (16/72)	25.6 (11/43)	17.2 (5/29)	1.48 (0.58 to 3.82)	0.40
7-day dose	10.6 (7/66)	12.2 (5/41)	8.0 (2/25)	1.52 (0.32 to 7.27)	0.70 Fisher exact test



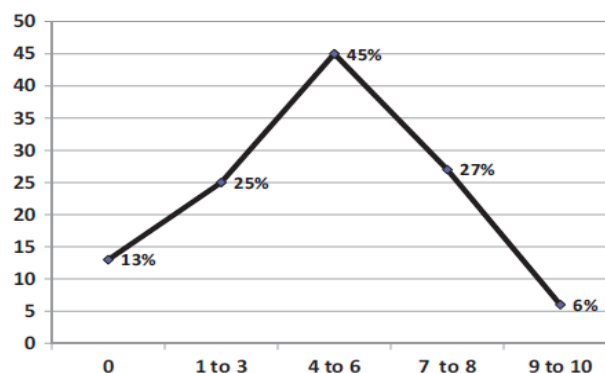
These data suggest that the vaginal environment associated with BV at the time of TV treatment in some way partially protects the organism (TV) from the effects of single-dose MTZ but over a more prolonged period of time the effect is largely lost. The influence of the vaginal environment on TV treatment has been alluded to in other studies. Over 40 years ago, Nicol et al.<sup>89</sup> reported the possibility that inactivation of MTZ might be the cause of multiple treatment failures in a woman

infected with a TV strain that was susceptible to the drug in vitro. A few years later, McFadzean et al.<sup>90</sup> described inactivation of MTZ by bacteria isolated from 16 samples taken from 84 women with TV vaginitis. Ten of the organisms were Gram-positive cocci including 7 cases of *Streptococcus (Enterococcus) faecalis*. The six others were Gram negative rods including *Escherichia coli*, *Proteus* spp. and *Klebsiella* spp. More recently, several papers have reported that various members of the genus *Enterococcus* are capable of inactivating MTZ<sup>91-93</sup>. Deep sequencing studies have not found many of these organisms in patients with BV<sup>94-96</sup> but it could be that even minor populations of certain organisms or combinations of organisms may have the ability to inactivate MTZ clinically.

Martin et al. found that TV prevalence was highest in the women with intermediate Nugent scores (fig. 3) confirming the observations of Hillier et al.<sup>97</sup> and Gatski<sup>88</sup>. A heat map analysis of on pyrosequencing data showed that the vaginal flora of 18/30 TV + women had a similar unique microbiota characterized by high abundance of *Mycoplasma* spp or *Ureaplasma* spp. and relatively low abundance of *Lactobacillus* spp. and *Gardnerella* spp<sup>98</sup> suggesting that TV directly influences microbial environment and confirms the potential importance of interactions between TV and vaginal microbiota.

Our findings show that BV has no effect on TV treatment outcome in women taking the 7 day course of MTZ treatment, the recommended dose for BV, clearly implying that drug inactivation by BV flora is not complete. In our previous studies, we proposed that MTZ treatment induces a state of latency in TV to the extent that many treatment failures are not detectable by culture shortly following treatment but are detectable after longer time intervals<sup>22,99</sup>. As the effect of BV on MTZ treatment outcome is only manifested soon after treatment there must be other mechanisms such as intrinsic relative resistance to MTZ in play to explain the fact that BV is not significantly associated with late single dose treatment failures. Whether BV influences TV treatment outcomes among HIV-negative women is unknown and will be explored in aim 2. In the preliminary work, we did not measure BV at the follow-up visits, so we could not determine if resolution of BV improves treatment effect of multi-dose MTZ. This present study will measure BV at all visits to determine if the BV was resolved and compare the effects of resolved and unresolved BV on TV treatment.

**Figure 3. TV prevalence by Nugent Score**



## 2.3 Potential Risks and Benefits

Every measure will be taken to protect the rights of subjects, protect confidentiality, and minimize the risk.

### 2.3.1 Potential Risks

Participants will be recruited from the patients receiving care at participating clinics. Because of the nature of the study, very personal information regarding sexual behaviors and STIs will be collected. The potential risk for these subjects fall into three categories: psychological risk, confidentiality risk, and physical safety risks.

*Psychological risk* – Many of the questions are highly personal in nature (e.g. sexual activity, substance use). These questions could evoke feelings of embarrassment, anxiety, fear or remorse. Through the biological testing, the participant could discover that they have an STI or are pregnant. There is also a potential that these biological tests could have a false positive or false negative result.

*Protection against psychological risk* - The participants will be told that they are not required to be in the study to receive a clinical exam and STI testing and/or treatment as per standard clinical protocols. Each clinical site will provide these services as part of their standard of care. A response of “refuse to answer” will be included on all survey questions. Participants will be told that she may choose not to answer any questions that make her feel uncomfortable. All surveys will take place in a private setting. Clinical protocols will be followed in case of a positive culture result at follow-up visits; therefore she will receive counseling and treatment as per typical clinic visit. Participants will be counseled that not every biological test is 100% accurate but the tests used for this study have very low error rate. If the participant needs further counseling, clinical protocols will be followed to refer her to a mental health counselor, social worker or other specialist.

*Confidentiality risk* –The interviews and surveys will elicit information on sexual behaviors and partnerships. They may feel vulnerable to breach of confidentiality answering personal questions about themselves and their partners. Contact information will be collected in order to conduct follow-up visits.

*Protection against confidentiality risk* –

- A Certificate of Confidentiality has been obtained from the Food and Drug Administration. This can be utilized to legally refuse to disclose information that may identify participants in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings, for example, if there is a court subpoena. When possible, this certificate will be utilized to resist any demands for information that would identify participants. The Certificate cannot be used to resist demand for information from

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personnel of the United States Government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

- Databases are housed on Tulane's HIPAA compliant servers. Access to these databases is password protected and is limited to authorized personnel (including select site staff, the Lead PI and the Tulane staff who handle data). All authorized personnel have undergone training in the handling of confidential data and have signed a confidentiality form.
- Survey data are temporarily located on site computers that are encrypted and password protected and are only accessed by authorized site personnel. These computers are provided by the study and are encrypted and maintained by Tulane's Informational Technology staff. Survey data is transferred to Tulane at least weekly after which Tulane staff confirms receipt of the data and requests that the data is deleted from the site computers. Data deletion is confirmed by communication between Tulane staff and the site staff who transferred the data and is checked at the next data transfer by Tulane staff. On site visits, confirmation that no data is stored on site computers is confirmed by the person conducting the site visit. Site computers are secured kept in a locked space when not in use.
- Any contact information collected by the interviewers will only be accessible to study staff at the site where it was collected, the Principal Investigator and authorized study staff at Tulane University. Once the study follow-ups are completed or the participant is considered lost to follow-up, their contact information is archived on a password protected computer in a password protected database at the Tulane study office in case of need to contact participants.
- Original paper contact forms and CRFs are kept in study folders at the sites along with the signed consent forms and are stored in secure file cabinets. Copies of CRFs are kept at Tulane University in secured cabinets.

*Physical risk* - Women may have some discomfort during her specimen collection; they may also be at risk for abuse if she discloses her TV status to the partner. The MTZ multi dose is not approved by the FDA for treatment of TV; however, it is a commonly used medical dose and is listed as an alternate treatment for non-pregnant and non-lactating women in STD treatment guidelines from the Centers for Disease Control and Prevention (CDC)

[\[http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6403a1.htm\]](http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6403a1.htm). Women may suffer increased side effects or adverse reactions from this dose. Metronidazole is a Class B medication that has been used safely in the first trimester of pregnancy but pregnancy outcomes have not been systematically described in prior studies and thus remain contraindicated for use in the first trimester as per the final product label. If a pregnant woman tests negative for pregnancy at enrollment and takes metronidazole there is a potential for danger to the fetus. If a subject becomes pregnant while in this study, the clinician may request permission to follow her during the pregnancy and to follow her child after delivery to document any possible adverse outcomes of exposure during pregnancy. Breast feeding women will not be enrolled in the study, however,

if a woman does not disclose that she is breast feeding and enrolls, the medication may pass through her breast milk to her nursing child and there is a potential for metronidazole-related medical problems with that child.

*Protection against physical risk* - For the specimen collection, women will be instructed on proper collection techniques and will collect specimens in a private and clean room which will be maintained at a comfortable temperature. Women will be counseled about the investigational dose provided and potential side effects and adverse reactions will be described. Participants will be advised to contact their provider if any such events occur. At any time a woman wishes to discontinue participation in the study, she may with no consequences to her primary health care. For the danger of abuse from a sexual partner, women will be reminded that if they feel that any of their partners will have such a reaction, they should not tell them about her TV infection; she will also be referred to additional counseling as needed per clinic protocol.

Pregnancy and breastfeeding are exclusion criteria. Women of child bearing potential who have not been tested for pregnancy during her clinic visit on the day of enrollment will be tested for pregnancy by study staff (post-consent and pre-randomization) to ensure that she is not pregnant. Women who have had a hysterectomy or are post-menopausal for at least two years will not be required to take a pregnancy test. Staff will ask women about their breast feeding status prior to enrollment. Any woman who is breast feeding will not be eligible for the study. Women will be advised to use two forms of birth control while taking study medications.

### **2.3.2 Potential Benefits**

There are a few benefits. The potential benefit to the woman is that she will be receiving more intensive follow-up of her TV infection. She will also have the opportunity to have her specimen tested for drug resistance (which is not routinely done at the clinic).

Information from this research study will be used to improve treatment options for TV-positive women. Given the high prevalence of TV, the absence of a national screening program, the deleterious reproductive outcomes associated with TV and the potential for a TV infection to increase HIV transmission, reducing repeat TV infections is an important targeted public health approach. The overall goal of this project is to determine the influence of index treatment and host factors on repeat TV infections among HIV-negative women in two specific objectives. The ultimate goal is improved reproductive health for women, particularly minority women, and the reduction of the potential for HIV transmission, fitting well with NIAID's mission.

### **3. STUDY OBJECTIVES**

#### **3.1 Primary Objective**

1. To determine if women receiving multi-day MTZ will be less likely to test TV positive at TOC compared to women who receive the single dose MTZ.
2. To examine if this effect (H1) will only be observed among women who have BV (Nugent score  $\geq 7$ ).

#### **3.2 Secondary Objectives**

To determine more precisely the origin of a TOC positive result using the results of the genotyping and the sexual histories and to determine if most of the infections are treatment failure, re-exposure, sexual exposure to a new partner or organism lack of susceptibility to MTZ.

#### **3.3 Exploratory Objectives**

Examination of self-reported adherence to medication, subject knowledge of partner treatment, sexual exposure and the nature of the sexual act and sexual partnering, and condom use will be possible using the ACASI survey data. These factors will be assessed by arm to determine if any confounding occurred and if so, these analyses will be adjusted for these factors.

### **4. STUDY DESIGN**

#### **4.1 Description of the Study Design**

To achieve the 2 aims, we will conduct a phase III randomized clinical trial among TV infected HIV-negative women (N=1664) attending clinics in New Orleans, LA, Birmingham, AL and Jackson, MS. These public clinics serve mostly African American, low income women, the demographic group most highly affected by TV<sup>26</sup>. Informed by our prior RCTs, women will be randomized to a single dose or multi-dose arm. Test of cure will be conducted at 4 weeks post treatment completion. If at TOC, a subject is NAAT TV+/InPouch TV- she may be asked to come back 4 weeks post TOC for an 8 week follow up. Viable specimens of women who are TV+ at TOC will undergo MTZ susceptibility testing and TV genotyping (to compare baseline and follow-up types). A baseline, TOC, and 8 week follow up specimen will be stored for future microbiome studies of the vaginal flora for women who consent to long term specimen storage and use. The information obtained from this study will be used to refine treatment recommendations for TV. Given the strong multi-centered, RCT design, and the large sample size, results should rapidly inform treatment guidelines thus having high impact. The follow-up survey and specimen collection will be repeated for those who return for an 8-week follow-up visit.

## 4.2 Study Endpoints

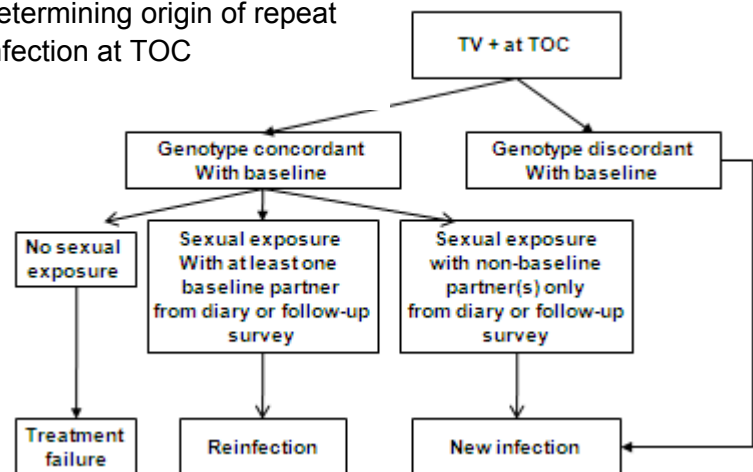
### 4.2.1 Primary Endpoint

The primary endpoint for both aims is a TV infection identified by culture or NAAT at the TOC visit.

### 4.2.2 Secondary Endpoints

The study is powered for repeat infections. Since we will have information on sexual exposure and genotyping, we can also refine this outcome measure to examine treatment failure (H1 & H2) and will use the algorithm depicted in figure 4.

**Figure 4:** Algorithm for determining origin of repeat infection at TOC



### 4.2.3 Exploratory Endpoints

Examination of index adherence to medication, index knowledge of partner treatment, sexual exposure and the nature of sexual acts and sexual partners, and condom use will be possible using the ACASI survey data. These factors will be assessed by arm to determine if any confounding occurred and if so, these analyses will be adjusted for these factors or stratified by these factors.

### 4.2.4 Substudy Endpoints

N/A

## 5. STUDY POPULATION

### 5.1 Description of the Study Population

TV-positive women (N=1664) will be enrolled from three sites, Crossroads Clinic in Jackson, MS, STD Specialty Clinic in Birmingham, AL and CrescentCare Health and Wellness Center and in

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New Orleans, LA. Approximately 95% of the clinics' populations are African American. Potential participants will be patients at the sites who have tested positive for TV by standard clinical screening during their routine clinic appointment and who meet the inclusion/exclusion criteria.

### **5.1.1 Participant Inclusion Criteria**

To be eligible for the study, all subjects must meet the following inclusion criteria: female; English speaking; age of majority; test TV positive during their clinical visit; have the ability to agree to refrain from all alcohol use for 24 hours after taking oral MTZ; be willing to take MTZ treatment; and be willing to be randomized to either arm of the study.

### **5.1.2 Participant Exclusion Criteria**

Subjects who meet any of the following exclusion criteria will not be able to participate in the study: HIV-infected; pregnant, breast feeding; treated by their provider for BV at visit; previously enrolled in the study; incarcerated; have medical contraindications to MTZ (such as currently taking disulfiram, alcoholism or known liver damage, taking anticoagulants/blood thinners); have been treated with any meds used to treat TV or BV including MTZ, tinidazole, seconidazole, acetarsol, boric acid, furazolidone, and paromomycin within the previous 14 days; unable or unwilling to provide informed consent; unwilling to return for a follow-up visit 4 weeks post treatment completion (3-13 weeks post treatment completion).

## **5.2 Strategies for Recruitment and Retention**

Women who are TV positive per clinical screenings will be referred to study staff by their clinical providers. Eligibility criteria will be reviewed by study staff and informed consent obtained. During consent, follow up visits will be described to the participant. At baseline, detailed subject contact information and preferred methods of contact will be obtained and reviewed. Study staff will remind participants of their follow up visits via text messages, email, phone calls, or mail depending on the preference of the participants. If a participant misses her scheduled follow up appointment, study staff will contact her to reschedule.

Prior to their follow up visits, women will be contacted by study staff via their preferred method (i.e., cell phone, text message, email) as a reminder. If they miss their scheduled visit, study staff will attempt contact at least five times to reschedule. In our prior research, we found that 95% of women have either daily internet or text messaging use, so these methods of enhancing follow-up will be used if patients prefer them. All contact attempts will be noted in the participant's file. At follow up visit, contact information and preferred methods of contact will be updated in the case that the TOC specimens are positive.

## **6. STUDY AGENT/INTERVENTIONS**

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## **6.1 Study Agent Acquisition**

### **6.1.1 STUDY AGENT/INTERVENTION # 1 -- Metronidazole**

#### *6.1.1.1 Formulation, Packaging, and Labeling*

Generic MTZ will be provided on site at all clinics. Treatment will be given by the study provider (RN, NP, MD). Medication that is not consumed in the clinic will be in containers with child-proof caps, labeled with dose, instructions, and contact information for the clinic and the study personnel. Also warning labels will be on the container (for multi-dose) which will instruct users to refrain from alcohol consumption and to not take if there are known allergies to MTZ.

Subjects receiving the single dose will be provided the information verbally. Participant dose will be determined at randomization. Half of the women (n=832) will be randomized to receive the single dose and the other half (n=832) will be randomized to receive the 7 day dose.

MTZ 2 g single oral dose - (CDC recommended treatment regimen) Following randomization, women assigned to the single dose arm will be given the 2 g oral dose of MTZ under direct observation. If for any reason a participant is unable to take the medication DOT, this will be recorded in their study chart and adherence questions will be asked at the follow up visit.

MTZ 500 mg oral dose BID for 7 days - (CDC alternative treatment regimen) Following randomization, women assigned to the 7 day arm will be given the 500 mg oral dose BID for 7 days with the first dose given under direct observation. If for any reason a participant is unable to take the first dose DOT, this will be recorded in their study chart and adherence questions will be asked at the follow up visit.

### **6.1.2 Co-enrollment Guidelines**

Participants of this study are eligible to co-enroll in any other studies being conducted at the clinical sites during their enrollment in this study excluding studies that require treatment with metronidazole.

### **6.1.3 Preparation, Administration, Storage, and Dosage of Study Agent(s)/Intervention(s)**

Metronidazole will be stored at room temperature and protected from light. Doses will be packaged at the clinic by approved pharmacists/clinicians. Expiration dates will be noted and inventory checked by study coordinators and lot numbers will be documented. This is not a blinded study therefore no placebo pills will be given to women in the single dose arm.

#### *6.1.1.3 Study Agent Accountability Procedures*

Metronidazole for the study will be obtained by the investigators following approved guidelines at each clinical site. Each site will follow a pharmacy plan that is approved by their institutional IRB(s) and acknowledged by Tulane IRB. Metronidazole will be stored at room temperature, and away from heat, moisture, and light. Procedures for medication dispensing will follow

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approved pharmacy plans. An inventory of all medication received and distributed will be maintained by the study coordinator/nurse.

#### **6.1.4 STUDY AGENT/INTERVENTION #2**

N/A

### **6.2 Assessment of Participant Compliance with Study Agent(s)/Intervention(s)**

Participants randomized to the single dose of MTZ will be asked to take their medication at the clinic under direct observation. If there are any complications taking the medication, it will be noted on her study folder.

Those randomized in the 7 day arm will be asked to take their first dose under direct observation at the clinic and instructed to take the additional doses over the following six days.

At the follow up visits, participants will be asked to report adherence to medication on their ACASI surveys.

### **6.3 Concomitant Medications and Procedures**

We will capture concomitant medications and co-occurring STIs at baseline on the CRF when this information is available.

### **6.4 Precautionary and Prohibited Medications and Procedures**

#### **6.4.1 Prohibited Medications and Procedures**

None

#### **6.4.2 Precautionary Medications and Procedures**

If the participant was not tested for pregnancy during her clinical visit and she is of child bearing potential, she will be tested by study staff after consent and pre-randomization to ensure that she is not pregnant. Women who have had a hysterectomy or are post-menopausal for at least two years will not have pregnancy testing. If it is determined that an enrolled participant is pregnant per her study pregnancy test, she will receive her baseline compensation and be administratively withdrawn from the study. These women will not receive a study ID number. Participants will be advised that alcohol should be avoided because metronidazole and alcohol together can cause severe nausea, vomiting, cramps, flushing, and headache. Metronidazole

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can increase the blood thinning effects of warfarin (Coumadin) and increase the risk of bleeding probably by reducing the breakdown of warfarin. Cimetidine (Tagamet) increases blood levels of metronidazole while cholestyramine reduces blood levels of metronidazole by reducing its absorption. Metronidazole blocks the breakdown of propylene glycol in the liver leading to accumulation of propylene glycol in blood. Accumulation of propylene glycol could cause seizures, increased heart rate, and lead to kidney failure. Metronidazole increases the blood levels of carbamazepine (Tegretol, Tegretol XR, Equetro, Carbatrol), lithium (Eskalith, Lithobid) and cyclosporine through unknown mechanisms. Serious reactions may occur if these drugs are taken with metronidazole

## **6.5 Prophylactic Medications and Procedures**

N/A

## **6.6 Rescue Medications**

N/A

# **7. STUDY PROCEDURES/EVALUATIONS**

## **7.1 Clinical Evaluations**

Baseline clinical evaluation will be provided during regularly scheduled clinical exam prior to enrollment including screening for TV.

At enrollment, women will be asked to provide self-collected vaginal swabs for InPouch culture, NAAT, Gram stain testing for bacterial vaginosis (BV) and storage for future microbiota studies (FMS). The same procedure will be done at 4 week and 8 week follow up visits, however, at 4 weeks the sites will have the option to obtain an additional vaginal swab for wet prep testing for TV.

At each visit, medical records will be abstracted (when those records are available) for clinical data which may include signs/symptoms and co-infections.

If a participant is positive by InPouch culture at her Test of Cure visit, and she has a viable organism, it will be tested for metronidazole sensitivity.

## 7.2 Laboratory Evaluations

### 7.2.1 Clinical and Research Laboratory Evaluations and Specimen Collection

Vaginal secretions will be used for Gram stain, InPouch testing, NAAT, TV genotyping, metronidazole resistance testing, wet prep, and FMS for participants who consented for FMS.

There will be eight laboratory activities: 1) InPouch Culture readings, 2) InPouch culture processing for TV+ specimens, 3) Gram stain readings, 4) MTZ susceptibility testing, 5) storage of FMS swabs, 6) NAAT testing, 7) wet prep readings (where applicable) and 8) prepping of genotyping specimens. All laboratory activities and staff expertise and procedures will be reviewed as required by the participating laboratories (i.e., LSUHSC, UAB, UMMC, CDC and NYU).

**Biohazard Containment:** Laboratory personnel will follow their institutional requirements for biosafety procedures training. Universal Precautions for blood and body fluids will be followed. Study personnel will routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with specimens. 1. Gloves will be worn handling specimens and handling items or surfaces soiled with body fluids. Gloves will be changed after contact with each patient. 2. Hands and other skin surfaces should be washed immediately and thoroughly if contaminated with body fluids. Hands will be washed immediately after gloves are removed. 3. Any items used for the collection or storage of biological specimens will be disposed of using the clinics' biohazard containment and disposal systems.

### 7.2.2 Specimen Preparation, Handling and Shipping

All specimens will be labeled with the study id number and date of collection.

InPouch cultures will be read at the clinic's corresponding laboratory. Any positive baseline cultures will be processed and frozen for further testing. Frozen specimens will be stored at the site laboratories until shipment is required. Positive test of cure specimens may also be shipped live to the CDC for sensitivity testing.

Wet prep at TOC will be an option for clinical sites that are able to perform wet preps for TV. Wet prep reads will be performed by study staff in order to have a point of care for repeat TV infections.

FMS swabs will be frozen and stored at the clinic's corresponding laboratory until shipment is requested. FMS shipments to the LSUHSC lab will occur annually. Notation of consent to use this swab for future use is found in REDCap.

NAAT specimens will be collected at the sites and shipped to the LSUHSC or UAB laboratory for processing at least once per month

Handling and shipping - A shipment manifest will be emailed prior to and included with all shipments. This manifest will be checked by the receiving laboratory staff for completeness of shipment. If any discrepancies arise, the receiving laboratory will contact the shipping laboratory for clarification. Any aberrations in shipment will be reported to the PI.

There will be six types of specimen shipments.

- 1) Transport to local labs- All specimens collected from the clinical sites (except TOC wet preps which will be read at the clinical site) will be couriered to their corresponding laboratories for processing. The specimens will be contained in biohazard packaging and carried in a climate controlled container to the labs.
- 2) Live specimens for metronidazole resistance testing at TOC will shipped to Dr. Evan Secor, of the CDC's parasitology laboratory, from all laboratory sites. These specimens contain only study ID and no other personal identifiers. Dr. Secor will perform susceptibility testing on the specimens of women who have a repeat infection at test of cure follow-up. Positive live cultures will be kept incubated until ready for shipment, and then shipped to the CDC laboratory via overnight shipping. If necessary, the pouch may be inoculated with fresh culture medium if it cannot be immediately shipped (weekends or holidays) or if the parasites are at risk of dying because of length of time in the incubator before positive read. Shipment of InPouch will follow all standard regulations for shipment of Category B biological substances. InPouch will be securely closed after all fluid is pushed down into the lower chamber. The InPouch will be labeled with date of collection and study identification number. The InPouch will be wrapped in an absorbent material and placed into a sealable leak proof bag. The bag will be placed inside of a rigid container and sealed. A UN3373 label will be placed on the container before being placed into the Clinical Pak.
- 3) Frozen specimens (processed cultures) will be shipped from the three laboratories to NYU laboratory for genotyping. If a live specimen shipment to the CDC (#2) is not a viable option, a frozen TOC specimen may also be shipped to CDC. Frozen specimens will be shipped according to established laboratory protocols for Category B shipment of frozen specimens using dry ice. The boxes will be shipped overnight. Cryoboxes with frozen specimens will be surrounded with absorbent material and placed in a sealed plastic bag. This bag will be placed in an insulated shipment box with dry ice.
- 4) Gram stain specimens from UMMC and UAB laboratories will be batch shipped to LSUHSC for reading; specimens collected at the New Orleans clinical sites will be delivered to the LSUHSC lab by courier at least weekly.
- 5) FMS frozen specimens will be batched shipped from the UMMC and UAB laboratories to LSUHSC laboratory annually. These specimens will be stored for future microbiota studies; specimens collected at the New Orleans clinical sites will be delivered to the LSUHSC lab by courier at least weekly.

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6) NAAT specimens will be batch shipped to the LSUHSC or UAB for testing. Specimens should be labeled directly on the manufacturer label with a fine point Sharpie marker and each tube placed in individual Ziploc bags prior to being placed in a larger biohazard bag for shipment.

### **7.2.2.1 Instructions for Specimen Storage**

InPouch cultures will be stored in an incubator (37°C) at the clinical and laboratory sites until final result is recorded. Positive baseline specimens will be processed and stored frozen (-70 °C or colder) at each laboratory site until shipment to NYU. Gram stain slides are stored at room temperature at the laboratory sites until shipment to LSUHSC lab for reading. Gram stains will be read at LSUHSC. TV specimens for future microbiota studies (FMS) will be stored frozen (-70 °C or colder) at each laboratory site until annual shipment to LSUHSC where they will be stored frozen (-70 °C or colder) until future microbiota research resumes. Participants will have the ability to opt out of their FMS specimen storage/usage during the consent procedures. There will be a paragraph in the consent that outlines storage and use of frozen specimens, participants may choose to not have their specimens stored for long term use and still be able to participate in the study. If the participant opts out of future use of specimens, this will be indicated in REDCap and laboratories will be alerted to that status. At the end of the study, specimens of women who did not consent for specimen storage will be disposed of.

### **7.2.2.2 Specimen Shipment Preparation, Handling and Storage**

InPouch Culture – InPouch cultures will be collected at the clinic sites and processed at the clinics' corresponding laboratories. Once collected, the culture will be placed in an incubator at the clinics with a regulated temperature of 37°C.

InPouch specimens are couriered to the corresponding laboratories where trained technicians will be required to obtain at least 3 readings separated by at least 24 hours within a 7 day period or until a positive TV pouch result. A diagnosis of TV will be made after the first positive pouch reading. If all InPouch readings within the 7 day period are negative, the woman will be considered TV negative. The pouches will be labeled with the study identification number and date of collection. Results from all reads will be kept in a laboratory log at each site.

Gram stain - Once the Gram stain slides are received at the LSUHSC they will be examined under low power magnification to find areas that have a concentrated amount of material to yield accurate readings. Then under oil immersion, 5 noncontiguous fields are examined that are representative of the material seen on the slide. These data are entered into the laboratory data form for each field. Results are entered into REDCap which calculates the Nugent score. Gram stain results will not be used for the clinical diagnosis of BV; they will only be used for research purposes.

T. vaginalis susceptibility testing - Dr. Evan Secor, of the CDC's parasitology laboratory, will

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perform susceptibility testing on the viable specimens of women who have a repeat infection at follow-up. Upon arrival at the CDC, parasites will be prepared according to CDC protocol<sup>100</sup>. In brief, they will be cultured in modified Diamond's TYM medium at 37°C and transferred every 2 days until an axenic culture is obtained. Duplicate assays for drug resistance will be performed and isolates will be cryogenically preserved in Diamond's TYM medium supplemented with 5% dimethyl sulfoxide (DMSO) and agar. Metronidazole susceptibility will be assessed separately for each isolate in this study, after log growth is achieved. In brief, a stock solution (32 mg/ml) of metronidazole will be prepared in DMSO. Trichomonads ( $10^4$ ) will be added to two-fold serial dilutions (400-0.2 µg/ml) of metronidazole in 96-well plates. After incubation, the plates will be examined using an inverted microscope to determine the MLC, the lowest drug concentration at which no motile trichomonad was observed. Metronidazole susceptibility is defined by a MLC from 0.2 to 25 µg/ml. Low to moderate metronidazole resistance was defined by a MLC from 50 to 100 µg/ml. High metronidazole resistance was defined by a MLC 200 µg/ml or greater. The prevalence of in-vitro drug resistance will be compared by treatment study arm using chi-square test to determine if there is any indication drug resistance could account for some of the treatment failure. If the rates are similar by arm, we will ignore this potential confounder.

Prepping of InPouch specimens for TV genotyping- All positive InPouch cultures will be allowed to grow to a minimum of 50 parasites/field for up to 7 days. Then, 3 ml of the InPouch culture will be removed from the pouch and spun down. The pellet will then be resuspended in 2ml of 10% DMSO in fresh Diamond's media supplemented with 10% Horse serum. The suspension will then be split between two internally-laced cryotubes and stored at -70°C or colder until shipped. The stabilates will be sent to NYU on dry ice overnight and shipments will follow all standard regulations for shipment of Category B biological substances. Each clinical site laboratory will perform the specimen prep and batch freeze. Frozen specimens will be shipped to NYU according to a schedule determined by the PI.

TV Genotyping methods - Until recently, genotyping of *T. vaginalis* was limited by the crudeness of the genotyping techniques, e.g. RAPDs and RFLPs<sup>101-105</sup>. These methods are highly sensitive to contaminating DNA or to slight variations in assay conditions, which influence their ability to accurately characterize *T. vaginalis* isolates. A recently developed panel of 21 *T. vaginalis*-specific microsatellites<sup>106</sup> now provides a sensitive, reproducible, highly discerning set of genetic markers to accurately determine isolate-specific haplotypes and mixed genotype infections. Because of the high polymorphism of these markers and the number of loci that can be assayed, minor variations in haplotypes can be detected, allowing for high-resolution discrimination between related strains. Dr. Carlton's laboratory at NYU will genotype baseline and repeat infection specimens from all patients testing positive for TV at follow-up (~40 per year, ~120 total over 3 yrs). Frozen stabilates will be sent to NYU overnight and shipments will follow all standard regulations for shipment of Category B biological substances. Both isolates from each individual will be genotyped at 11 MS loci as described<sup>106</sup> with minor modifications. Briefly, microsatellite-specific NAAT reactions will be performed for each locus using fluorescently labeled primers, and fragment length polymorphisms at the loci will be measured

by capillary electrophoresis on an ABI 3130xl sequencer and scored using GeneMapper 4.0.

The haplotypes determined for the baseline and repeat infection will be compared to make inferences about the origin of reinfection. In addition, ~120 non-repeat infections (~40 each year) will also be genotyped to identify local population genetic characteristics and address the issue of confounding population structure.

NAAT swabs will be placed in a GenProbe vaginal collection tube and stored at 2-30°C. Swabs will be couriered daily from the clinic to the clinical laboratories and shipped at least monthly to the LSUHSC or UAB laboratory. Shipment will follow all Category B shipping restrictions. Upon arrival, DNA isolation will be performed using published methods and nucleic acid diagnostic results will be returned within one week.

Future microbiome study (FMS) swab – The vaginal swab will be placed in a tube containing 100ul of nucleic acid preservative. The tube will be maintained at room temperature following the manufacturer's recommendations until transport to the laboratory where it will be frozen at -70°C or colder until processing for the extraction of DNA

### **7.3 Substudies**

There are presently no sub-studies but the FMS may be added, pending provision of funding.

## **8 STUDY SCHEDULE**

### **8.1 Screening**

Per standard clinic protocol at each site, a patient's clinical examination will include testing for TV. Pregnancy testing by the clinic will follow their standard clinical care guidelines. If the patient self-reports that she is HIV negative, is not pregnant as determined by clinical protocol, not treated for BV and TV positive by wet prep, she will be referred by her clinician to the study personnel for potential enrollment. At sites where NAAT screening is performed, women may be referred because of a NAAT TV+ result. Study staff will discuss the study with the patient, review eligibility criteria and obtain informed consent.

### **8.2 Enrollment/Baseline**

The study will be described to potential participants by study staff and any questions will be answered and documented in the study records. Each site has an eligibility checklist for the study staff to use to confirm eligibility. As part of her eligibility checklist, staff will determine if a potential participant was given a pregnancy test at her clinical visit and confirm that the test was negative or that she had a hysterectomy or is post-menopausal for at least two years. If the woman is willing to participate and is eligible for the study, staff will obtain informed

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consent and provide a copy to participant. After consent is obtained, if a woman did not provide a urine pregnancy test as part of her clinical visit or meet the other criteria to determine she was not pregnant, she will be asked to provide a urine sample for testing. If the test is positive, women will be told that she is ineligible to continue with the study protocol since pregnancy is an ineligibility criterion. Study staff will provide her with her baseline compensation and refer her for pregnancy counseling as per each clinic's standard of care. The participant will be withdrawn prior to randomization and her randomization number will be used for the next participant. A participant may also be withdrawn from the study prior to randomization if she is found to meet other ineligibility criteria post-consent and pre-randomization. In these cases, she would receive her compensation and her randomization number would be used for the next participant.

If the participant is negative for pregnancy, she will undergo the audio computer assisted self-interview (ACASI). This survey will take approximately 30-45 minutes to complete and will be conducted after the clinical exam and written informed consent is obtained but before randomization to treatment. Information elicited will include detailed information about participants' demographics, socioeconomics, STI risk behavior and symptoms, substance use, and birth control methods, as well as partner specific information about sexual behavior, condom usage, and partnership status.

Women will be asked to self-collect vaginal swabs for Gram stain testing, In Pouch, TV NAAT and FMS. The participant will be given three or four sterile swabs and tubes and instructed on how to self-swab, place the swab in the tubes and return to the study office.

Study staff will process the specimens: for Gram stain, the swab will be carefully rolled by study staff over a predefined area of a glass slide, air dried, and stored in a slide cover; for FMS, the swab will be placed in a tube with preservative; for InPouch, the swab will be placed in the culture medium; for NAAT, the swab will be placed in the GenProbe tube. The specimens will be labeled with study identification numbers and dates of collection.

She will then be randomized to treatment arm (Single dose or 7 day dose). A blocked randomization scheme will be used to avoid "runs" in the randomization process. Pre-numbered, sealed envelopes will be selected sequentially and opened for each participant who will then be enrolled in the indicated arm.

If a woman is randomized into the single oral dose MTZ arm, she will be given the 2 g dose of MTZ and asked to ingest it under direct observation. If a woman is randomized into the 7 day oral dose MTZ arm, she will be given the 500 mg BID for 7 days and asked to ingest the first dose under direct observation.



All participants will receive the same counseling: to refrain from unprotected sexual intercourse until 1 week after they and their partner(s) complete the medication, to refrain from alcohol consumption while taking the medication and for 24 hours after completion; MTZ-related adverse events will be discussed including rash, dizziness, headache, diarrhea, nausea, vomiting, fatigue, upset stomach, dark red-brown urine, and change in taste sensation (metallic taste) or dry mouth. It will also be explained that MTZ can cause urine to darken in color but that this effect is not harmful. Participants in the 7-day arm will be given additional counseling on the importance of taking all doses of the medication.

If a participant's clinical TV positive test result is not confirmed TV-positive by InPouch culture or NAAT, she will be withdrawn from the study. These testing procedures and de-enrollment potential will be fully explained in the consent form. Women will receive \$20 compensation after completion of the baseline enrollment visit. Women will receive a study contact card that has all contact information for study personnel so that she may contact staff in case of appointment change, questions, or adverse events. She will be instructed to keep this card during the course of the study period. If she is withdrawn, she will keep her baseline compensation but told she will not need to attend her follow-up visits.

### **8.3 Follow-up**

Participants will be scheduled for a Test of Cure (TOC) follow up visit 4 weeks after treatment completion with a window of 3-12 weeks post-baseline for the 2 gm MTZ dose and 4-13 weeks post-baseline for the 7 day MTZ dose. Prior to the follow-up visit, women will be contacted by study staff via their preferred method (i.e., cell phone, text message, or email) to remind them of their study visit. If they miss their scheduled visit, study staff will attempt contact at least five times. All contact attempts will be noted in the participant's file.

At follow-up visits, she will undergo the ACASI survey, and testing for TV and BV. For specimen collection, women will be reminded of vaginal self-swab procedures and asked to collect up to five vaginal swabs, place them in the sterile tubes and return to study office. Study staff will place one swab in the InPouch, one in the FMS tube, one for NAAT and use the other swab to prepare a Gram stain slide as described above. For sites that will perform a wet prep at TOC, a swab will be prepared for wet prep and read by the study staff. Specimens will be labeled with study identification number and date of collection. Procedures for processing these specimens and reporting of results will be same as described for baseline procedures. Any positive InPouch or wet prep will be reported to the participant's provider at the clinic to arrange for treatment. The study does not provide medication for participants after baseline enrollment and initial treatment. In the case of a TV positive result at follow up, clinical staff will provide treatment in accordance with clinic protocols. Gram stain results are for study evaluation only and will not be used as a diagnostic tool for providers. After completion of visit, participant will receive \$50 compensation; contact information and preferred methods of

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If a woman is TV positive by InPouch culture at her TOC visit, she will have concluded her follow up visits. If, however, a woman is TV negative by InPouch but TV positive by NAAT testing, she may be asked to return for a follow-up visit 4 weeks post TOC visit (3-8 weeks after her TOC visit) for further evaluation. At the follow-up visit 4 weeks post TOC, she will be asked to self-collect up to five vaginal swabs and complete an ACASI survey as in her TOC visit. She will be given \$50 compensation and contact information and preferred methods of contact will be updated. If she tests TV positive by InPouch at this visit, results will be provided to clinical staff in order for her to receive treatment as standard clinical protocol.

### **8.4 Final Study Visit**

The final study visit will either be the TOC visit at 4 weeks post treatment completion or a follow-up visit 4 weeks post TOC visit (if she was NAAT+/InPouch- at her TOC visit and asked to return).

### **8.5 Early Termination Visit**

After providing informed consent and undergoing randomization, it is possible that a woman will refuse the intervention. If this happens, she will still receive her baseline compensation for dedicating time to the study up to that point (the randomization is near the end of the baseline visit). She will be referred back to her clinician for treatment of TV as per clinical protocol. Her self-collected vaginal swabs will be discarded.

### **8.6 Pregnancy Visit**

Women will not be enrolled if they are pregnant. If they become pregnant during the course of the study, it will not affect study enrollment because they would have already been treated for TV. If it is determined that the participant was pregnant at the time of treatment, the woman and her child will be followed according to the site specific regulatory requirements.

### **8.7 Unscheduled Visits**

Since the study is housed within the clinics, there may be times when a participant has a clinic visit and is not scheduled for a study visit. If this occurs and the participant inquires about completing their study visit during their clinic time, study staff will review the participants file to see if she is within her follow up window, if so, she can complete her follow up visit, if not, she will have to return at her scheduled date/time.

If a participant has persistent symptoms or other complaints and comes to the clinic for an unscheduled visit, she will be referred to the clinic staff by the study staff for medical evaluation

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and treatment. She will keep her regularly scheduled TOC visit and her interim clinic visit will be noted.

The following is a schedule of visits and subject compensation at each visit

#### Study Visit Schedule

Visit Number	When is it?	Acceptable window of time to complete	Compensation
Visit 1	Date subject enrolls in study	N/A	\$20
Visit 2	4-weeks after scheduled completion of the medicine	If 1-day dose: 3 weeks-12 weeks after visit 1	\$50
		If 7-day dose: 4 weeks-13 weeks after visit 1	\$50
Visit 3 *only if applicable	4-weeks after visit 2	3 weeks-8 weeks after visit 2	\$50

\*You may be scheduled for this visit **only** if the NAAT is positive **and** the culture is negative.

## 9. ASSESSMENT OF SAFETY

### 9.1 Specification of Safety Parameters

Metronidazole in the 2gm dose is an FDA approved treatment for TV. The 7 day 500mg BID dose is recommended in the CDC treatment guidelines for TV, however, is not currently FDA approved.

There are three main safety concerns:

- 1) Adverse drug reactions including, allergy, hypersensitivity reaction, or other adverse reaction to metronidazole, alcohol use within 24 hours of taking medication, and contra-indications with other medications.
- 2) Pregnancy in a woman who tested negative at entry.
- 3) That discussing their infection with partners may put participants at risk for verbal or physical abuse by disclosing their STI to their partners.

## 9.2 Definition of an Adverse Event (AE)

Vomiting within 2 hours of consumption of the medication with no other signs of allergic reaction to MTZ will not be considered an adverse event but will be documented. Adverse events include eye pain, sudden vision changes, sore throat, persistent fever, unusual bleeding/bruising, severe stomach pain, persistent nausea/vomiting, fatigue, hypersensitivity reaction or other adverse reaction to MTZ.

Any form of intimidation, verbal or physical abuse will be considered an adverse event.

## 9.3 Definition of a Serious Adverse Event (SAE)

An adverse event is considered “serious” if in the view of the Investigator, it results in any of the following outcomes: death, a life threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Serious adverse events include seizures, encephalopathy, aseptic meningitis, optic and peripheral neuropathy, leukopenia, and a hypersensitivity reaction including Stevens-Johnson syndrome and toxic epidermal necrolysis.

## 9.4 Methods and Timing for Assessing, Recording, and Analyzing, Managing Safety Parameters

MTZ has been used for the treatment of TV for over 40 years. However, we will monitor adverse reactions in an ongoing basis and by the DSMB every 6 months.

The most serious adverse reactions reported in patients treated with MTZ have been convulsive seizures, encephalopathy, aseptic meningitis, optic and peripheral neuropathy, the latter characterized mainly by numbness or paresthesia of an extremity. Since persistent peripheral neuropathy has been reported in some patients receiving prolonged administration of MTZ, patients should be specifically warned about these reactions and should be told to stop the drug and report immediately to their physicians if any neurologic symptoms occur. The most common adverse reactions reported have been referable to the gastrointestinal tract, particularly nausea reported by about 12% of patients, sometimes accompanied by headache, anorexia, and occasionally vomiting; diarrhea; epigastric distress; and abdominal cramping. Constipation has also been reported. The following reactions have also been reported during treatment with MTZ: *Mouth*: A sharp, unpleasant metallic taste is not unusual. Furry tongue, glossitis, and stomatitis have occurred; these may be associated with a sudden overgrowth of *Candida* which may occur during therapy. *Hematopoietic*: Reversible neutropenia (leukopenia); rarely, reversible

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thrombocytopenia. *Cardiovascular*: Flattening of the T-wave may be seen in electrocardiographic tracings. *Central Nervous System*: Encephalopathy, aseptic meningitis, convulsive seizures, optic neuropathy, peripheral neuropathy, dizziness, vertigo, incoordination, ataxia, confusion, dysarthria, irritability, depression, weakness, and insomnia. *Hypersensitivity*: Urticaria, erythematous rash, Stevens-Johnson Syndrome, toxic epidermal necrolysis, flushing, nasal congestion, dryness of the mouth (or vagina or vulva), and fever. *Renal*: Dysuria, cystitis, polyuria, incontinence, and a sense of pelvic pressure. Instances of darkened urine have been reported by approximately one patient in 100,000. Although the pigment which is probably responsible for this phenomenon has not been positively identified, it is almost certainly a metabolite of metronidazole and seems to have no clinical significance.

*Other*: Proliferation of *Candida* in the vagina, dyspareunia, decrease of libido, proctitis, and fleeting joint pains sometimes resembling "serum sickness." If patients receiving MTZ drink alcoholic beverages, they may experience abdominal distress, nausea, vomiting, flushing, or headache. A modification of the taste of alcoholic beverages has also been reported. Rare cases of pancreatitis, which generally abated on withdrawal of the drug, have been reported. Crohn's disease patients are known to have an increased incidence of gastrointestinal and certain extra-intestinal cancers. There have been some reports in the medical literature of breast and colon cancer in Crohn's disease patients who have been treated with metronidazole at high doses for extended periods of time. A cause and effect relationship has not been established. Crohn's disease is not an approved indication for MTZ.

See Section 10 for a greater description of the monitoring process and <http://www.rxlist.com/flagyl-drug.htm> (medication insert) for more details on side effects.

### **9.4.1 Methods and Timing for Assessment**

Adverse events reported by participants will be recorded and reported to the institutional IRBs according to their reporting requirements. At each follow up visit, staff will inquire if any metronidazole was taken since last visit and if there were any adverse events from taking the medication. This information will be recorded in study data.

#### **9.4.1.1 AE/SAE Grading and Relationship Assignment**

Serious adverse events to metronidazole by participants will be recorded by severity and relation to dosing at each clinical site. These will be immediately reported to the Lead PI, other Investigators, and institutional IRBs according to their reporting requirements. DSMB will be notified.

### **9.4.2 Recording/Documentation**

A standard event reporting form will be used by all sites. In the event of an adverse event, it will be mandated that this form must be filled out by study personnel within 24 hours of the

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reporting of the event and sent to the investigators for review. A complete description of the adverse event will include time of onset, length of event, relation to metronidazole, and resolution and severity of event.

### **9.4.3 Analysis/Management**

Adverse events will be considered in the analysis of the endpoint in cases where they affect adherence to the drug.

## **9.5 Reporting Procedures**

The Lead PI and the site PI(s) will discuss the reported event and decide if it meets the standard for institutional IRB reporting of adverse events. If so, they will report the event according to their IRB reporting requirements. The DSMB will also be notified of the AE or SAE by the Lead PI. The site investigators will be notified to discuss the event and resolution with the patient to make certain the participant is treated appropriately.

### **9.5.1 Specific Serious Adverse Event Requirements**

All serious adverse events will be recorded on the case report form as SAE. Investigators at the clinical site who are the study physicians will work with the Principal Investigator to resolve the SAE. The study physical will follow up with participants and report resolution success to the PI.

Any AE that meets the division's or protocol-specific expedited (or serious) adverse event reporting criteria must be submitted to the NIAID's pharmacovigilance contractor, at the following address, using the appropriate form (if applicable):

DMID

Clinical Research Operations and Management Support (CROMS) 6500 Rock Spring Dr, Suite 650

Bethesda, MD 20814, USA

SAE Hot Line: 1-800-537-9979

SAE FAX: 1-800-275-7619

SAE Email Address: PVG@dmidcroms.com

The study clinician will complete an Expedited or Serious Adverse Event Form within the following time guidelines:

All deaths and immediately life threatening events, whether related or unrelated, will be recorded on the Expedited or Serious Adverse Event Form and faxed/electronically communicated within 24 hours of site awareness.

Adverse events other than death and immediately life threatening events, that meet expedited reporting criteria, regardless of relationship, will be faxed/electronically communicated by the site within 72 hours of becoming aware of the event.

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Other supporting documentation of the event may be requested by the pharmacovigilance contractor and should be provided as soon as possible.

All reportable AEs will be followed until satisfactory resolution or until the Principal Investigator or Sub-investigator deems the event to be chronic or the participant to be stable.

## REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER NIAID-SPONSORED IND

Following notification from the investigator, the IND sponsor/Lead PI, will report events that are both serious and unexpected and that are associated with Study Agent(s)/Intervention(s) to the FDA and other applicable health authorities within the required timelines as specified in 21 CFR 312.32: fatal and life threatening events within 7 calendar days (by phone /fax/electronic communication) and all other serious adverse events in writing within 15 calendar days. All serious events designated as “not associated” to Study Agent(s)/Intervention(s), will be reported to the FDA at least annually in a summary format.

### **9.6 Reporting of Pregnancy**

Interventions are given at baseline visit only. All participants found to be TV positive at a follow up visit, will be referred to clinic providers to be retreated at the provider’s discretion. The study does not treat participants or their partners with medication at follow up visits. Women who become pregnant during the study remain eligible for study participation. Pregnancy will not be recorded as an adverse event but will be noted in study charts and the pregnant participant and their child may be followed according to the site IRB regulations.

### **9.7 Type and Duration of the Follow-up of Participants after Adverse Events**

Because this is a phase III study of medications that are being used for their intended purpose, we anticipate that serious adverse events will be a rare occurrence. Participants who experience unresolved nausea and/or vomiting or allergic reaction will be referred to the clinic providers and they will provide care according to clinic protocol.

### **9.8 Modification of Study Agent(s)/Intervention(s) for a Participant**

Metronidazole in both treatment doses is the CDC recommended treatment for TV. The study will treat participants at baseline only. There will be no need for modification of study agents unless the participant is unable to complete a dose. Clinical protocols will be followed in case of severe reaction to MTZ.

### **9.8.1 Dose / Schedule Modifications for a Participant**

If the participant is unable to complete the dose of MTZ, she will be referred to her clinical provider for alternative treatment.

## **9.9 Halting Rules for the Protocol**

Since the study is taking place at public clinics, the investigators are physicians at the clinics, the drug intervention is recommended as treatment by the CDC, and the investigators have extensive experience, it is expected that SAEs will be minimal. If, however, during the course of the study, the number of side effects exceeds what is considered normal, the DSMB will recommend whether to continue without modification, continue with specified modification, discontinue one or more arms of the study, discontinue enrollment at one or more clinical sites or halt or modify the study until more information is available. If a major change is recommended, the DSMB will report this directly to the NIH Program Officer who will contact the PI and collectively make a determination on how to proceed. All DSMB recommendations and proposed changes to the protocol will be reported to the IRB. All adverse events will be reported to medical provider responsible for the participant and followed clinically to assure that the participant experiences the minimal amount of sequelae possible. Unexpected adverse events are reported to the participating institutions per their respective protocols.

## **9.10 Stopping Rules for an Individual Participant/Cohort**

Criteria for Discontinuation of Study Agent(s)/Intervention(s):

- if they are randomized to an arm and are unable to tolerate the dose the study agent will be discontinued.

Criteria for Withdrawal of a Participant:

- if they become incarcerated and are unable to attend follow up visits

- if any clinical adverse event, laboratory abnormality, concurrent illness, other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.

- if a woman is found to be pregnant after consent but prior to randomization

- if a woman is found to be ineligible after consent but prior to randomization

- if a participant initial TV positive wet prep result is not confirmed by either InPouch culture or TV NAAT test from her enrollment visit



## **9.11 Premature Withdrawal of a Participant**

Voluntary withdrawal is always an option for participants without affecting their clinical care. Women may choose to withdraw from the study at any time. If they choose to withdraw the data that has been previously collected will be included in the analyses.

## **9.12 Replacement of a Participant Who Discontinues Study Treatment**

There is no plan in the protocol to replace women who discontinue the study. Enrollment will continue up until 2 months prior to the study end date.

# **10. Clinical Monitoring Structure**

## **10.1 Site Monitoring Plan**

The Principal Investigator and Tulane study staff under the direction and supervision of the PI, will conduct regular site monitoring to ensure human subject protection and that study procedures are being conducted as per protocol and Manual of Procedures as outlined in section 12.0. For the first 4 months of enrollment, weekly conference calls will be held with investigators and study personnel at sites to ensure start up activities are following protocol, any questions can be answered, and clinic flow and specific clinical issues can be addressed. The PI and Program Manager are available at any time via email or cell phone to address any urgent issues. Quarterly site visits will be conducted by the PI, or her designee. At these visits, at least 10% of study charts will be pulled and reviewed, all consent forms will be reviewed and consent procedures will be discussed. There will also be a systematic review of enrollment statistics (i.e., number of eligible patients, refusal log, ineligibility log). Weekly data transfer to the research office at Tulane University will allow the program manager to review data collected for any errors or discrepancies and report back to sites to resolve any inconsistencies.

Women will be systematically asked if they experienced any of the common side effects on the TOC CRF.

## **10.2 Safety Monitoring Plan**

The safety of the subjects is paramount. AE reports are monitored on an ongoing basis by the Principal Investigator at monthly meetings and by the DSMB. All SAEs are reviewed by the site and Lead PI (or designee) within 2 working days of receipt. Both AEs and SAEs are reported to the IRB according to each institution's regulations including the Tulane IRB. The DSMB reviews these at their bi-annual meetings. Events will be reported to the FDA in accordance with 21CFR312.32 guidelines.

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### **10.2.1 Safety Review Plan by the DSMB / SMC**

The DSMB will be responsible for monitoring all aspects of the study, including those that require access to blinded data, and will follow the procedures detailed in the DSMB charter (see appendix). A brief description of the DSMB activities is as follows.

The “convening authority” for the DSMB is NIH-NIAID. The DSMB will consist of three experts who are not otherwise affiliated with the study. The DSMB will meet semiannually and may meet more frequently if requested by members of the DSMB. These meetings will consist of three sessions: Open Session, Closed Session, and Closed Executive Session. The Data Coordinating Center (DCC) representative will be responsible for preparing and presenting up-to-date statistical reports on the progress of the study at these meetings. These reports will include data on recruitment, randomization, adherence, adverse drug responses as well as statistical tests and special analyses requested by the DSMB. The Open Sessions will be attended by DSMB members, a FDA representative, a DCC representative, the principal investigator and members of the study team as needed. All voting will occur during the Closed Executive Session, which is only attended by DSMB members.

During the active recruitment phase, the DSMB will monitor the progress of recruitment and the random allocation of participants to the various treatment arms and may recommend modifications in (or termination of) the trial if the study design goals are not being met. The DSMB may recommend discontinuation of any of the treatment arms for safety reason or a very low probability of successfully addressing the study hypotheses within a feasible time frame, because of inadequate recruitment, compliance, drug response, effect size of outcome, etc. All votes will be decided by a simple majority. The NIAID will make the final decision on whether or not to accept the DSMB's recommendation to discontinue any component of the study.

The approval of the DSMB will also be required for any significant changes in the protocol recommended during the course of the study.

## **11. STATISTICAL CONSIDERATIONS**

### **11.1 Overview and Study Objectives**

The overall goal of this project is to determine the influence of index treatment and host factors on repeat TV infections among HIV-negative women in two specific objectives: 1) To determine if the multi-dose (alternate) MTZ is superior to single dose (recommended) for the treatment of TV among HIV-negative women. 2) To determine if altered vaginal flora (measured by Nugent's score) interferes with the MTZ treatment of TV for the single dose but not the multi-dose.

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## **11.2 Study Population**

English speaking, HIV negative women, who are not pregnant or breastfeeding, and are of the age of majority, who attend a clinical exam at 4 public clinics and are found to be positive for *Trichomonas vaginalis*.

## **11.3 Description of the Analyses**

The TV positive rate will be compared between the two arms for superiority. Additional analyses will be conducted to compare the rates stratified by BV status.

## **11.4 Measures to Minimize Bias**

### **Enrollment/ Randomization/ Masking Procedures**

After eligibility is assessed, informed consent is obtained and the woman has completed the ACASI survey and self-swab collection, she will be randomized to treatment arm. A blocked randomization scheme will be used to avoid “runs” in the randomization process. Each site will have its own blocked randomization scheme. Pre-numbered, sealed envelopes will be selected sequentially and opened for each participant who will then be enrolled in the indicated treatment arm. Because we are conducting a phase III trial and behaviors associated with the interventions are important, we will not attempt to make the single dose mimic a 7-day dose by supplementing with placebo. The trial will, therefore, be un-blinded. There will be no replacement of participants who discontinue early. Enrollment will continue through two months prior to study end.

Study personnel will know which arm the participant was randomized to. This information will not be disclosed to laboratory personnel. The Program Manager will periodically ensure that all participants were randomized to the correct treatment arm as indicated in the randomization scheme. Any discrepancies will be immediately discussed with investigators to prevent further error.

## **11.5 Appropriate Methods and Timing for Analyzing Outcome Measures.**

The primary outcome measure is TV infection. All women will be TV positive at baseline and will be screened for TV using InPouch culture and NAAT at follow up visits. Binary (TV positive or TV negative)

Key secondary outcomes are:

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BV which will be measured by self-collected vaginal swabs and Gram stain screening at all visits. Categorical (Scores of 7 -10 are considered to indicate BV). This measure will add information about the primary outcome measure by indicating whether BV affects TV repeat infections.

Re-exposure, which will be self-report by the participant for each partner. This data will be collected on the behavioral survey at follow up visits. Binary (partner treated and /or no sexual exposure prior to screening vs. partner not treated and sexual exposure prior to screening, This measure will allow us to adjust for the confounding effects of sexual exposure/re-exposure.

## **11.6 Study Hypotheses**

From the two aims, there are two hypotheses:

H1: Multi-dose (alternate) MTZ is superior to the single dose (recommended) for the treatment of TV among HIV-negative women.

H2: Altered vaginal flora (measured by Nugent's score) interferes with the MTZ treatment of TV for the single dose but not the multi-dose.

## **11.7 Sample Size**

### **Considerations**

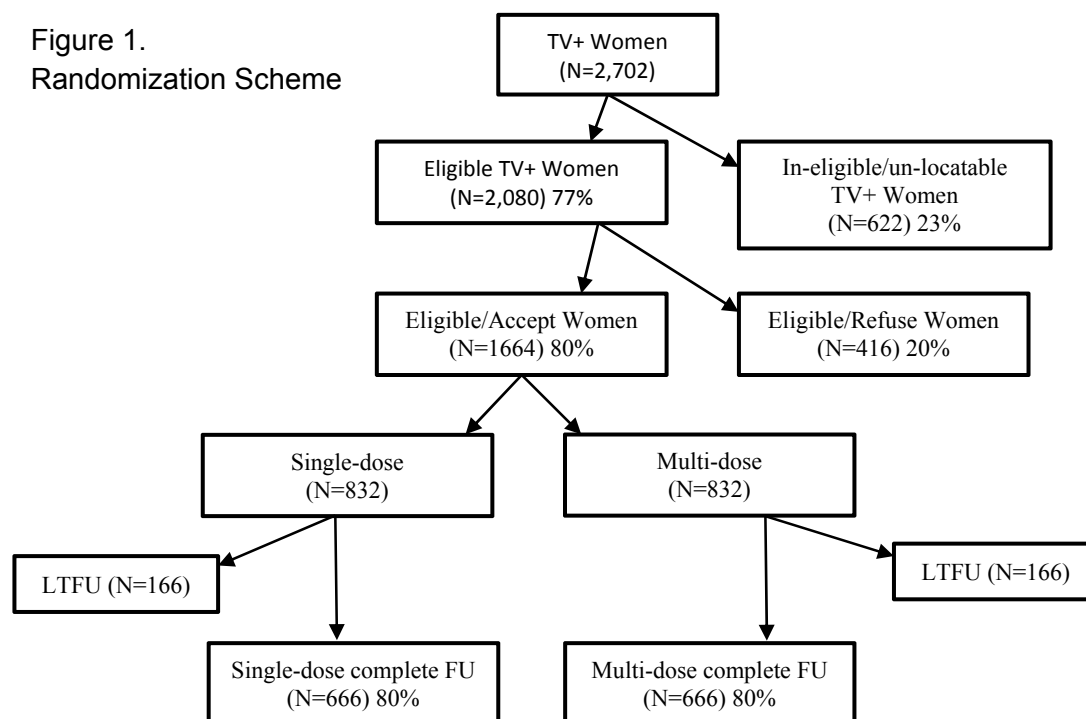
This RCT will allow for evaluation of index dosing of MTZ by BV status. This study examines two hypotheses simultaneously:

H1: Women receiving multi-day MTZ will be less likely to test TV positive at TOC compared to women who receive the single dose MTZ.

H2: This effect (H1) will only be observed among women who have BV (Nugent score  $\geq 7$ ).

From our pilot work, we found the rates of repeat infection at TOC to be 16.2% for single dose and 10.7% for multi dose at a power of 0.80, we will need 1206 subjects. If we account for a 5% Inner Class Correlation (ICC) for correlation of the subjects within the 4 sites using the formula  $1=(M-1)*ICC$ . We will need to inflate by 15%. We will also inflate by 20% to accommodate for our anticipated follow-up for a required sample size of 1664. We will have 1332 evaluable unites at TOC, which will provide adequate power taking into consideration the ICC.

Figure 1.  
Randomization Scheme



## 11.8 Maintenance of Trial Treatment Randomization Codes

Randomization will occur at the end of each baseline study visit. Randomization codes will be determined by a block randomization strategy at each site. Sealed envelopes will contain a study identification number on the outside and the randomization arm on the inside. Study staff will choose randomization envelopes sequentially and only open the card after enrollment, survey and specimen collection has occurred. There will be a separate staff member who will create and number the envelopes thereby shielding clinic study staff from knowing the randomization scheme. No study staff at the clinic will have access to the randomization database or numbering scheme. Every six months, a randomization interim analysis will be performed by Tulane staff for DSMB review to ensure that the randomization strategy is successful. The randomization arm will be noted on the study file and in the participant's data once the baseline visit is completed. No laboratory or clinical staff will have access to the randomization database.

## 11.9 Participant Enrollment and Follow-Up

Women will be enrolled from three public clinics in New Orleans, LA (CrescentCare Health and Wellness, Birmingham, AL (JCDH STD Specialty Clinic) and Jackson, MS (Crossroads Clinic). Each will continue to enroll up until two months from study end to allow sufficient time for follow up; therefore anticipated enrollment duration will be 30 months. The clinics' census is adequate to achieve sample size.

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### **11.10 Planned Interim Analyses**

The DSMB will be responsible for monitoring all aspects of the study, including those that require access to blinded data, and will follow the procedures detailed in the DSMB charter. The Data Coordinating Center (DCC) representative will be responsible for preparing and presenting up-to-date statistical reports on the progress of the study at semiannual meetings. These reports will include data on recruitment, randomization, adherence, adverse drug responses, as well as statistical tests and special analyses requested by the DSMB. During the active recruitment phase, the DSMB will monitor the progress of recruitment and the random allocation of participants to the various treatment arms and may recommend modifications in (or termination of) the trial if the study design goals are not being met.

### **11.11 Safety Review**

Investigators and the DSMB may recommend discontinuation of any of the treatment arms for safety reasons or a very low probability of successfully addressing the study hypotheses within a feasible time frame, because of inadequate recruitment, compliance, drug response, effect size of outcome, etc. This discontinuation may apply to one or all sites. All votes will be decided by a simple majority. The NIAID will make the final decision on whether or not to accept the DSMB's recommendation to discontinue any component of the study.

### **11.12 Immunogenicity or Efficacy Review**

Since this is a Phase III trial of medications that are being used for their intended use, there should be no need to conduct interim analyses.

### **11.13 Final Analysis Plan**

Analyses will be conducted in an intent-to-treat manner with variables categorized per protocol. To assure that randomization has been achieved, selected demographic, clinical and behavioral (including medication adherence and sexual re-exposure) factors will be compared by study arms using chi-square, t-test or ANOVA, where applicable. If randomization was found to be successful (as indicated by even distribution of potential confounders) then analyses will be conducted with no need to adjust for confounding. If randomization was not achieved, then associations will be explored for potential confounding and if found these factors will also be included in the model. From the two aims, there are two hypotheses:

H1: Women receiving multi-day MTZ will be less likely to test TV positive at TOC compared to women who receive the single dose MTZ.

H2: This effect (H1) will only be observed among women who have BV (Nugent score  $\geq 7$ ).

Analysis: The research questions will be answered using a multivariable logistic regression analysis to examine the effect of single versus multi-dose (H1) and the differential impact of BV on this effect (H2). This primary logistic model contains two main effects (i.e. treatment and BV)

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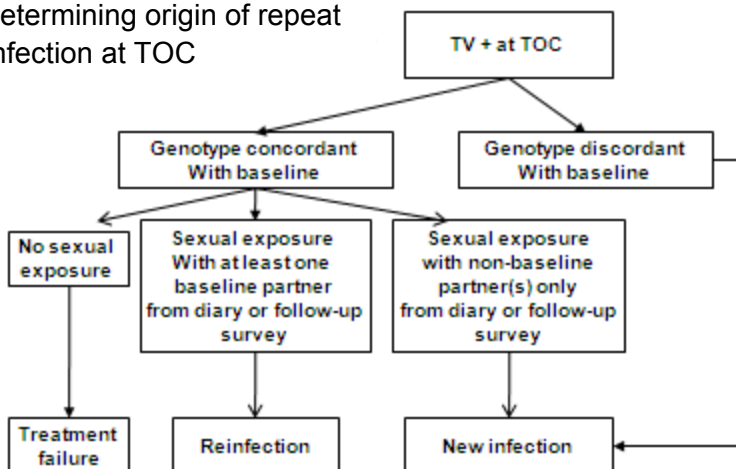
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and 1 first order interaction (BV by index treatment)). The model (1) will be:  $g(Y_i) = \beta_0 + \beta_1 + \beta_2 + \beta_1 \cdot \beta_2$  where:  $g(Y_i)$  = log odds of repeat infection,  $\beta_0$  = coefficient for the intercept,  $\beta_1$  = (Factor 1) single versus multi-dose,  $\beta_2$  = (Factor 2) BV at time of visit

All analyses will be conducted in SAS by Tulane staff under the direction of Drs. Kissinger and Myers. Because we will use strict protocols and employ tight supervision of the study sites and the study populations are similar (mostly African American and underserved) we do not anticipate site variations but we will examine for any differences and if found, we will include a site variable in the models. We do not anticipate a three-way interaction, and our simulations (see below) support that belief. We will, however, run a fully parameterized model including all main effects, first order interactions, and second order interaction before running the primary analysis model (1) above. We will consider any p-value less than 0.05 to be significant and any p-value less than 0.10 as near-significant. If there is unexpected significant or near-significant interaction, we will stratify on BV status and assess the impact of index treatment (single vs. multi-dose) and any possible interaction separately for BV positive and BV negative women.

**Additional analyses**– The ACASI will allow for examination of index adherence to medication, index knowledge of partner treatment, sexual exposure and the nature of the sexual act and sexual partner, condom use. These factors will be assessed by arm to determine if any confounding occurred and if so, these analyses will be adjusted for these factors or stratified by these factors.

**Figure 4:** Algorithm for determining origin of repeat infection at TOC



**Outcome measures** – The study is powered for repeat infections. Since we will have information on sexual exposure and genotyping, we can also refine this outcome measure to examine treatment failure (H1 & H2) and reinfection and will use the algorithm depicted in figure 4.

All analyses will be conducted in SAS by the Tulane staff under the direction of Drs. Kissinger and Myers. As the data collection instruments are designed to

minimize missing data we do not anticipate an excessive amount. However, imputation techniques will be utilized to compensate for missing data (e.g. multiple imputation and maximum likelihood). Sensitivity analyses will be conducted to ensure that the method chosen to account for missing data does not bias the study's results. Additionally, the characteristics of those who complete the study will be compared to those who drop out to determine whether drop out is informative. If not, multiple imputation methods will be used for initial analyses. If,

instead, data are non-ignorably missing, weighted regression methods will be explored but extreme caution will be used in interpreting results. Regardless of missingness mechanism, complete case analysis will also be performed for comparison.

## **12. QUALITY CONTROL AND QUALITY ASSURANCE**

Dr. Kissinger will serve as the study PI and all co-investigators will be accountable to her. She will be overseen by the DSMB and the NIAID Project Officer. There are three clinical sites: the CrescentCare Health and Wellness in New Orleans, the JCDH STD Specialty Clinic in Birmingham, AL and Crossroads Clinic in Jackson. All provide STI services. Drs. Lillis, Muzny, and Mena will serve as site PI's, respectively. The Program Manager will supervise the site Study Coordinators who will, screen, enroll, and follow all participants. Dr. Kissinger and Tulane study staff, under the direction and supervision of Dr. Kissinger, will conduct site visits at least once a quarter to assure that all study activities adhere to the study protocol. In all site laboratories, a trained lab technician will read TV InPouches and prepare specimens for storage and shipment to the appropriate labs. The study protocol, manual of operations, currently approved consent forms and other study related materials will be posted in a shared study Box.com project folder that is password protected. Site study staff/co-investigators will prepare monthly recruitment and follow-up statistics that will be presented at monthly conference calls. Lab PIs will supervise their technicians.

The REDCap system will be used for data entry of clinical visits, results and contacts. Contact data will be kept in a separate REDCap database and will be site specific, such that sites cannot see other sites' contact databases. Tulane study staff, under the direction and supervision of Dr. Kissinger, will have access to this information for related study activities. The REDCap system will be programmed for skip patterns and out of range checks. De-identified REDCap data will be immediately available to Tulane study staff who will run descriptives to check for aberrations, under the direction of Drs. Kissinger and Myers. Any abnormalities will be discussed with the site staff during site calls or via email. Participants may be contacted by site staff to provide missing data or to clear up any inconsistencies in the data. Any data corrections will be logged and dated and reviewed at the monthly conference calls with sites. Individual ACASI/CAPI data files will be merged into databases by site and study visit (baseline, test of cure, 8 week). For analyses purposes, these databases will be merged into comprehensive SAS databases for index and partner levels. Data will be stored on an encrypted Tulane server that is dedicated for study use only.

## **13. ETHICS/PROTECTION OF HUMAN SUBJECTS**

### **13.1 Institutional Review Board/Ethics Committee**

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The study (i.e., protocol, consent forms, surveys, and study forms) will be reviewed by all IRBs associated with Tulane University and participating clinics which include University of Mississippi Medical Center, Mississippi State Department of Health, University of Alabama at Birmingham and Louisiana State University Health Sciences Center. No sites will start enrollment until Tulane IRB approval has been obtained. The study will not be implemented at any individual site until IRB approval has been received from that site and acknowledged by the Tulane University IRB.

## **13.2 Informed Consent Process**

After the clinic provider refers a potential participant to the study staff, the study staff will provide a description of the study and what participation involves and check eligibility criteria. If a woman is eligible and interested in enrolling, informed consent will be obtained. Study staff, who are trained in consent procedures and have participated in an institutional human subjects protection course will obtain consent. First, there will be a more detailed discussion of what the study involves. Next, staff will review the consent form including the procedures, risks, and benefits that may be anticipated. Staff will answer any questions that the woman may have and make certain she understands that she does not have to be in the study if she chooses not to be and non-participation will not affect her care at the clinic. Non-English speakers are excluded from participation in this study. If a woman has a low literacy level, there is a provision and signature line that the consent form has been fully read to the participant. The consent form will be signed and a copy will be given to the participant. The subjects should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the subjects for their records.

A consent form template with suggested language for the clinical sites' consent forms will be approved by Tulane IRB. Site specific consent forms will be IRB approved at the study site by their specific the clinical institution's IRB (i.e. Louisiana State University Health Sciences Center, University of Mississippi Medical Center, the Mississippi State Department of Health and University of Alabama at Birmingham) and acknowledged by Tulane University IRB.

### **13.2.1 Assent or Informed Consent Process (in Case of a Minor)**

N/A

## **13.3 Exclusion of Women, Minorities, and Children (Special Populations)**

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Women and minorities are eligible for enrollment. Children less than 18 years of age are ineligible. This is a study of *Trichomonas vaginalis* which mainly affects an older, sexually active population.

### **13.4 Participant Confidentiality**

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects. No names or identifying information such as addresses and phone numbers will be linked to study data. Clinical and laboratory staff will not have access to contact information collected by study staff. Contact information will only be used by the study coordinators or PIs to remind participants of appointments, reschedule appointments, or to notify them of any relevant changes to the study or information needed. All files will be kept in locked file cabinets with access limited to study staff. All databases will be password protected and kept on computers that are secure and accessible only by authorized study staff. Any data files that are emailed will be coded and encrypted.

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic or hospital as permitted by the clinical study site IRB) and pharmacy records for the participants in this study. Copies of CRFs will be transferred and stored at the Lead Site.

### **13.5 Study Discontinuation**

In the event that the study is discontinued, participants can choose to continue care at the clinical site.

## **14. DATA HANDLING AND RECORD KEEPING**

### **14.1 Data Management Responsibilities**

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The web based data system we will be using is REDCap (Vanderbilt University). The program will be used for web based data entry from clinics and laboratories. REDCap provides a high level of data privacy and security regarding data storage, transmission, backup, and access, as well as server protection. REDCap secure servers are registered with site certificates at Tulane University. The servers are stored in a locked, well-ventilated room in locked server cabinet/racks with 24/7 alarm security. Remote security is accomplished with Watchguard firewall hardware. Server backups are conducted daily. They are encrypted and streamed over on a private network to a secure offsite location. Access to data is available only to authorized personnel with specified username and password. Access privileges can be specified for each member of the research team with access to data, granting more or less access depending on their job description. All authorized personnel will be CITI certified for human subjects' research. There are four different data that will be entered into the system:

1. Provider assessment forms will be filled out by providers at each site, as permitted data will be abstracted by study staff and then data entered into REDCap. The encrypted database will only contain study identification numbers and dates of visits; they will not contain any other identifying data.
2. Laboratory results will be entered into REDCap by study staff. Results may be delivered from the laboratory to study staff by email or courier. Participants will only be identified by their study numbers. These databases will be archived and merged into a single database for analyses purposes.
3. Medication logs will be kept on site and follow guidelines of site pharmacies. These logs will contain medications distributed to participants and their partners. The logs will be reviewed during site visits by the PI and study coordinator. Copies of these logs may be provided to the Lead site.
4. Contact data will be entered at the sites and will only be accessible to their site personnel, the Lead PI and Tulane study staff.

All sites will transfer data to the data manager. The data manager at the study offices of Tulane University will be responsible for all data merging and cleaning. S/he will do routine review and cleaning of databases and provide feedback to site staff. S/he will maintain merged study databases and also create data coding dictionaries for all databases. These coding dictionaries will include variable names, types, and descriptions. This dictionary will be updated as needed. All databases will be reviewed quarterly by program manager and PI. Any discrepancies or errors will be discussed and resolved. Tulane study staff will also be responsible for producing monthly reports to investigators that include enrollment and other study statistics by site (blinded for randomization).

All source documents and laboratory reports must be reviewed by the study and clinical staff at each site, who will ensure that they are accurate and complete. Adverse Events must be graded, assessed for severity and causality and reviewed by the site Principal Investigator or

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study manager. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site Principal Investigator. During the study, the Investigator will maintain complete and accurate documentation for the study.

The analysis plan will be reviewed and revised by the PI and the Biostatistician. The analyses will be conducted by Tulane study staff under the direction and supervision of the PI with consultation with the Biostatistician. The PI will lead manuscript development and reporting.

## **14.2 Data Capture Methods**

There will be five types of data collected:

1. Survey data: QDS software to create an ACASI survey. All data will automatically be entered into a database. In the event of computer complications, a paper survey will be available as a backup. If a paper survey is used, the data will be entered from the survey into the QDS software once the computer issue is resolved. Collection will be ongoing; data will be batched and sent to main study office at Tulane from site locations.
2. Contact information: Collected on paper and entered into REDCap. Collection will be on-going and revised at follow up visits.
3. Provider form CRF: Collected on paper and entered into a REDCap database. Once entered, the paper copy will be kept in the study records. Collection will be on-going.
4. Laboratory data: Will be recorded on paper/electronic results forms and/ or entered directly into REDCap. Gram stain results from LSUHSC lab will be de-identified and sent via email to study staff who will enter the results into REDCap
5. Adverse event CRF: Will be collected on paper and stored at the clinic offices. These forms will be on-going as events occur and sent to main study office via fax or electronic scan. Sent to main study office at Tulane from site locations

## **14.3 Types of Data**

Behavioral, laboratory, clinical, and safety data will be collected. Safety data will be kept in the database with clinical data.

## **14.4 Source documents and Access to Source Data/Documents**

Source data may include laboratory results, clinical records, contact forms and behavioral surveys. Research records will be housed at the four clinic sites and at the main study office at

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Tulane University in the PI offices. Access to research records and data at each site will be limited to trained study personnel at each site (i.e. site investigators, site coordinators, research assistant) and Tulane study staff, under the direction and supervision of the PI during site visits. Access to research records and data at the main study offices will be limited to Tulane study staff and the Biostatistician under the direction and supervision of the PI. These records and data may also be accessed by members of the IRBs, DSMB, and an authorized representative of NIAID upon request.

## **14.5 Timing/Reports**

Daily – data at each site will be reviewed by study coordinators for accuracy

Weekly – data will be transferred to main study office and reviewed for accuracy by Tulane study staff under the direction and supervision of the PI.

Monthly – conference calls will be held with the full research group to review enrollment statistics and study flow. Tulane study staff will create monthly reports to distribute to investigators under the direction and supervision of the PI.

-PI and program manager will review any safety reports

Semiannually – The PI will provide reports to members of the DSMB which will include data on recruitment, randomization, adherence, adverse drug responses, as well as statistical tests and special analyses requested by the DSMB.

Outcome data is measured at each visit. These data are monitored by site coordinators, data manager, program manager and PI. Data collection may be postponed or discontinued if severe unexpected adverse events occur. Coding of data will be ongoing. Data analysis will take place at the end of data collection when the randomization blind will be lifted.

## **14.6 Study Records Retention**

At the end of the study, copies of the clinical sites' CRFs and the archived contact database will be stored at the Tulane study office. Clinical sites will retain their study folders. These records will be kept according to FDA and NIH requirements (whichever is longer). The PI will follow all mandates regarding records retention of NIAID and the participating institutions.

## **14.7 Protocol Deviations**

Deviations from the IRB approved protocol are not allowed. Thorough monitoring of adherence to the study protocol will be maintained by investigators. Any deviations will be reported to the

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site PI, the Lead PI, the DSMB, the IRBs, NIAID and FDA the in the time frame and manner required by each institution. Corrective action will be taken.

Since adherence to the study medication (metronidazole) is an outcome that will be evaluated by the study, missed doses will be documented but not considered a protocol deviation. It is preferred that that subjects on both arms take their first dose of medication under direct observation but it is not mandatory. If this happens, it will be documented in the study records, but is not considered a protocol deviation.

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with Good Clinical Practice (GCP ICH E6) Sections:

Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3

Quality Assurance and Quality Control, section 5.1.1

Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations according to the guidelines.

## 15. PUBLICATION POLICY

Publication of the results of this trial will be governed by NIAID publication policies. Any presentation, abstract, or manuscript will be made available for review by the NIAID supporters prior to submission.

### **Additional Considerations for the above section:**

Following completion of the study, the investigator may publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as [ClinicalTrials.gov](http://ClinicalTrials.gov), which is sponsored by the National Library of Medicine.



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