The Microbicide Tenofovir Does Not Inhibit Nucleic Acid Amplification Tests for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Urine Samples\(^\text{v}\)

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Received 19 September 2007/Returned for modification 5 November 2007/Accepted 19 November 2007

The potential inhibitory effects of tenofovir and a placebo were examined using the Becton Dickinson ProbeTec, Gen-Probe Aptima Combo 2, and Roche Amplicor tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Concentrations of 5% to 0% of tenofovir and the placebo were added to dilutions of *C. trachomatis* and *N. gonorrhoeae*. No appreciable inhibition was observed.

*Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections are among the top 10 reported diseases in the United States (8). Approximately 976,000 *C. trachomatis* infections were reported in the United States in 2005. During this period, the rate of chlamydia infection in women was nearly three times greater than the rate of infection in men. Approximately 340,000 cases of *N. gonorrhoeae* infection were reported during 2005; the number of infected women was 1.1 times greater than that of infected men (4). Most *C. trachomatis* and many *N. gonorrhoeae* infections are asymptomatic. Thus, many infections are left untreated, which can lead to sequelae such as pelvic inflammatory disease, chronic pelvic pain, tubal factor infertility, and ectopic pregnancy (4).

Vaginal microbicides may help prevent the spread of sexually transmitted infections (STIs) and provide women with more control of their sexual health (2, 10, 11, 17, 18). Tenofovir, a human immunodeficiency virus (HIV) treatment drug, has been proven to be effective in blocking the transmission of simian immunodeficiency virus in animal models when given systemically as pre- or postexposure prophylaxis or when applied as an intravaginal gel (19, 20). Intravaginal use of tenofovir may be more feasible due to its long intracellular half-life (15). A 2-week course of 1% tenofovir vaginal gel used twice daily has been shown to be well tolerated in sexually abstinent and sexually active HIV-negative and HIV-positive women (12).

Microbicides must be safe and effective for vaginal or rectal administration and should cause slight or no genital symptoms after use (6). These agents should not interfere with diagnostic testing for STIs, such as nucleic acid amplification tests (NAATs) for the detection of *C. trachomatis* and *N. gonorrhoeae* in urine samples. Research is ongoing to evaluate the efficacy of vaginal microbicides and their effect on NAATs (1, 3, 5, 14, 17, 18). NAATs, highly sensitive and specific assays, are important tools in the detection of asymptomatic chlamydia and gonorrhea infections (8, 9). The objective of this study was to investigate the possible inhibitory effects of tenofovir in a microbicide gel formulation and a placebo gel without drug on the detection of *C. trachomatis* and *N. gonorrhoeae* by the use of three types of NAATs.

(Results of this study were presented in part at the International Society for Sexually Transmitted Diseases Research, July 2005, Amsterdam, The Netherlands.)

Tenfold serial dilutions yielding final concentrations of 10^5 to 0 inclusion-forming units (IFU) per ml of *C. trachomatis* and 10^3 to 0 CFU/ml of *N. gonorrhoeae* were prepared using pretested, uninfected urine specimens obtained from healthy laboratory staff. Tenofovir was then added to these specimens by a checkerboard titration method to achieve final microbicide concentrations of 5%, 2%, 1%, 0.5%, 0.1%, and 0% per ml. The same procedure was followed for the placebo. Based on the expert opinions of microbicide scientists, a starting concentration of 5% was chosen as the maximum concentration of theoretical contamination of urine by a vaginal microbicide gel. Assay performance controls were as follows: 5 dilutions of each organism containing no tenofovir, 5 dilutions of each organism containing no placebo, 5 dilutions of tenofovir containing no organisms, and 5 dilutions of the placebo containing no organisms. Specimens were tested in duplicate for *C. trachomatis* and *N. gonorrhoeae* by use of the Becton Dickinson ProbeTec strand displacement amplification (SDA) (Sparks, MD), Gen-Probe transcription-mediated amplification (Aptima Combo 2) (San Diego, CA), and Roche Amplicor (PCR) (Indianapolis, IN) assays by following the manufacturers’ directions. The tenofovir gel and the placebo gel with no drug were provided by Gilead (Foster City, CA).

The *C. trachomatis* results showed no inhibition in detection for all concentrations of tenofovir or the placebo and at all concentrations of the organism when tested by the SDA, Aptima Combo 2, and PCR assays (Table 1). It is unknown why urine specimens containing high levels of *C. trachomatis* organisms but no tenofovir demonstrated PCR inhibition, but it is well documented that urine often shows inhibition in PCR assays. It is possible that tenofovir abrogated the inhibition in the PCRs containing tenofovir. No inhibition was observed for...
TABLE 1. Effects of various concentrations of tenofovir and placebo on detection of C. trachomatis by the SDA, Aptima Combo 2, and PCR assays

| Microbicide or placebo and concn (%/ml) | Detection of C. trachomatis at indicated concn of organisms by a,b |
|----------------------------------------|---------------------------------------------------------------|
|                                        | SDA              | Aptima Combo 2 | PCR            |
|                                        | 10^5  | 10^4  | 10^3  | 10^2  | 10^1  | 10^0  | 10^5  | 10^4  | 10^3  | 10^2  | 10^1  | 10^0  |
| Tenofovir                              | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 5                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 2                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 1                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 0.5                                    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| Placebo                                | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 5                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 2                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 1                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 0.5                                    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 0                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |

**Notes:**
- a: In, detection inhibited; +, detection positive; -, detection negative; +/Low+, detection positive for first replicate and low positive for duplicate test.
- b: IFU/ml.

All concentrations of tenofovir or the placebo in the detection of the N. gonorrhoeae organisms at concentrations of 10^5, 10^4, and 10^3 CFU/ml (Table 2). For the lower N. gonorrhoeae concentrations (≤10^2 CFU/ml) that contained tenofovir or the placebo, results varied, some positive, while others were equivocal or negative (Table 2). The high number of equivocal results noted with PCR is of concern.

The limit of detection for each assay was determined by testing quality controls of various concentrations of C. trachomatis and N. gonorrhoeae organisms that contained no tenofovir or placebo. The limit of detection for C. trachomatis was 10^3 IFU/ml for the SDA, Aptima Combo 2, and PCR. The limits of detection for N. gonorrhoeae varied slightly. The SDA and PCR measured N. gonorrhoeae at concentrations of ≥10^3 CFU/ml, while the Aptima Combo 2 measured N. gonorrhoeae at concentrations as low as 10^2 CFU/ml. This assay performance variation was expected, since the methodologies differ. Test results for positive and negative quality controls were as expected: all tests containing only C. trachomatis or N. gonorrhoeae were positive, with some variation in the levels of detection of the different assays at the lower concentrations, and all tests containing neither C. trachomatis nor N. gonorrhoeae were negative (Tables 1 and 2).

In many countries, a lack of economic and social power may leave women unable to negotiate condom use (7, 13, 16). Microbicides may provide women with more control over their sexual health and an opportunity to prevent the acquisition and spread of STIs and HIV (2, 11, 17). Today, there are approximately 60 vaginal microbicides in various phases of development (3, 13). Only a few of these products have made it to

TABLE 2. Effects of various concentrations of tenofovir and placebo on detection of N. gonorrhoeae by the SDA, Aptima Combo 2, and PCR assays

| Microbicide or placebo and concn (%/ml) | Detection of N. gonorrhoeae at indicated concn of organisms by a,b |
|----------------------------------------|---------------------------------------------------------------|
|                                        | SDA              | Aptima Combo 2 | PCR            |
|                                        | 10^5  | 10^4  | 10^3  | 10^2  | 10^1  | 10^0  | 10^5  | 10^4  | 10^3  | 10^2  | 10^1  | 10^0  |
| Tenofovir                              | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 5                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 2                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 1                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 0.5                                    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 0                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| Placebo                                | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 5                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 2                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 1                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 0.5                                    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 0                                      | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |

**Notes:**
- a: +, detection positive; -, detection negative; Low+, detection low positive; Eq, equivocal results; In, detection inhibited. Results separated by a slash symbol indicate two different results in the duplicate tests.
- b: CFU/ml.
clinical trials where the de novo effects are being examined (3). Crucitti et al. and Rizzo-Price et al. reported some microbicide inhibition for various NAATs in their studies with BufferGel, Pro 2000, and cellulose sulfate gel (5, 14). In this study, we observed no significant inhibition for tenofovir or the placebo in any of the three NAATs used to detect *C. trachomatis* and *N. gonorrhoeae*.

In summary, our findings indicate that tenofovir, in urine samples at various concentrations, does not interfere with diagnostically testing using NAATs for the detection of *C. trachomatis* and *N. gonorrhoeae* at multiple concentrations of the organisms. Additional studies of urine and vaginal samples from human clinical microbicide trials to further assess the effect on the detection of *C. trachomatis* and *N. gonorrhoeae* are warranted. Future studies might consider the concentration of microbicides present in the sample at the time of testing and its potential effect on the performance of NAATs. If the safety and efficacy of microbicides are proven in clinical trials, women should be able to use these products to protect themselves from STIs and HIV.

Funding was provided by the NIH, NIAID, HIV Prevention Trials Network (grant U01 AI 68613-01C). C. A. Gaydos acknowledges that she has received lecture honoraria fees and grants from Becton Dickinson and Gen-Probe to support her research.

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