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Vaginal microbicides can interfere with nucleic acid amplification tests used for the diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection

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Abstract

We confirmed findings from previous studies that cellulose sulfate gel can interfere with nucleic acid amplification tests used for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. We therefore recommend that the effects of microbicide gels on diagnostic assays of sexually transmitted infections be established before starting up clinical studies.

Keywords: Nucleic acid amplification test; Microbicides; Inhibition

Microbicides are antimicrobial products that are applied topically to offer a chemical barrier to HIV and sexually transmitted infections. Currently, 6 microbicides are being, or are planned to be, evaluated for effectiveness (Minnis and Padian, 2005).

The assessment of the effectiveness of microbicides in preventing the transmission of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) is a secondary objective in many of these trials. Highly sensitive and specific tests are used, such as nucleic acid amplification tests (NAATs), which are nowadays considered to be the gold standard tests for the detection of CT and NG.

In vitro studies of microbicides have shown inhibition of molecular amplification reactions (Young et al., 2001; Stamper et al., 2003; Suksripanich et al., 2002; Crucitti et al., 2004). Studies in Thailand assessed the effects of Carraguard® (3% carrageenan gel) and placebo. In vitro, Carraguard® inhibited both the COBAS Amplicor polymerase chain reaction (PCR) and the Gen-Probe nucleic acid hybridization test. No inhibition was observed with the placebo gel. Specimens collected from women who had used Carraguard® did not inhibit the COBAS Amplicor PCR, and less than 3% of the specimens inhibited the Gen-Probe assay (Suksripanich et al., 2002).

In preparation for phase III clinical trials of 6% cellulose sulfate (CS) gel, we assessed the product's effect on NAATs.

The inhibitory activity of the CS gel and placebo was assessed on specimens of genital secretions, known to be positive for chlamydial infection and/or gonorrhea. The specimens were spiked separately with 10 mg of the CS and placebo. All specimens were tested with 2 commercial amplification tests, the Amplicor CT/NG PCR (Roche, Molecular Systems, Branchburg, NJ) and the strand displacement amplification (SDA) BDProbeTec ET CT/NG assay (Becton Dickinson, Sparks, MD), and with 2 in-house assays, the *MOMP* gene PCR for CT and the *cppB* gene PCR for NG. Inhibition of the in-house assays was assessed with the β_2-microglobulin gene PCR. Specimens were extracted and tested according to the manufacturer's instructions. For the in-house assays, extraction was performed using the NaOH extraction method (Gu et al., 1998), and published primer pairs were used for the amplification of *MOMP*, *cppB*, and β_2-microglobulin gene (Dutilh et al., 1989; Ho et al., 1992; Tabrizi et al., 1997). Specimens were tested undiluted and diluted in 10% phosphate-buffered saline (10% PBS) at 2- and 4-fold dilutions.

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Table 1
Inhibition of NAATs by vaginal specimens spiked with CS gel

Gel spiked in	Amplicor CT/NG PCR assay	SDA BDProbeTec ET CT/NG assay	*MOMP* PCR	*CppB* PCR	β_2-Microglobulin PCR
Positive control	Inhibition	+	Inhibition	Inhibition	Inhibition
Negative control	Inhibition	–	nt	nt	nt
CT specimen	Inhibition	+	Inhibition	nt	Inhibition
CT specimen 1/2 diluted	Inhibition	+	Inhibition	nt	Inhibition
CT specimen 1/4 diluted	Inhibition	+	Inhibition	nt	Inhibition
NG specimen	Inhibition	+		Inhibition	Inhibition
NG specimen 1/2 diluted	Inhibition	+		Inhibition	Inhibition
NG specimen 1/4 diluted	Inhibition	+		Inhibition	Inhibition

nt = not tested; + = positive test result; – = negative test result; CT specimen = genital secretion specimen positive for CT; NG specimen = genital secretion specimen positive for NG.

No inhibition of any of the NAATs was detected with the placebo; however, the CS gel interfered with all NAATs except the SDA assay. The inhibitors were also active at the 2- and 4-fold dilutions of the specimens (Table 1).

To assess the inhibitory effect of CS gel at concentrations equivalent to residual product, we conducted a study with healthy consenting female volunteers. The placebo was not tested in this set up because, in vitro, no effects on the NAATs were demonstrated. This study was approved by the local ethics committee. The study was designed in 2 parts: the 1st part aimed to confirm the inhibitory effect of the microbicide on the NAATs, and the 2nd part aimed to explore whether accumulation of the residual product has an incremental effect on inhibition of the NAATs.

In part 1, 10 women were asked to submit self-collected vaginal specimens before applying the gel (day 0), as well as 24 h (day 1) and 96 h (day 4) after a single application of the CS gel.

In the 2nd part, 20 women were asked to apply the gel daily for 4 consecutive days, and to self-collect vaginal specimens on the 1st day before gel application (day 0) and 24 h after every application (days 1, 2, 3, and 4). The self-administered specimen was collected with a BD culture EZ swab (Becton Dickinson), and stored dry within 30 min of collection at −20 °C until testing. Gel application and specimen collection were performed by the participant under the supervision of a clinician.

The specimens collected in both parts were tested with the same NAATs as described hereinabove. Inhibition was determined by a negative result of the amplification control or β_2-microglobulin gene PCR. Specimens inhibiting the NAATs were spiked with CT L2 strain (7.5×10^6 elementary bodies) and NG (25×10^6 CFU) and tested diluted at 1:4 and 1:10 in 10% PBS. Negative results obtained in the CT and/or NG NAAT were considered to be due to inhibition.

In the 1st part, inhibition of the Amplicor assay was detected in 3/10, 5/10, and 2/10 specimens collected on day 0, 1, and 4, respectively. Inhibitory activity persisted at the 4-fold dilution in most specimens collected on day 1. There was no inhibition of the SDA or of the in-house assays.

Three specimens inhibited the Amplicor assay before gel was used. Possibly, the inhibition of these cases was caused by residuals of hygiene products that could have been present despite the instruction not to use any 24 h before study entry.

In the 2nd part, inhibition of the Amplicor assay started to appear in specimens collected 24 h after gel application (Table 2). More than half of the specimens (41/80) collected on days 1, 2, 3, and 4 inhibited the Amplicor assay, and inhibition persisted at the 4-fold dilution of the specimens. For 1/10 specimen collected on day 1 and 3/11 specimens collected on day 4, the 10-fold dilution was not sufficient to eliminate the inhibitors.

The inhibition initially present in the specimens collected at day 1 was also present in the subsequent collected specimens. However, for 3 participants, no Amplicor assay inhibitors were detected in the specimen collected at days 2, 3, and 4.

Inhibition appeared for 1 participant in the specimens collected at days 3 and 4, and in another participant in the specimen collected at day 4 only.

Inhibition of the in-house PCR assays by specimens collected on days 1, 2, 3, and 4 after gel use persisted in more than half of the cases after 4- and 10-fold dilution.

This study has some limitations. First, our study populations were healthy women at low risk for sexually transmitted infections compared with the high-risk women participating in the effectiveness clinical trial. These women will apply the

Table 2
Inhibition of NAATs by vaginal specimens self-collected after daily application of CS gel, $N = 20$

	No. of specimens inducing inhibition (%)				
	Day 0	Day 1	Day 2	Day 3	Day 4
Amplicor CT/NG PCR assay	0	10 (50)	10 (50)	10 (50)	11 (55)
SDA BDProbeTec ET CT/NG assay	0	0	0	0	0
β_2-Microglobulin gene PCR	3 (15)	9 (45)	8 (40)	13 (65)	8 (40)

Day 0 = self-collected vaginal specimen before the application of the CS gel; days 1, 2, 3, and 4 = self-collected vaginal specimen after 24, 48, 72, and 96 h of CS gel application, respectively.

gel before every sexual act, which might be more frequent than once daily. Second, samples in this study were stored at −20 °C within the 30 min of collection. In the effectiveness trial, endocervical specimens will be collected by the clinician, and depending on the study site, the specimens may have to be transported to a more distant laboratory.

However, our findings confirm that residual microbicide gel in vaginal specimens can inhibit NAATs. We found that CS gel inhibited the Amplicor PCR, the in-house PCRs, but not the SDA assay. Only the SDA assay uses an Exo-Bst polymerase. This polymerase shows resistance to the inhibitory activity of acidic polysaccharides, such as the active compound of the 6% CS gel, in contrast to the Taq polymerase used in the other assays (Monteiro et al., 1997).

In conclusion, because of these results, the SDA BDProbeTec ET CT/NG assay is used for the detection of CT and NG in 2 ongoing phase III trials of the CS vaginal gel.

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References

Crucitti T, Cuylaerts V, Jespers V, De Deken B, Buvé A (2004) Inhibitory effect of vaginal microbicides on molecular amplification assays. Microbicides 2004 Conference London, Abstract 02652.

Dutilh B, Bébéar P, Rodriquez P, Vekri A, Bonnet J, Garret M (1989) Specific amplification of a DNA sequence common to all *Chlamydia trachomatis* serovars using the polymerase chain reaction. *Res Microbiol* 140:7–16.

Gu XX, Rossau R, Jannes G, Ballard R, Laga M, Van Dyck E (1998) The rrs(16S)–rrl(23S) ribosomal intergenic spacer region as a target for the detection of *Haemophilus ducreyi* by a heminested-PCR assay. *Microbiology* 144:1013–1019.

Ho BSW, Feng WG, Wong BKC, Egglestone SI (1992) Polymerase chain reaction for the detection of *Neisseria gonorrhoeae* in clinical samples. *J Clin Pathol* 45:439–442.

Minnis AM, Padian NS (2005) Effectiveness of female controlled barrier methods in preventing sexually transmitted infections and HIV: current evidence and future research directions. *Sex Transm Infect* 81:193–200.

Monteiro L, Bonnemaison D, Vekris A, Petry KG, Bonnet J, Vidal R, Cabrita J (1997) Complex polysaccharides as PCR inhibitors in feces: Helicobacter *pylori* model. *J Clin Microbiol* 35:995–998.

Stamper PD, Theodore ML, Reynolds SJ, Quinn TC, Gaydos CA (2003) Inhibitory effects of vaginal microbicide products on nucleic acid amplification testing of urine for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. ISSTDR congress Ottawa, presentation 0586.

Suksripanich O, Kilmarx PH, Chaowanachan T, Wasinrapee P, Chaikummao S, Thongpun J, Young NL (2002) Do topical microbicides interfere with *Chlamydia trachomatis* or *Neisseria gonorrhoeae* testing? Microbicides Congress Antwerp, Abstract A-232.

Tabrizi SN, Paterson B, Fairley CK, Bowden FL, Garland SM (1997) A self-administered technique for the detection of sexually transmitted diseases in remote communities. *J Infect Dis* 176:289–292.

Young NL, Kilmarx PH, Borchardt K, Chaowanachan T, Wasinrapee P, Suksripanich O (2001) Inhibitory effect of vaginal microbicides on COBAS Amplicor and Gen-Probe *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, and InPouch TV *Trichomonas vaginalis* tests. *Int J STD AIDS* 193:12S2.