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Can mailed swab samples be dry-shipped for the detection of *Chlamydia trachomatis, Neisseria gonorrhoeae,* and *Trichomonas vaginalis* by nucleic acid amplification tests?☆

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ABSTRACT

Dry-shipped and mailed vaginal swabs collected at home have been used in research studies for the detection of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC), and *Trichomonas vaginalis* (TV) by nucleic acid amplification tests (NAATs) in screening programs. A verification study was performed to compare the limit of detection of CT, GC, and TV on swabs that were dry-shipped to paired swabs that were wet-shipped in transport media through the US mail. The Centers for Disease Control and Prevention prepared inocula in sterile water to mock simulated urogenital swabs with high to low concentrations of CT and GC. Replicate swabs were inoculated with 100 μL of dilutions and were dry transported or placed into commercial transport media ("wet") for mailing for NAAT testing. The University of Alabama prepared replicate concentrations of TV, which were similarly shipped and tested by NAAT. All paired dry and wet swabs were detectable for CT. For GC, all paired dry and wet swabs were detectable for GC at concentrations $\geq 10^3$. At 10^2 and 10 CFU/mL, the 10 replicate GC results were variably positive. For TV, wet and dry shipped concentrations $>10^2$ TV/mL tested positive, while results at 10 TV/mL were negative for dry swabs. Holding replicate dry swabs at 55 °C 5 days before testing did not affect results. NAATs were able to detect CT, GC, and TV on dry transported swabs. Using NAATs for testing home-collected, urogenital swabs mailed in a dry state to a laboratory may be useful for outreach screening programs.

1. Introduction

Chlamydia trachomatis (CT) is the most commonly reported communicable disease in the United States, occurring most often among adolescent and young adult females. The Centers for Disease Control and Prevention (CDC) estimates there are approximately 3 million new cases each year (CDC, 2010a). Acute chlamydia infections often have no symptoms, leaving many cases untreated. Serious sequelae such as pelvic inflammatory infections can be associated with untreated CT infections. The US Preventive Services Task Force and major medical professional organizations recommend an annual screening test for chlamydia for all sexually active adolescents and young women 25 years of age and under, for all pregnant women, and for women at high risk (U.S. Preventive Services Task Force, 2007). Yet chlamydia screening remains an underutilized clinical preventive service with less that 50% of eligible women in commercial or Medicaid health plans being screened annually (National Committee for Quality Assurance, 2010).

Likewise, *Neisseria gonorrhoeae* (GC) infections are highly prevalent with over 700,000 new infections every year in the United States and are also associated with the serious sequelae as CT (Centers for Disease Control and Prevention, 2010a). *Trichomonas vaginalis* (TV) infections are highly prevalent with estimates of 7–8 million infections annually in the United States and 180 million globally (WHO, 2010), representing the most common curable STI in sexually active women (Van Der Pol et al., 2005; Weinstock et al., 2004). Trichomonas infections have been associated with poor reproductive outcomes such as low birth weight and premature birth (Cotch et al., 1997; Schwebke and Burgess, 2004). Screening for all of these infections in women of reproductive age can impact these epidemics but many barriers exist, which could be overcome by offering home collection of urogenital samples to women.

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Despite national screening recommendations and concerted efforts at control, rates of CT and GC among young women remain high, particularly among young minority women. Expanded outreach screening programs, beyond traditional clinics, are needed to reach young sexually active persons at risk. The advent of noninvasive specimens for CT and GC detection using NAAT technology has enabled the expansion of screening to nonclinical settings such as schools, mobile vans, and other settings outside the clinic. Because of the increasing prevalences of CT and GC, public health programs are needed that can implement novel screening programs to improve access to testing. Although there are no national surveillance data available for TV, because these infections are not reportable to CDC by law, research studies have demonstrated high prevalences in both young and older populations, as well as in minority groups (Van Der Pol et al., 2005; Weinstock et al, 2004).

Home collection of urogenital samples has been promoted by public health officials (Hobbs et al., 2008). Submission of such self-obtained vaginal samples (SOVS) collected at home could complement screening programs, as well as remove barriers that women and men face in getting tested for STIs (Gaydos et al., 2009; Gaydos and Rompalo, 2002). Since neither home-collected nor dry-shipped urogenital swab samples are cleared by the Federal Drug Administration (FDA) for use with commercial nucleic acid amplification tests (NAATs), we performed a verification study to compare the limit of detection of CT, GC, and TV titered concentrations of organisms on swabs that were dry-shipped versus matching swabs that were transport media (wet)–shipped through the US mail to a laboratory for testing.

2. Methods

2.1. Chlamydia and gonorrhea organisms

C. trachomatis genotype E was selected for this study based on genetic analysis of the outer membrane protein gene at the CDC and is a strain commonly found in genito-urinary tract infections among women (Millman et al., 2004). A single strain was tested since this was a pilot study and this strain is a very common serotype. The CT strain was grown in cultured buffalo green monkey kidney cells and harvested by differential centrifugation to remove cellular debris. The strains were serially diluted in sucrose–phosphate–glutamic acid buffer and titrated for determination of the infectious end point by calculation of inclusion-forming units (IFU). Inocula to spike simulated urogenital swabs were prepared in sterile water and represented dilutions ranging from 10^7 to 1 IFU/mL, which were estimated by CDC to be representative of most clinical infections (Michel et al., 2007).

A clinical isolate of *N. gonorrhoeae* was selected on the basis of biochemical reference testing at the CDC. A single clinical strain was selected as being representative of current circulating strains since this organism is always genetically changing. The *N. gonorrhoeae* strain was grown on modified Thayer–Martin selective medium incubated at 35 °C for 24 h under an atmosphere containing 5% CO_2. The strain was serially diluted in Mueller–Hinton broth and titrated for determination of the infectious end point by calculation of colony forming units (CFU). Inocula were prepared in sterile water and represented dilutions ranging from 10^7 to 10 CFU/mL.

2.2. Swabs

Becton Dickinson, BBL culture EZ, #220144 swabs were used. Presently, self-sampling swabs collected at clinics are placed into manufacturers' liquid ("wet") transport media for shipment to the laboratory. We refer to these swabs for standard techniques as "wet." There are many research studies that have successfully used shipment of urogenital swabs in the dry state without any transport media (Gaydos et al., 2009; Gaydos et al., 2002; Gaydos et al., 2006; Masek et al., 2009). We refer to these as "dry" swabs. For CT, there were replicate (2) dry swabs and matching wet swabs of each concentration (10^7, 10^5, and 10^3 IFU/mL, and 10 duplicates of 10 and 1 IFU/mL). For GC, there were replicate (2) dry and matching wet swabs of each concentration (10^7, 10^5, and 10^3 IFU/mL, and 10 duplicates of 10 IFU/mL). Replicate swabs were inoculated with 100 μL of appropriate CT and GC concentrations. Thus, 100 μL of the 10^7 /mL concentration yielded a swab representing 10^6 organisms per swab for that test. One replicate swab was placed in the holding tube for dry transport, 1 replicate swab was placed into the holding tube for "heated transport," and the matching swab made from the same concentration was placed into specimen transport media (STM) for wet transport (Aptima Combo 2, Gen-Probe, San Diego, CA, USA) (Schachter et al., 2005). The replicate dry-shipped swabs were held at 55 °C for 5 days before testing (see below). Also included were 8 swabs with only STM as negative controls.

Additionally, for GC, additional experiments with sets of the lower concentrations (10^2 and 10 CFU/mL) were repeated and transported 2 additional times.

2.3. Trichomonas organisms

Likewise, the University of Alabama at Birmingham (UAB) laboratory prepared concentrations of a clinical strain of TV, representing currently circulating strains, which was grown in Diamonds Media (Remel, Lenexa, Kansas). TV organisms were counted in a hemocytometer counting chamber, and dilutions were prepared to achieve various concentrations by serially diluting TV 10-fold in Diamonds Media and titrating for determination of the TV organisms per milliliter. Inocula were used to mock 2 simulated vaginal swabs from 10^7 to 10^2 organisms per milliliter. There were 4 replicate swabs for concentrations of 10^1 and 10 replicates of 1 TV per milliliter. Amount placed on each swab was 100 μL of each concentration. Negative control swabs containing STM transport media (Gen-Probe) were included for testing. The replicate dry-shipped swabs were held at 55 °C for 5 days before testing, as for the CT and GC experiments.

2.4. Shipping conditions

Matching swabs containing the various concentrations of organisms (2 dry and 1 wet swab of each dilution) were placed in sealed biohazard bags and then into individual mailing envelopes at the CDC in Atlanta (CT and GC) or UAB (TV) and sent via United States Postal Service, first class, to The International STD Research Laboratory, Johns Hopkins University, Baltimore, MD, USA.

2.5. Testing

Upon receipt, one of the dry swabs was placed into Aptima Combo 2 transport media for testing. These simulated urogenital swabs (wet and 1 dry transported) were tested for CT and GC at the same time according to the routine Aptima Combo 2 (Gen-Probe) protocol. Similarly, the same algorithm was followed for the TV swabs, which were tested by the ASR (analyte specific reagents) Aptima TV assay (Gen-Probe).

2.6. High-temperature environment

Upon receipt, for all 3 organisms, the replicate set of identical dry-shipped swabs with the various organism concentrations was heated at 55 °C in an incubator for 5 days before being placed into