Meatal Swabs Contain Less Cellular Material and are Associated with a Decrease in Gram Stain Smear Quality Compared to Urethral Swabs in Men

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Running Title: Meatal swabs perform poorly in Gram stain testing

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Abstract:

Urethral swabs are the test of choice for point-of-care Gram stain testing to diagnose Neisseria gonorrhoeae (NG) and non-gonococcal urethritis (NGU) in men. As an alternative to urethral swabs, meatal swabs have been recommended for collection of urethral discharge to diagnose NG and Chlamydia trachomatis (CT) in certain populations by nucleic acid amplification testing (NAAT), as they are a less invasive collection method. However, as meatal swabs could be sampling a reduced surface area and result in fewer collected epithelial cells when compared to urethral swabs, the...
adequacy of meatal swab specimens to collect sufficient cellular material for Gram stain testing remains unknown. We enrolled 66 men who received either a urethral swab or a meatal swab and compared the cellular content and Gram stain failure rate. We measured the difference in swab cellular content using the Cepheid Xpert® CT/NG sample adequacy control crossing threshold and determined the failure rate of gram stain smears (GSS) due to insufficient cellular material. Meatal smears were associated with a significant reduction in cellular content ($P = 0.0118$), which corresponded with a significantly higher GSS failure rate compared to urethral swabs (45% vs. 3% respectively, $P <0.0001$), in the absence of discharge. When discharge was present, there was no difference between urethral and meatal swabs. Therefore, if GSS testing is being considered for point-of-care diagnosis of NG or NGU in men, meatal swabs should be avoided in the absence of a visible discharge.

Introduction

*Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) infections continue to rise with 1.4 million and > 350,000 cases reported, respectively, in the United States in 2015 (1); though non-gonococcal urethritis (NGU) remains the most common form of urethritis in men (2). For screening at-risk individuals, the Centers for Disease Control and Prevention (CDC) recommends highly sensitive nucleic acid amplification testing (NAAT) of urine or a urethral swab of urethral secretions (3). Meatal swabs have been suggested as a less invasive alternative to urethral swabs for specimen collection for NAAT testing and are amenable to patient collection by eliciting less discomfort (4). Although not yet FDA-approved or CDC-recommended for NAAT testing in men, meatal
swabs are recommended for NAAT testing in prepubertal boys with discharge, given concerns about urethral trauma from urethral swabs (3,7). In men, self-obtained meatal swabs appear to be equivalent to clinician-collected urethral swabs for diagnosing CT/NG infection by NAAT, although there are mixed reports of the sensitivity of this sample type (5, 6). Currently, no rapid (< 30 minutes) point-of-care NAAT test is available to diagnose NG or NGU; and Gram stain smear (GSS) testing of urethral secretions remains the test of choice in settings where rapid diagnosis is needed and microscopy can be performed. The 2015 Sexually Transmitted Diseases Treatment Guidelines specify that GSS testing of male urethral secretions is appropriate for diagnosing NG or NGU, though no preferred swab type is indicated for specimen collection. Meatal swabs are recommended as an alternative for collecting urethral secretions in specific populations (7), though they have yet to be approved for NAAT testing in men. In contrast to NAAT, which amplifies nucleic acids from lysed cells, GSS testing requires collection of intact cells. As meatal swabs may sample a smaller surface area than urethral swabs, and also sample a higher proportion of cornified squamous epithelium from the meatus, it is possible that the number of intact cells collected using a meatal swab may be insufficient to reliably diagnose NG or NGU by GSS testing. To address this concern, we enrolled both symptomatic and asymptomatic men and systematically assigned them to receive either a meatal or urethral swab and measured the swab cellular content using the Cepheid Xpert® CT/NG sample adequacy control crossing threshold (SACCT) (8) and also determined the failure rate of GSS testing for each swab type. The SACCT is an internal control of the Xpert® CT/NG assay and denotes the cycle number at which human hydroxymethylbilane synthase, a
single-copy housekeeping gene, is first detected by real-time PCR amplification. Included in each assay to ensure specimen sample adequacy (9), the SAC\text{CT} is inversely proportional to the amount of cellular material present in the specimen. The primary outcome of our study was to determine if meatal swabs were associated with a lower cellular content and a higher GSS failure rate, compared with urethral swabs. A secondary objective was to determine how the meatal swab was influenced by the presence or absence of discharge.

Results

66 men were included in this study (Table 1). Participants were 20 to 69 (mean 29) years of age, and 56 (89%) were black. 27 men (41%) had visible discharge on genital examination. Swab collection (meatal vs. urethral) was alternated such that 33 men provided a urethral swab and 33 men had a meatal swab taken. No difference in age, race, symptoms, prior STI history, or number of sex partners in the last 30 days was identified between the groups. 9 (14%) men had Gram-negative intracellular diplococci (GNID) present on GSS and 20 men (30%) were diagnosed with NGU. The Xpert® CT/NG assay, performed on both swab and voided urine specimens, diagnosed 20 men with CT and/or NG: 8 men were positive for CT alone, 4 men for both CT and NG, and 8 men for NG alone.

To assess whether meatal swabs were associated with a decrease in cellular content, we compared the SAC\text{CT} of meatal swabs to urethral swabs. Meatal swabs were associated with significantly higher SAC\text{CT} values, indicating they contained less cellular material, compared to urethral swabs (mean 25.6 vs 23.9, \(P = 0.0026\), Table 1).
This difference in cellular content did not affect the performance of the NAAT since the swab sample NAAT results were 100% concordant with the urine NAAT results (data not shown).

We then assessed the failure rate of GSS prepared using urethral and meatal swabs, with failure defined as an absence of cellular material on microscopy (i.e. “quantity of cells not sufficient” [QNS]). The GSS QNS rate of meatal swabs was significantly higher compared with urethral swabs (45% vs. 3%, \( P < 0.0001 \)). Despite no difference in the frequency of signs or symptoms of urethritis between the two groups, meatal swabs were associated with significantly lower number of GSS with PMN between 2-5 (3% vs. 33%, \( P = 0.0011 \), Table 1) than urethral swabs, which is reflected in the high GSS failure rate.

To determine if the increased QNS rate in meatal GSS was associated with less cellular content, we stratified the SAC\textsubscript{CT} results by QNS status. As shown in Figure 1A, Gram stains identified as QNS were associated with a higher meatal swab SAC\textsubscript{CT} (mean 27.4 vs 24.1, \( P = 0.0002 \)), indicating that the meatal swabs used to prepare the GSS contained less cellular material.

Given our finding that meatal swabs are associated with significantly less cellular material and a higher GSS failure rate compared with urethral swabs, we were interested in establishing how meatal swabs performed when sampling discharge. As shown in Figure 1B, in the absence of discharge, meatal swabs collected significantly less cellular material than urethral swabs (mean SAC\textsubscript{CT} 27.0 vs 24.4, \( P = 0.0003 \)). If discharge was present, the meatal swab collected significantly more cellular material than a meatal swab from men without discharge (mean SAC\textsubscript{CT} 23.7 vs 27.0, \( P = 0.0003 \)).
Further, in the setting of visible discharge, no difference in the cellular content collected comparing the meatal or urethral swabs was identified (mean SAC\textsubscript{CT} 23.7 vs 23.3, \( P = 0.4789 \)). Evaluating the GSS failure rate of swabs in the presence or absence of discharge, the highest QNS rates were seen in meatal swabs from men without discharge compared to meatal swabs from men with discharge (68\% vs 15\%, \( P = 0.0022 \), Figure 1C) and compared to urethral swabs in men without discharge (68\% vs 6\%, \( P < 0.0001 \), Figure 1C). In the presence of discharge, meatal swabs were associated with a slight non-significant increase in the QNS rate compared to urethral swabs (15\% vs 0\%, \( P = 0.1373 \), Figure 1C).

We then compared the diagnoses (using both Xpert\textsuperscript{®} and GSS results) of all QNS results to assess the number of NG or NGU diagnoses that could have been missed by GSS failures. Of the 16 QNS GSS results, none were NG diagnoses, but three NGU diagnoses (one CT diagnosed by NAAT and two non-CT NGU [defined by discharge on exam]) were included, which highlights that GSS failures from meatal swabs could delay the time to effective treatment (\textit{i.e.,} missed opportunity to evaluate PMNs in point-of-care testing) in the absence of discharge (data not shown).

Discussion

Evaluation by GSS of urethral secretions from a urethral or meatal swab remains the test-of-choice for rapid diagnosis of NG or NGU in men with urethritis symptoms, though no preferred swab type is recommended by the 2015 STD Treatment Guidelines for collecting urethral secretions (7). In this study, we compared meatal and urethral swabs from men and used the Cepheid Xpert\textsuperscript{®} CT/NG SAC\textsubscript{CT} to measure the
difference in the amount of swab cellular material and determine the failure rate of GSS testing for each swab type. Not surprisingly, we found that meatal swabs collected significantly less cellular material, compared with urethral swabs, in the absence of urethral discharge. Moreover, meatal swabs were associated with a high GSS failure rate, eliciting a 12-fold increase in QNS GSS rates, compared with urethral swabs, in men without discharge. In the presence of urethral discharge, there was no difference in the cellular content or GSS failure rate between the swab types, indicating that the collection quality of meatal swabs is highly susceptible to the sampling surface area of the urethral meatus and/or may be collecting increased numbers of non-intact cells; limitations that may be overcome by urethral sampling. Additionally, the limitations of meatal swabs may only significantly affect Gram stain testing, which is dependent on the collection of intact cells to evaluate for the presence of GNID and PMNs, whereas NAAT testing is much more sensitive and less likely to be significantly influenced by changes in the swab cellular content. In fact, NAAT testing comparing urine and either swab type demonstrated a 100% concordance rate for the diagnosis of CT and/or NG in our study, despite the meatal swabs containing less cellular material.

Although the NAAT performance of self-collected meatal swabs appears comparable to clinician-collected urethral swabs (5) and superior to urine for diagnosis of CT, NG, and trichomonas (6), no study has compared the performance of meatal swabs to urethral swabs for Gram stain point-of-care testing to evaluate urethritis (7). To our knowledge, our study is the first to demonstrate that in the absence of urethral discharge, meatal swabs are inferior to urethral swabs at collecting cellular material, which increases the failure rate of GSS testing. In addition, although there was no
difference in the signs or symptoms between the swab groups, meatal swabs were associated with significantly fewer numbers of GSS with PMN 2-5 (Table 1), suggesting that under-diagnosis of NGU cases may also result when meatal swabs are used for GSS testing.

Our findings have important implications for the use of meatal swabs to collect urethral secretions, a practice that may be increasing given that meatal swabs have several advantages over urethral swabs for NAAT testing. Meatal swabs are likely better tolerated than urethral swabs as demonstrated in a comparison study of men who received both a urethral and meatal swab, which found that 76% of men preferred the meatal swab (4). Another study attempted to quantify the discomfort associated with urethral swab collection using a visual analogue pain scale and found that collection of a urethral swab using standard technique (inserting a swab 2 cm into the distal urethra) elicited a median pain score of 52 mm (on a 100-mm pain scale), which correlated with moderate (>30 mm) to severe (>54 mm) pain (10). Furthermore, multiple studies have identified discomfort from urethral swabbing as a major factor that causes men to delay seeking health care (11-13) and participants in adolescent focus groups expressed strong negative emotions when asked about the urethral swab testing process (12, 13).

In addition, the ease of obtaining a meatal swab facilitates self-collection for NAAT testing, allowing patients to self-collect specimens at home or in a clinical setting where regular interval screening of asymptomatic, high-risk men is performed. Given that 77% of men preferred self-collection of a meatal swab to provider-collection (14) and over 90% of men report self-collection of a penile-meatal swab as “easy” or “very easy” (5, 14), it is likely that NAAT testing of self-collected meatal swabs may play an important
future role in screening asymptomatic men in busy, high-flow clinical settings or non-
clinical settings.

Our study has several limitations. We could not evaluate the performance of
meatal swabs versus urethral swabs for the diagnosis of NGU or NG by Gram stain as
our study wasn’t powered to evaluate that outcome. Also, the majority of men in our
study were African American, which is reflective of demographic characteristics of
clients attending the STD clinic and our results may not be generalizable to other, non-
STD Clinic populations. Additionally, the Gram stains were prepared by multiple
clinicians, all highly proficient at preparing and reading Gram stain smears. Although no
difference in either swab cellular content or the rate of QNS GSS results was associated
with any individual clinician (data not shown), we cannot rule out the possibility that
differences in provider collection techniques may have influenced our results.

In conclusion, in the absence of visible urethral discharge, the use of meatal
swabs for point-of-care diagnosis of NG or NGU by GSS testing was associated with a
significant decrease in swab cellular content and an increase in GSS failure rates,
compared to urethral swabs. Therefore, in the absence of discharge, meatal swabs
should be avoided when considering point-of-care testing for NG or NGU in men.

Materials and Methods

We recruited Men ≥ 19 years old from the Jefferson County Department of
Health (JCDH) STD clinic in Birmingham, Alabama, excluding only men who had voided
within the past 60 minutes and those who had received antibiotics with CT/NG activity
within the past 30 days. After informed consent was obtained, a directed physical exam
was performed for the presence or absence of discharge and men were alternately assigned to receive either a urethral or meatal swab for specimen collection using a dacron swab. To collect a urethral swab, highly experienced clinicians inserted the swab 2cm into the urethra and rotated during insertion and extraction. For meatal swab specimen collection, the swab was positioned perpendicular to the urethral meatus and rolled back and forth several times across the meatus for 5 seconds. Following specimen collection, swabs were immediately rolled onto the surface of a glass microscope slide in preparation for Gram stain of urethral secretions and then placed in an Xpert® CT/NG (Cepheid, Sunnyvale, CA) specimen transport container and stored at 4°C. Following meatal or urethral swab collection, an initial void urine specimen was collected for CT and NG testing.

Gram stain of the smear was performed using standard laboratory procedure and all smears were read by a highly-experienced clinician (JRS), blinded to the physical exam findings and Xpert® CT/NG assay results. After identifying an area of high cellularity using low magnification, the presence or absence of Gram negative intracellular diplococci (GNID) and average number of leukocytes from five contiguous high power fields were determined and recorded using high magnification with oil immersion (1000x). GSS were labeled “Quantity cells not sufficient” (QNS) if no cells were identified by scanning either low or high power magnification. NGU was diagnosed if no GNID were seen and either (1) a discharge was present on exam or (2) ≥2 PMN’s per HPF were seen by GSS testing.

Xpert® CT/NG testing was performed on both urethral and meatal swab specimens and post-swab urine specimens within 7 days of collection as described in
the package insert. For quantification of specimen cellularity, the Xpert® CT/NG sample adequacy control crossing threshold (SAC_CT) was used. We also tested the performance of each swab type to prepare a GSS by determining the failure rate of GSS testing, defined as lacking sufficient number of cells to evaluate the specimen. This study was approved by the institutional review board of the University of Alabama at Birmingham and the research review committee at JCDH. Bivariate comparisons were evaluated using an unpaired T-test while Fisher’s Exact or Chi-square tests were used to test differences between groups. Significance was reported as $P < 0.05$ using Prism software (v7.0b; Graphpad Software, Inc., San Diego, CA).

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Figure Legend:

**FIGURE 1.** Comparison of cellular content and percent QNS in meatal versus urethral swabs in the presence or absence of urethral discharge. (A) Meatal swabs that resulted QNS by GSS are associated with a significantly higher SAC<sub>CT</sub>, compared with satisfactory GSS, indicating they contain less cellular material. (B) In the absence of discharge, meatal swabs have a significantly higher SAC<sub>CT</sub> compared to urethral swabs from men without discharge or meatal swabs from men with discharge. In the presence of discharge, there was no difference in the SAC<sub>CT</sub> between meatal or urethral swabs. (C) In the absence of discharge, meatal swabs were significantly more likely to result QNS by GSS compared to urethral swabs from men without discharge or meatal swabs from men with discharge. In the presence of discharge, there was no difference between the swab types. Horizontal lines and whiskers denote the mean and 95% CI, respectively. SAC<sub>CT</sub> values were obtained from the Xpert® CT/NG assay.


8. Jordan SJ, Van Der Pol B, Hook EW. 2017. Utilization of the Cepheid Xpert® CT/NG sample adequacy control to determine the influence of the urethral swab


<table>
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<th>Characteristic</th>
<th>Total (n = 66)</th>
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<th>Meatal Swab (n = 33)</th>
<th>P-value</th>
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<td>29 (20 – 60)</td>
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Abbreviations: AA, African American; STI, sexually transmitted infection; GNID, Gram-negative intracellular diplococci; PMN, polymorphonuclear neutrophil; QNS, quantity cells not sufficient; CT, chlamydia; NG, gonorrhea

*a3 missing

Significance evaluated using Fisher’s Exact test or T-test, as appropriate.