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Publication date

01-11-2008

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Citation for this work (American Psychological Association 7th edition)

Alexander, S., Ison, C., Parry, J., Llewellyn, C., Wayal, S., Richardson, D., Phillips, A., Smith, H., & Fisher, M. (2008). *Self-taken pharyngeal and rectal swabs are appropriate for the detection of Chlamydia trachomatis and Neisseria gonorrhoeae in asymptomatic men who have sex with men* (Version 1). University of Sussex. <https://hdl.handle.net/10779/uos.23313683.v1>

Published in

Sexually Transmitted Infections

Link to external publisher version

<https://doi.org/10.1136/sti.2008.031443>

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Article (Unspecified)

Alexander, S., Ison, C., Parry, J., Llewellyn, Carrie, Wayal, S., Richardson, D., Phillips, A., Smith, Helen and Fisher, M. (2008) Self-taken pharyngeal and rectal swabs are appropriate for the detection of Chlamydia trachomatis and Neisseria gonorrhoeae in asymptomatic men who have sex with men. Sexually Transmitted Infections, 84. pp. 488-492. ISSN 1368-4973

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Sex Transm Inf 2008;84:488-492
doi:10.1136/sti.2008.031443

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Self-taken pharyngeal and rectal swabs are appropriate for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in asymptomatic men who have sex with men

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Accepted 12 August 2008

ABSTRACT

Introduction: Self-taken specimens from men who have sex with men (MSM) could be important in reducing high levels of demand on sexual health services. The performance of self-taken specimens for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) from both pharyngeal and rectal sites in asymptomatic MSM was assessed.

Methods: MSM were examined according to clinic protocol: a rectal and pharyngeal swab for GC culture and a rectal swab for the CT strand displacement assay. An extra set of nurse-taken and self-taken pharyngeal and rectal specimens were also requested and were tested using the Aptima Combo 2 assay and the result compared with the routine clinic result, which was considered the gold standard.

Results: A total of 272 MSM was recruited and the sensitivity and specificity of nurse-taken and patient-taken swabs, respectively, was as follows: rectal GC: 94.9% and 90.1% (nurse); 92.3% and 87.9% (patient); pharyngeal GC: 88.2% and 91.8% (nurse); 100% and 87.8% (patient); rectal CT: 80.0% and 99.6% (nurse); 91.4% and 98.2% (patient). No significant difference in sensitivity or specificity was observed between the nurse-taken and the patient-taken rectal swabs for either GC or CT. For the detection of GC from the pharynx, comparable sensitivities were achieved between nurse-taken and patient-taken swabs ($p = 0.5$); however, a significant difference in specificity was observed ($p = 0.006$). This was due to a higher number of false GC-positive self-taken pharyngeal swabs from patients with high rates (90.9%; 10/11) of confirmed concurrent GC infection in different anatomical sites.

Conclusions: MSM are able to collect self-taken rectal and pharyngeal swabs that are comparable to those taken by clinicians.

The incidence of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) infections continues to rise in men who have sex with men (MSM), in England and Wales.¹ Some of the key factors in onward transmission include high rates of undiagnosed asymptomatic CT and GC, delays in being able to access healthcare due to increasing demand on genitourinary medicine (GUM) services and increasing levels of high-risk behaviour.^{2,3} If recent trends in the prevalence of sexually transmitted infections (STI) are to be reversed, novel intervention strategies, which enable the rapid detection of

pathogens and the administration of appropriate therapy, will be necessary.

Non-invasive specimens such as urine and vulvovaginal swabs have been shown to be an acceptable alternative to clinician-taken urethral and endocervical specimens and are the principal success behind screening programmes, which are primarily targeted at young heterosexual individuals. The self-taken specimen (which may contain lower levels of target agent) is, however, only of value when examined using a nucleic acid amplification test (NAAT), which has superior sensitivity when compared with traditional methods of diagnostics such as enzyme immunoassays and bacteriological culture. However, the appropriateness of using NAAT in MSM populations has additional problems, because of the unvalidated nature of dual CT and GC NAAT in extragenital sites such as the rectum and the pharynx. Consequently, the potential of self-taken specimens to detect CT and GC in MSM has yet to be fully explored.

The high specificity of all CT NAAT platforms has been established in previous studies, and although these tests have not been formally approved by the Food and Drug Administration for use with non-genital specimens, they have been validated and are now widely used for testing both pharyngeal and rectal swabs.^{4,5} In contrast, the specificity of GC NAAT are very variable between commercial platforms: with reports of crossreactivity with other *Neisseria* spp when using both the Cobas Amplicor CT/NG test (Roche Diagnostics, West Sussex, UK) and CT/GC strand displacement assay (SDA; Becton Dickinson, Oxford, UK) tests.⁶ To date, the only currently available NAAT in which the GC component has not as yet been reported to crossreact with other *Neisseria* spp are the Genprobe Aptima Combo 2 (AC2) and GC mono-specific assays. However, these two assays have not been as rigorously evaluated as some of the other platforms and further studies to determine their specificities are warranted.^{6,7}

It was the aim of this study to compare the performance of self-taken versus nurse-taken specimens for the detection of both CT and GC from both pharyngeal and rectal sites, in asymptomatic MSM patients attending GUM clinics, using the AC2 test.

Table 1 The concordance between AC2-generated GC results for both nurse-taken and patient-taken swabs from rectal and pharyngeal sites and comparison with the routine GC culture result

GC culture result	Nurse-taken swab AC2 GC result	Patient-taken swab AC2 GC result	Number*
Rectal specimens (262 patients)			
–	–	–	188
+	+	+	35
–	+	+	17
–	–	Equ	3
–	+	–	3
–	+	Equ	2
–	–	+	10
+	+	–	1
+	Equ	+	1
+	+	Equ	1
+	–	–	1
Pharyngeal specimens (262 patients)			
–	–	–	211
+	+	+	15
–	+	+	19
–	+	–	1
–	Equ	–	1
–	–	+	11
–	–	Equ	2
+	Equ	+	1
+	–	+	1

Equ, equivocal test result; –, negative test result; +, positive test result.

*The number of participants with this combination of test results.

GC, *N gonorrhoeae*.

METHODS

Subjects and recruitment

Subjects were recruited from MSM attending a single GUM clinic in Brighton, UK, between October 2005 and May 2007. Cases were defined as individuals asymptomatic at either or both pharyngeal and rectal sites with confirmed infection with either GC or CT identified on routine clinical testing (SDA for rectal CT, culture for GC) within the past 6 weeks who had not received antibiotics with activity against either organism during that time period. A small number of patients was also recruited to the study (as cases) on their initial clinic visit because they were positive for GC at the urethra by microscopy, but were asymptomatic at both the pharynx and the rectum. Controls were defined as MSM attending during the same time period who were asymptomatic and negative on the same tests outlined above at either or both sites.

Recruitment was initially performed at first presentation in order to minimise the risk of any untreated infection or the impact of any empiric antibiotic usage on study uptake. After a midway review of study recruitment (December 2006), when the number of controls far outnumbered the number of cases, subsequent recruitment was purposively of cases until the required number for powering the study was achieved, with a random inclusion of controls (a minimum of one control to every case) to maintain laboratory blinding (see below). Patients were eligible to be included into the study more than once either as a control or as a case if they had been appropriately treated and a period of 6 weeks had elapsed between their last inclusion date.

Study procedures

Subjects were re-tested by the above routine clinical methods performed by a trained GUM nurse (analysed at the local

laboratory) and then underwent further sampling tests from both the pharyngeal and rectal sites by a nurse (nurse-taken) and by the subject after provision of a detailed instruction sheet (self-taken). These specimens were sent to the Sexually Transmitted Bacteria Reference Laboratory (STBRL) where they were tested using the AC2 assay. The laboratory team were blinded to the identification of study subjects as either cases or controls and if the specimen was either nurse or self-taken. Any specimen that generated either a positive or an equivocal result for either CT or GC was repeated using the AC2 platform and the specimens were then stored at 4°C. At the end of the study after unblinding and comparison with the routine gold standard result if any discrepancy was identified in the CT or the GC results between the nurse-taken and the self-taken swab results and/or the routine clinic result specimens were retested with the CT or GC mono-specific test (Gen-Probe, San Diego, California, USA).

Statistical methods and power calculation

The specificity and sensitivity of the nurse and self-taken specimens were calculated by comparing the results generated with the standard routine clinic result (SDA for CT detection; culture for GC detection). For the purposes of this study the routine clinic result was considered the gold standard. In sensitivity and specificity calculations equivocal results were regarded as negative. Both the sensitivity and specificity of nurse versus patient-taken specimens were evaluated using a McNemar's test, in STATA 10.

It was anticipated that a minimum of 212 patients would need to be enrolled in the study (56 true positive cases of gonorrhoea and 56 true controls) in order to detect a sensitivity and specificity of 95% (rejecting with 80% power a sensitivity and specificity as low as 80% and a level of significance of 0.05) and 40 true positive cases of chlamydia and 60 true controls would be required to give 80% power to reject the test if sensitivity falls below 80% (against gold standard of 95%) and specificity falls below 90% (against gold standard of 100%).

Table 2 The concordance between AC2-generated CT results for both nurse-taken and patient-taken swabs from rectal and pharyngeal sites and comparison with the routine clinic SDA result

SDA result	Nurse-taken swab AC2 CT result	Patient-taken swab AC2 CT result	Number*
Rectal specimens (258 participants)			
–	–	–	218
+	+	+	28
–	+	+	1
–	Equ	–	1
–	Equ	+	1
+	Equ	+	4
–	–	+	2
+	–	Equ	1
+	–	–	2
Pharyngeal specimens (265 participants)			
–	–	–	257
+	+	+	3
–	–	+	2
+	+	Equ	1
+	+	–	2

Equ, equivocal test result; –, negative test result; +, positive test result.

*The number of participants with this combination of test results.

CT, *C trachomatis*; SDA, strand displacement assay.

Table 3 The sensitivity and specificity of self-taken and nurse-taken swabs for the detection of CT and GC

	Method of collection	Sensitivity (95% CI)	p Value (no of true positives)	Specificity (95% CI)	p Value (no of true negatives)
Rectal GC* (n = 262)	Nurse	94.9% (82.7 to 99.4)	1.00 (39)	90.1% (85.4 to 93.7)	0.30 (223)
	Patient	92.3% (79.1 to 98.4)		87.9% (82.9 to 91.9)	
Pharyngeal GC* (n = 262)	Nurse	88.2% (63.6 to 98.5)	0.50 (17)	91.8% (87.7 to 94.9)	0.006 (245)
	Patient	100% (80.5 to 100)		87.8% (83.0 to 91.6)	
Rectal CT† (n = 258)	Nurse	80.0% (63.1 to 91.6)	0.13 (35)	99.6% (97.5 to 100)	0.25 (223)
	Patient	91.4% (76.9 to 98.2)		98.2% (95.5 to 99.5)	

*Nurse and patient-taken swabs (tested by AC2) compared with GC culture results.

†Nurse and patient taken swabs (tested by AC2) compared with clinic strand displacement assay (SDA) result.

CT, *C trachomatis*; GC, *N gonorrhoeae*.

Consent and ethics

Approval for the study was obtained from the Brighton and Mid-Sussex Ethics Committee. Individuals were only recruited into the study after giving informed consent according to Good Clinical Practice Guidelines.

RESULTS

A total of 272 study participants (made up of 267 individual patients) was included in the study and a full set of specimen results were generated and returned for: 262 participants (rectal GC), 262 participants (pharyngeal GC), 258 participants (rectal CT) and 265 participants (pharyngeal CT). The number of study specimens positive for each infection by the “gold standard” (see above) methods were: GC rectum 39 cases, GC pharynx 17 cases and CT rectum 35 cases. Therefore, according to the initial power calculations, although the number of GC culture-positive cases (total 56) recruited was met, this study fell short of the anticipated number of CT cases, despite recruiting more patients than planned.

GC rectal and pharyngeal results

The combination of results generated from examining the three different specimens (a GC culture and a self and nurse-taken swab tested by AC2), from both rectal and pharyngeal sites, are displayed in table 1. Completely concordant results were obtained for 85.1% (223/262: rectal) and 86.3% (226/262: pharyngeal) of specimen sets tested. Concordant AC2 results (nurse and self-taken specimens) but GC culture-negative specimens accounted for 6.5% of rectal (17/262) and 7.3% of pharyngeal (19/262) specimens and for the purposes of analysis this specimen set combination was not considered discordant. The remaining 8.4% (22/262) rectal and 6.5% (17/262) pharyngeal specimen sets were discordant for GC in various combinations between the culture result and/or the two AC2 results, but it is notable that positive or equivocal GC specimens were more common in the patient-taken than the nurse-taken specimen (table 1). Interestingly, of the 22 discordant rectal specimens, 15 (68.2%) had been sourced from patients, with a GC culture-positive result from another site (either urethral or pharynx). Of the 17 patients who generated a pharyngeal discordant result, 15 (88.2%) had GC culture positive from another site (either urethral or rectal).

CT rectal and pharyngeal results

A total of 258 study participants had a full set of rectal specimen results returned (routine SDA swab and self and nurse-taken for AC2) and the combination of results generated are presented in

table 2. It was not routine clinic practice to perform a SDA CT test on pharyngeal specimens, but the result of the self and nurse-taken swabs from 265 study participants are displayed. In 95.3% (246/258) of rectal and 98.1% (260/265) of pharyngeal specimen sets, concordant CT results were generated. However, 12 rectal and five pharyngeal specimen sets produced discordant results in various combinations (table 2).

Comparison of nurse versus patient-taken swabs

The final sensitivity and specificity of both the nurse and self-taken swabs was compared with the gold standard routine clinic result, which for GC was culture and for CT was the SDA result (table 3). No significant difference in either the sensitivity or specificity was observed between the self and nurse-taken rectal swab for the detection of either GC or CT (table 3). Similarly, although no significant difference in sensitivity was observed between the nurse and self-taken pharyngeal swabs for GC, the difference in specificity was found to be significant (table 3). Due to the absence of a gold standard clinic result for pharyngeal chlamydia these data could not be presented.

DISCUSSION

Non-invasively collected specimens such as self-taken vaginal swabs and first catch urines have enabled population-based prevalence studies and community screening programmes for CT in heterosexual individuals. This study was undertaken to determine if self-taken rectal and pharyngeal specimens were appropriate for the detection of GC and CT in MSM. No significant difference in sensitivity was observed between the nurse-taken and the patient-taken swabs for the detection of either GC or CT in the rectum and GC in the pharynx, when using the AC2 test (table 3). Clearly, if provided with detailed instructions, MSM are as equally capable of collecting rectal and pharyngeal specimens as clinic staff.

Interestingly, when examining the specificity of the pharyngeal GC specimens, a significant difference was observed between the nurse and patient-taken swabs, with the patient-taken swabs having a higher number of false GC-positive results (tables 1 and 3). It is possible that high rates of concurrent GC infection in different anatomical sites in these specific individuals may explain this, as 90.9% (10/11) of patients who generated a false GC-positive self-taken pharyngeal specimen were culture positive at either the rectum or the urethra. It could be speculated that patients were more likely to cross-contaminate the specimens between sites because of inexperience in handling the collection materials, but the clinical implications of this are probably minimal. However, this,

combined with concerns raised by previous studies that highlight that CT nucleic acids are present at detectable levels in the genitourinary environment, suggest that issues regarding specimen purity should be reinforced to patients before self-collection kits are distributed.⁸

The high sensitivities and conversely low specificity values generated for the GC component of this study (table 3) is a direct consequence of comparing the AC2 result with the gold standard GC bacteriological culture, which is inevitably less sensitive. The reduced sensitivity of GC culture in the rectum and pharynx has previously been documented and it has also been recommended that molecular tests may be more appropriate for the detection of GC infection in these sites.⁹ In reality, how a laboratory actually provides a highly sensitive and specific service for GC detection in extragenital sites is actually extremely problematical because of the higher numbers of commensal *Neisseria* spp in these sites and the low specificity of some GC NAAT platforms.⁶ The significant number of asymptomatic patients identified in this study with negative GC cultures but positive AC2 GC results highlights the problems associated with detecting GC in rectal and pharyngeal sites, and indeed the high number of patients who will go undetected and untreated if culture alone is the only method used for diagnosis. It should also be reiterated that GC NAAT platforms are not equal in performance. In this study the AC2 assay was used, which has been shown to be highly specific and caution should be applied before extrapolating the data presented here to other GC NAAT tests.

Interestingly, in this study the sensitivity of the CT component of the AC2 test, when compared with the SDA result, was lower (tables 2 and 3). This is in sharp contrast to many previous studies, all of which have shown the AC2 to be a more sensitive assay than SDA when testing urine, cervical swabs and vaginal swabs.^{10–12} Participants who had a positive SDA result but were AC2 CT negative were not repeated during this study, but were re-examined using the CT monospecific test at the end when the results were unblinded. Upon re-examination, seven AC2 CT-negative specimens (five nurse and two self-taken) generated a positive using the monospecific CT test and were therefore given a CT equivocal status (table 2). In our sensitivity and specificity calculations (using a very conservative approach) equivocal results were regarded as negative and therefore this explains the anomaly in AC2 sensitivity. One possible reason for seven clinical specimens initially being CT AC2 false negatives is that, despite the high throughput nature of the AC2 testing work performed in the study, it was conducted by hand pipetting rather than using a robot and each AC2 test does not contain an internal control. It is recognised that the introduction of automation not only improves turnaround times, and reduces staff time, but also improves the reliability of clinical results.¹³ Such a problem was not highlighted when examining the GC results because the GC data were compared with a less sensitive, more forgiving, gold standard.

This is the first study to provide conclusive data that self-taken swabs from both rectal and pharyngeal sites are comparable to those taken by the nurse for the detection of both CT and GC, when using the AC2 test. It is hoped that the findings from this study will enable the development of non-GUM community STI screening programmes within MSM populations, thereby improving both individual and public health. This strategy is currently being evaluated by this research group.

Key messages

- ▶ STI disproportionately affect MSM and novel strategies for testing are needed.
- ▶ NAAT provide a technology that can facilitate sampling in non-clinical settings.
- ▶ This is the first study to evaluate self-taken specimens against a gold-standard clinical test at both pharyngeal and rectal sites for CT and GC.
- ▶ Self-taken specimens perform with similar sensitivity to clinician-taken specimens from both sites in MSM.
- ▶ Evaluation of self-taken specimens in MSM as a means of improved access to testing and earlier diagnosis is warranted.

Acknowledgements: The authors would like to acknowledge Matthew Hankins and Tom Nicols for their statistical input into the project and Mariangela Bizzarri and Melese Tedla for technical assistance with sample processing.

Funding: This study was funded by a grant awarded from the Medical Research Council (MRC) Sexual Health and HIV Research Strategy Committee. The views expressed in this manuscript are those of the authors and not necessarily those of the MRC or the health department.

Competing interests: None.

Ethics approval: Approval for the study was obtained from the Brighton and Mid-Sussex Ethics Committee.

Patient consent: Individuals were only recruited into the study after giving informed consent according to Good Clinical Practice Guidelines.

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Contributors: JP, CI, CL, DR, HS and MF were responsible for the design, progress and management of the study. AP and DR were responsible for patient recruitment and AP was responsible for data collection. SW was responsible for study management and implementation and critical appraisal of the manuscript. SA performed the AC2 testing at STBRL and was responsible for the data analysis and production of the first draft of the manuscript.

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