ORIGINAL ARTICLE

Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* from pooled rectal, pharyngeal and urine specimens in men who have sex with men

David John Speers,[1,2] I-Ly Joanna Chua,[1] Justin Manuel,[3] Lewis Marshall[2,3]

[1]Department of Microbiology, PathWest Laboratory Medicine WA (LMWA), Queen Elizabeth II (QEII) Medical Centre, Nedlands, Western Australia, Australia
[2]School of Medicine and Pharmacology, University of Western Australia, Crawley, Western Australia, Australia
[3]M Clinic, WA AIDS Council, West Perth, Western Australia, Australia

Correspondence to
Clinical Associate Professor David John Speers, Department of Microbiology, PathWest Laboratory Medicine WA (LMWA), Queen Elizabeth II Medical Centre, Hospital Avenue, Nedlands, WA 6009, Australia; david.speers@health.wa.gov.au

Received 8 June 2017
Revised 24 September 2017
Accepted 8 October 2017
Published Online First 24 October 2017

To cite: Speers DJ, Chua I-LJ, Manuel J, *et al*. *Sex Transm Infect* 2018;**94**:293–297.

ABSTRACT

Objectives Screening of men who have sex with men (MSM) for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) requires sampling from anorectal and pharyngeal sites in addition to urogenital sampling. Due to the cost of testing multiple anatomical sites individually testing of pooled specimens has potential merit. The Cepheid GeneXpert CT/NG assay (GeneXpert), which also has potential for point-of-care nucleic acid testing in the sexual health clinic, has not been assessed for pooled specimen testing.

Methods We prospectively compared GeneXpert testing of pooled pharyngeal and rectal swabs with urine samples to standard of care testing of individual specimens from 107 participants using the Roche cobas 4800 CT/NG assay (cobas) for CT and NG in high-risk MSM attending an inner city sexual health clinic.

Results We found testing of pooled pharyngeal, rectal and urine samples by the GeneXpert to have 100% agreement for NG and 94% overall agreement for CT when compared with individual specimen testing by cobas. For CT testing, 14 cases were detected for both tests, 4for cobas only, 2 for GeneXpert only and 89 participants were negative for both tests.

Conclusions Pooled specimen CT and NG testing by the GeneXpert was accurate when compared with single specimen testing and has potential for screening MSM for CT and NG. The role of pooled specimen testing with the GeneXpert as a point-of-care nucleic acid test in MSM requires further investigation.

INTRODUCTION

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (NG) are the most common STIs in men who have sex with men (MSM) with rates that continue to rise.[1] Since these infections are often asymptomatic, accurate diagnosis in MSM is critical to limit the transmission of CT and NG by ensuring early and appropriate treatment and to help reduce the risk of acquiring HIV infection.[2 3] Nucleic acid amplification tests (NAAT) are now recommended by international guidelines to screen MSM for extragenital CT and NG infections.[4 5]

The Centers for Disease Control and Prevention has reported rectal prevalence in MSM for chlamydia of 8.9% and 5.4% for gonorrhoea, with a pharyngeal prevalence of 1.6% and 5.3%, respectively.[2] Due to this high prevalence, it is recommended that rectal and pharyngeal specimens are collected in addition to urethral or urine specimens to avoid missed infections.[5] While there are no NAATs licensed for use with extragenital specimens, these platforms reliably detect pharyngeal and rectal CT and NG in MSM.[6–10] However, testing both genital and extragenital sites to determine the infection status is costly and requires more laboratory resources.[11]

Testing of pooled pharyngeal, urethral and rectal specimens is one potential cost-effective solution for diagnosing chlamydia and gonorrhoea in MSM, but only if there is no compromise in sensitivity and specificity. This is of particular concern for pooling specimens from extragenital sites due to the recognised lower CT and NG organism burden at these sites and the risk of false NG positives when using NAAT platforms due to the presence of non-gonococcal *Neisseria* spp.[12] There are limited data on the use of NAATs for the detection of CT and NG using pooled specimens. Sultan *et al*[11] studied pharyngeal, rectal and urine specimens in MSM by comparing single sample analysis with pooled remaining samples using the Aptima Combo 2 TMA assay (AC2) (Hologic, San Diego, CA, USA). For their cohort of 164 CT and 288 NG detections from single sample testing they found the sensitivity of pooled sampling to be slightly lower but not significantly different (p=0.167) for CT (92% vs 96%); however, pooled sampling was significantly lower (p<0.001) for NG (90% vs 99%), resulting in 13 missed CT infections and 31 missed NG infections. Interestingly, pooled testing detected six CT and four NG infections that were missed by single sample testing.

The GeneXpert CT/NG PCR assay (GeneXpert) (Cepheid, Sunnyvale, CA), which detects two chromosomal NG targets, NG2 and NG4, and one chromosomal CT target, CT1, is Food and Drug Administration approved for the detection of CT and NG from urogenital sites. This assay has potential application as a point-of-care (POC) NAAT[13] as it can be used with minimal operator training, has a small bench space footprint and provides results in a much shorter time frame than conventional testing (90 min). The GeneXpert was found to have a sensitivity of 10 NG genome copies per reaction and no false-positive results when 236 non-gonococcal *Neisseria* spp and closely related species were tested.[13] However, there are limited data on the use of the GeneXpert for extragenital specimens.[9 10]

When compared with the AC2, this test had lower sensitivity for the detection of pharyngeal and rectal CT and NG but the number of AC2 detections was relatively small.

The aim of this study was to evaluate the GeneXpert for testing of pooled rectal, throat and urine specimens from MSM attending an inner city sexual health clinic. The performance of the GeneXpert using pooled urogenital and extragenital specimens was compared with standard-of-care individual specimen testing using a second PCR assay, the cobas 4800 CT/NG assay (cobas) (Roche Molecular Diagnostics, Indianapolis, IN), in MSM at higher risk of chlamydia and gonorrhoea.

MATERIALS AND METHODS

Participants

The participant cohort was recruited from an inner city sexual health clinic managed by the Western Australia AIDS Council. Presentations of high-risk MSM, including contacts of partners diagnosed with NG and/or CT infection and symptomatic MSM, were invited to participate between January 2016 and December 2016 and consent obtained. Those who had recently received antibiotics within 1 month of presentation were excluded.

Specimen collection

Dual head swabs (Medical Wire & Equipment, Wiltshire, England) were employed for pharyngeal and rectal sampling to facilitate the prospective pooled specimen and the single specimen processing from a single collection and without the need to re-elute the swabs. After consent, one rectal swab and first void urine specimen were self-collected following verbal and written instruction by the trained clinic staff, and one throat swab was obtained by clinic staff from each participant. The dual head swabs were divided on receipt in the laboratory with one swab head used for pooled testing and the second for standard-of-care NG and CT laboratory single specimen testing. A 7 mL aliquot of urine was separated for pooled testing from the primary collection container prior to standard-of-care urine testing. The 7 mL volume was chosen as this was the manufacturer's recommended volume for urine processing using the GeneXpert Urine Collection Kit.

Pooling of specimens

The throat and rectal swab heads and 7 mL of urine were added into the GeneXpert Urine Specimen Collection Kit, vortexed for 30 s and then inoculated into the GeneXpert CT/NG test cartridge.

Laboratory methods

The GeneXpert was chosen for pooled specimen testing as this platform is a viable POC NAAT option for sexual health clinics and readily amenable to pooling of swab and urine specimens in the one cartridge, thus avoiding the reagent cost of testing three specimens from each client. To provide a more direct comparison of the GeneXpert as a pooled specimen NAAT to our standard laboratory platform (cobas) without the potential biases of POC testing, we compared pooled testing with the GeneXpert to single specimen testing using the cobas in a controlled laboratory environment.

All specimens were stored according to manufacturer's instructions. Single specimens were processed by the cobas method for NG and CT detection according to the manufacturer's instructions. Extragenital specimens that were positive on the cobas were confirmed using a validated in-house PCR assay. This assay employs real-time multiplex PCR detection of three targets for both CT and NG which has equivalent sensitivity and specificity to the previously published tandem PCR assay.[14] Pooled specimens inoculated into the GeneXpert Urine Collection Kit were tested according to manufacturer's instruction for urine testing. For the interpretation as positive for NG, both the NG2 and NG4 targets must be detected.

Interpretation of test results

Specimens positive or negative by both pooled and individual specimen testing were considered concordant. The in-house PCR assay was performed on all discrepant specimens. In addition, GeneXpert negative pooled specimens with a positive cobas result underwent repeat GeneXpert testing using the remaining unprocessed pooled primary specimen stored at 4°C. Retesting of GeneXpert positive but cobas negative swabs was not possible due to the processing of the swabs for primary testing.

Statistical methods

Statistical analysis was performed using GraphPad Prism software (GraphPad Software, San Diego, California, USA).

RESULTS

Participant clinical characteristics

The average age of the 107 participants was 32 years (range 17–71 years), of which 3 were confirmed and 1 self-reported to be living with HIV. There were 55 symptomatic presentations, 23 asymptomatic NG contacts, 16 asymptomatic CT contacts, 1 each of an asymptomatic contact of an HIV case and a syphilis case, and 13 asymptomatic screens. Two participants presented twice at least 3 months apart. There were 20 (19%) participants positive for CT by one (6) or both (14) methods, and 34 (31.8%) positive for NG by both methods. Five participants who had a throat and urine specimen but no rectal swab collected were included.

Performance of pooled testing

The comparative results of the pooled testing by the GeneXpert and the single specimen testing by the cobas are shown in table 1. There was complete agreement for the NG results. When comparing the CT results between the two methods the overall agreement was good at 94% (kappa coefficient 0.791, 95% CI 0.631 to 0.952) and not significantly different (p=0.683) by the McNemar's test. To further differentiate the discrepant CT results and to control for agreement by chance the positive percent agreement (PPA), the proportion of positive cobas results that were GeneXpert positive, and the negative percent agreement (NPA), the proportion of negative cobas results that were GeneXpert negative were compared. This showed a lower

Table 1 Comparison between GeneXpert pooled sample and cobas individual sample testing for detection of NG and CT

Organism			Assay comparison		
			cobas		
			Detected	Not detected	Total
Chlamydia trachomatis	GeneXpert	Detected	14	2	16
		Not detected	4	89	93
		Total	18	91	93
Neisseria gonorrhoeae	GeneXpert	Detected	34	0	34
		Not detected	0	75	75
		Total	34	75	109

CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*.

PPA (14/18=78%) for the CT results when compared with the NPA (89/91=98%).

Anatomical sites of infection

Overall, there were 51 NG and 19 CT specimen detections in 34 and 18 presentations, respectively, by cobas. Detection of NG was most common from throat swab (19 detections) compared with urine (17 detections) or rectal swab (14 detections). In contrast, CT was more commonly detected from rectal swab (nine detections) and urine (eight detections) compared with throat swab (two detections). There were 17 single site positives for NG, 12 dual site positives and 3 triple site positives by cobas. Of the 16 presentations with a single positive specimen for CT by cobas, 13 (81%) were detected by GeneXpert and 3 were negative. Of the eight presentations with only a positive rectal swab by cobas, there were six detections by GeneXpert. There were two presentations with two sites positive by cobas, both of which were detected by GeneXpert, and no presentations with all three sites positive for CT. There were 16 NG detections and 10 CT detections from rectal and/or throat swabs only by cobas.

The mean cycling threshold (Ct) value, which is inversely proportional to the genome copy number, for CT detections was 29.4 for positive GeneXpert pooled specimen testing and 33.3 (throat swab 30.2, urine 32.6, rectal swab 33.7) for positive cobas single specimen testing. The mean Ct for NG detections was also lower for positive pooled specimen testing (21.2 and 20.5 for target NG2 and NG4, respectively) than for positive single specimen testing (30.0). Urine detections for NG had the lowest mean Ct (25.7) compared with rectal swab (31.4) and throat swab (31.9) detections, despite the fact that throat swabs were more commonly positive.

Discrepant chlamydia result analysis

Of the 20 CT detections by either method, there were 6 discrepant results, 2 were detected by pooled specimen GeneXpert testing but not single specimen cobas testing, and 4 were detected by single specimen cobas testing but not pooled GeneXpert specimen testing. Samples from these participants were further analysed by in-house PCR testing and repeating the pooled specimen testing (table 2). For participant 1, the single specimens could not be retrieved for in-house PCR testing, such that only the remaining pooled specimen could be tested. This confirmed the detection of CT in the pooled specimen. Of the remaining five participants with discrepant CT results, four were positive only in the rectal swab specimen, three of which were detected in single specimen testing only and one was detected in pooled specimen testing only. One of the three single specimen detections was positive on repeat testing of the pooled specimen. The pooled specimen mean Ct for missed single specimen detection was higher than for overall CT detection (35.4 vs 29.4) and the single specimen mean Ct for missed pooled testing was also higher (38.0 vs 33.3). The remaining participant's throat swab was positive for chlamydia with a high Ct (40.4 vs 33.3) which could not be confirmed by in-house PCR testing.

Table 2 Analysis of the six discrepant CT participant results

Participant	GeneXpert	Specimen	cobas	In-house PCR
1	Detected	Urine	ND	Pooled
		Throat swab	ND	specimen
		Rectal swab	ND	Detected
2	Detected	Urine	ND	ND
		Throat swab	ND	ND
		Rectal swab	ND	Detected
3	ND (detected on repeat)	Urine	ND	ND
		Throat swab	ND	ND
		Rectal swab	Detected	Detected
4	ND (ND on repeat)	Urine	ND	ND
		Throat swab	ND	ND
		Rectal swab	Detected	Detected
5	ND (ND on repeat)	Urine	ND	ND
		Throat swab	ND	ND
		Rectal swab	Detected	Detected
6	ND (ND on repeat)	Urine	ND	ND
		Throat swab	Detected	ND
		Rectal swab	ND	ND

CT, *Chlamydia trachomatis*; ND, not detected.

DISCUSSION

Pooling of urine specimens from multiple individuals has been shown to be sensitive and specific for CT testing[15 16] and the effectiveness of pooling specimens from different anatomical sites within an individual for CT and NG detection has been demonstrated previously.[11] These workers tested throat and rectal swabs and urine samples in MSM using the AC2 assay and demonstrated high negative predictive values for pooled sample testing.

This study has further explored the possibility of testing for CT and NG using pooled specimens from MSM by comparing pooled samples tested by the GeneXpert with testing of single specimens using another commonly employed NAAT, the cobas. The GeneXpert has been evaluated against well-characterised bacterial strains and found to have high analytical sensitivity and specificity.[13] Moreover, in clinical studies it has demonstrated good performance in comparison to the AC2 and to the ProbeTec ET *C. trachomatis* and *N. gonorrhoeae* amplified DNA assay (Becton, Dickinson, Sparks, MD)[17] and to the cobas.[18 19] However, to our knowledge, there is no prospective analysis comparing the GeneXpert and cobas for urogenital specimens and extragenital specimens or pooled specimens in MSM.

In our inner city sexual health clinic setting, we found the performance of the GeneXpert for the detection of NG in pooled specimens to be equivalent to individual specimen testing by cobas. This agreement was unaffected by the actual site of infection or the number of anatomical sites infected. Fifteen of the 34 gonorrhoea infections involved multiple sites of infection; however, restricting testing to urine testing only would have missed 16 of these. Only 2 of the CT infections involved multiple sites, with 8 of the 18 participants having a positive urine specimen by cobas. There were fewer symptomatic CT infections than for those with NG, demonstrating the value in screening contacts without symptoms for CT infection. There were more NG detections than CT detections and the GeneXpert and cobas average Ct was lower for NG than for CT detection. This may represent either a higher NG organism burden at these sites or that the tests were more sensitive for NG.

The pooled specimen method for NG was in complete agreement with the single specimen testing, whereas for CT there was good overall agreement (94%) and no significant difference between pooled and single specimen testing results (p=0.683), but with better negative agreement (98%) than positive agreement (78%). There were six discrepant CT presentations, including four negative pooled testing results and two negative single specimen results. One specimen was CT positive by cobas in a throat swab which could not be confirmed by in-house

PCR testing. The Ct value for this sample was very high (40.4), suggesting the CT organism burden was close to the lowest limit of detection for all assays. Four of the remaining discrepant results occurred in participants with rectal CT infection only. Of interest, the average Ct for rectal CT positive specimens in the cobas assay was higher than for urine or throat swabs, indicating either a lower CT organism burden in these specimens or the presence of PCR inhibitors. However, this Ct differential was not seen between rectal and throat NG positive specimens suggesting rectal PCR inhibitors were not responsible. Previous studies using single rectal specimen testing have shown the GeneXpert missed 6 of 46[9] and 2 of 15[10] rectal CT when compared with AC2. Of note, in the former study, the AC2 also missed three rectal CT, likely reflecting a similar problem with sampling specimens with organism numbers at the lower limits of detection. Also, the residual AC2 buffer was diluted 1:15 into the GeneXpert buffer for GeneXpert testing which may have reduced its sensitivity.[9]

The added advantage of using the GeneXpert is its suitability as a POC NAAT for use in sexual health clinics. This would have the benefit of cost-effectiveness and could improve the management of chlamydia and gonorrhoea in MSM. Syndromic management of chlamydia and gonorrhoea can result in many infections going untreated, as well as overtreating others.[20] By providing an earlier diagnosis, more appropriate antimicrobial prescribing and more efficient contact tracing could be performed, potentially interrupting the transmission of CT and NG. This concept has been applied with success to CT and NG testing using the GeneXpert in several remote Australian indigenous communities.[21] In this setting of reduced access to timely pathology testing, POC testing has distinct advantages and the POC GeneXpert testing was found to be highly concordant with laboratory-based testing. Likewise, in MSM, POC testing could be of particular benefit due to the high rate of asymptomatic infections and the increased rates of chlamydia and gonorrhoea in MSM compared with other populations.

This study has several limitations. The relatively small sample size and testing of MSM with a high prevalence for both CT and NG means the performance of pooled testing would need to be further assessed in lower risk MSM and heterosexuals before it could be recommended. In addition, further work on optimising the pooled specimen testing would be worthwhile. It is possible that the dilutional effect of the urine in the pooled specimens was responsible for the apparent reduced sensitivity for the solitary rectal CT infections. Sultan *et al*[11] also found single site infections accounted for almost all of their missed CT and NG infections with pooled specimen testing, but found the throat swabs were less sensitive than the rectal swabs. Direct comparison of pooled testing and individual specimen testing using the GeneXpert would help address this. Not knowing the anatomical site of infection from pooled testing may have treatment implications for rectal CT infections. This could be mitigated by either immediately repeating the rectal swab in those that have a positive pooled specimen POC test result prior to treatment, or by the routine use of dual head swabs. Retesting the original swab would not be recommended as the previous elution of the swab would reduce the sensitivity on retesting. The assessment for lymphogranuloma venereum (LGV) in rectal swabs collected from MSM is of particular relevance due to the worldwide emergence of the LGV 2b strain.[22]

Laboratories are under increasing financial pressure and CT and NG testing contributes significantly to the laboratory's workload. Our findings suggest that pooled specimen testing of rectal, throat and urine samples from MSM with a high prevalence of CT and/or NG is accurate and has potential merit as a POC test method. If these results are confirmed, pooled specimen testing of such large volume tests may offer significant cost and labour savings.

Key messages

- Rectal and pharyngeal specimens should be collected in addition to urethral or urine specimens to avoid missed *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) infections in men who have sex with men (MSM).
- Testing of pooled specimens using the GeneXpert CT/NG assay was found reliable in high-risk MSM when compared with single specimen nucleic acid testing by cobas.
- Point-of-care nucleic acid testing of pooled specimens in sexual health clinics may provide a cost-effective option to improve early treatment rates and contact tracing.

Handling editor Catherine A Ison

Acknowledgements The authors acknowledge the WA AIDS Council (M Clinic) staff for the recruitment of the participants and collection of the samples.

Contributors JM and LM collected clinical data and samples. IJC performed the laboratory testing and DJS supervised the laboratory testing. IJC and DJS performed the analysis and interpretation of data. All authors contributed to the preparation and revision of the manuscript.

Funding The GeneXpert cartridges were supplied by Cepheid.

Competing interests None declared.

Ethics approval The study was registered as a research project through the Sir Charles Gairdner Hospital Governance, Evidence, Knowledge, Outcomes (GEKO) System (ID10704) and Human Research Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 PublicHealth England. Sexually transmitted infections and chlamydia screening in England, 2014. *Health Prot Rep* 2015;9:1–20.
2 Centers for Disease Control and Prevention (CDC). HIV prevention through early detection and treatment of other sexually transmitted diseases--United States. Recommendations of the Advisory Committee for HIV and STD prevention. *MMWR Recomm Rep* 1998;47:1–24.
3 Cohen MS. Sexually transmitted diseases enhance HIV transmission: no longer a hypothesis. *Lancet* 1998;351(Suppl 3):S5–7.
4 British Association for Sexual Health and HIV. Recommendations for testing for sexually transmitted infections in men who have sex with men. 2014 https://www.bashh.org/documents/BASHH%20Recommendations%20for%20testing%20for%20STIs%20in%20MSM%20-%20FINAL.pdf (accessed 13 Feb 2017).
5 Centers for Disease Control and Prevention. Recommendations for the laboratory-based detection of Chlamydia trachomatis and Neisseria gonorrhoeae-2014. *MMWRRecomm Rep* 2014;63(RR-02):1–19.
6 Koedijk FD, van Bergen JE, Dukers-Muijrers NH, *et al*. The value of testing multiple anatomic sites for gonorrhoea and chlamydia in sexually transmitted infection centres in the Netherlands, 2006-2010. *Int J STD AIDS* 2012;23:626–31.
7 Ota KV, Tamari IE, Smieja M, *et al*. Detection of Neisseria gonorrhoeae and Chlamydia trachomatis in pharyngeal and rectal specimens using the BD Probetec ET system, the Gen-Probe Aptima Combo 2 assay and culture. *Sex Transm Infect* 2009;85:182–6.
8 Benn PD, Rooney G, Carder C, *et al*. Chlamydia trachomatis and Neisseria gonorrhoeae infection and the sexual behaviour of men who have sex with men. *Sex Transm Infect* 2007;83:106–12.
9 Goldenberg SD, Finn J, Sedudzi E, *et al*. Performance of the GeneXpert CT/NG assay compared to that of the Aptima AC2 assay for detection of rectal Chlamydia trachomatis and Neisseria gonorrhoeae by use of residual Aptima Samples. *J Clin Microbiol* 2012;50:3867–9.
10 Geiger R, Smith DM, Little SJ, *et al*. Validation of the GeneXpert® CT/NG Assay for use with Male Pharyngeal and Rectal Swabs. *Austin J HIV AIDS Res* 2016;3:1021.
11 Sultan B, White JA, Fish R, *et al*. The "3 in 1" Study: Pooling Self-Taken Pharyngeal, Urethral, and Rectal Samples into a Single Sample for Analysis for Detection of

Neisseria gonorrhoeae and Chlamydia trachomatis in Men Who Have Sex with Men. *J Clin Microbiol* 2016;54:650–6.
12 Smith DW, Tapsall JW, Lum G. Guidelines for the use and interpretation of nucleic acid detection tests for Neisseria gonorrhoeae in Australia: a position paper on behalf of the Public Health Laboratory Network. *Commun Dis Intell Q Rep* 2005;29:358–65.
13 Tabrizi SN, Unemo M, Golparian D, *et al*. Analytical evaluation of GeneXpert CT/NG, the first genetic point-of-care assay for simultaneous detection of Neisseria gonorrhoeae and Chlamydia trachomatis. *J Clin Microbiol* 2013;51:1945–7.
14 Speers DJ, Fisk RE, Goire N, *et al*. Non-culture Neisseria gonorrhoeae molecular penicillinase production surveillance demonstrates the long-term success of empirical dual therapy and informs gonorrhoea management guidelines in a highly endemic setting. *J Antimicrob Chemother* 2014;69:1243–7.
15 Peeling RW, Toye B, Jessamine P, *et al*. Pooling of urine specimens for PCR testing: a cost saving strategy for Chlamydia trachomatis control programmes. *Sex Transm Infect* 1998;74:66–70.
16 Gomes JP, Ferreira MA, De Sá AB, *et al*. Pooling urine samples for PCR screening of C. Trachomatis urogenital infection in women. *Sex Transm Infect* 2001;77:76-a–7.
17 Gaydos CA, Van Der Pol B, Jett-Goheen M, *et al*. Performance of the Cepheid CT/NG Xpert Rapid PCR Test for Detection of Chlamydia trachomatis and Neisseria gonorrhoeae. *J Clin Microbiol* 2013;51:1666–72.
18 Le Roy C, Peuchant O, Hen L I, *et al*. Chlamydia trachomatis/neisseria gonorrhoeae screening in duplex: should we verify N.Gonorrhoeae positive results? *Sex Transm Infect* 2013;89(S1):A104.
19 Peuchant O, de Diego S, Le Roy C, *et al*. Comparison of three real-time PCR assays for the detection of Chlamydia trachomatis and Neisseria gonorrhoeae in young pregnant women. *Diagn Microbiol Infect Dis* 2015;83:335–7.
20 Dallabetta GA, Gerbase AC, Holmes KK. Problems, solutions, and challenges in syndromic management of sexually transmitted diseases. *Sex Transm Infect* 1998;74(S1):S1–11.
21 Natoli L, Maher L, Shephard M, *et al*. Point-of-care testing for chlamydia and gonorrhoea: implications for clinical practice. *PLoS One* 2014;9:e100518.
22 Van der Bij AK, Spaargaren J, Morré SA, *et al*. Diagnostic and clinical implications of anorectal lymphogranuloma venereum in men who have sex with men: a retrospective case-control study. *Clin Infect Dis* 2006;42:186–94.