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MAJOR ARTICLE

# Pooling Rectal, Pharyngeal, and Urine Samples to Detect *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Mycoplasma genitalium* Using Multiplex Polymerase Chain Reaction Is as Effective as Single-Site Testing for Men Who Have Sex With Men

**Thierry Prazuck,[1] Philippe Lanotte,[2] Gwénaël Le Moal,[3] Laurent Hocqueloux,[1] Simon Sunder,[4] Mélanie Catroux,[3] Magali Garcia,[5] Pascale Perfezou,[6] Guillaume Gras,[7] Chloé Plouzeau,[8] Nicolas Lévêque,[5] and Agnès Beby-Defaux[9]**

[1]Service des maladies infectieuses et tropicales, CHR Orléans, Orleans, France, [2]Service de bacteriologie-virologie, Centre Hospitalier Universitaire Tours, Tours, France, [3]Service des maladies infectieuses, Centre Hospitalier Universitaire Poitiers, Poitiers, France, [4]Service des maladies infectieuses, CH Niort, Niort, France, [5]Laboratoire inflammation tissus épitheliaux et cytokines EA 4331, Université de Poitiers, Poitiers, France, [6]Service des maladies infectieuses, CH Quimper, Quimper, France, [7]Service des maladies infectieuses, Centre Hospitalier Universitaire Tours, Tours, France, [8]Laboratoire de bactériologie, Centre Hospitalier Universitaire Poitiers, Poitiers, France, and [9]Laboratoire de virologie et mycobactériologie, Centre Hospitalier Universitaire Poitiers, Poitiers, France

***Background.*** Screening for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) at pharyngeal, urogenital, and anorectal sites is recommended for men who have sex with men (MSM). Pooling samples is a promising technique, but no data are available when pooled screening also includes *Mycoplasma genitalium* (MG). The main objective of this study was to examine the sensitivity of pooled samples for detecting CT, NG, and MG in MSM using nucleic acid amplification versus single-site testing.

***Methods.*** In this multicenter study, MSM with a positive result for CT, NG, or MG were recalled to the clinic for treatment and were asked to participate in this study. Separate samples were sent to a central virological department that proceeded to form the pooled samples. Testing was performed using the multiplex real-time polymerase chain reaction Allplex STI Essential Assay (Seegene, Seoul, Korea), which can simultaneously detect 7 pathogens.

***Results.*** A total of 130 MSM with at least 1 positive test for CT, NG, or MG were included. A total of 25.4% had a coinfection. The sensitivities of pooled-sample testing were 94.8% for CT, 97.0% for NG, and 92.3% for MG. Pooling failed to detect 8 infections, but pooled-sample analysis missed detecting only samples with a low bacterial load (cycle threshold >35).

***Conclusions.*** Pooling samples from MSM to detect CT, NG, and MG is as sensitive as individual-site testing for these 3 pathogens using the Allplex assay. Missed infections with a very low bacterial load could have a low impact on further transmission.

**Clinical Trials Registration.** NCT03568695.

**Keywords.** *Chlamydia trachomatis*; *Mycoplasma genitalium*; *Neisseria gonorrhoeae*; pooled samples; sexually transmitted infections.

*Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), and *Mycoplasma genitalium* (MG) are the most prevalent and treatable bacterial sexually transmitted infections (STIs), accounting for an estimated 213 million new STI cases worldwide in 2016 [1]. Both infections are increasing in high-income countries such as France [2], the United States, the United Kingdom [3], and Australia [3], particularly among men who have sex with men (MSM). The majority of extragenital CT and NG infections found in MSM are asymptomatic [4], and regular screening is required to diagnose and treat them to prevent ongoing transmission of these bacteria.

Most guidelines [4–7] recommend extragenital screening in MSM and women based on a reported sexual history of receptive anal sex and giving oral sex. However, several studies indicate that sexual history does not accurately identify those with extragenital infections, so universal genital and extragenital sampling is recommended [8].

Testing for CT and NG infections requires specimens from the anatomic sites of possible infection (pharyngeal, rectal, and urogenital anatomic sites). In traditional settings, samples are collected from 3 separate anatomical sites of possible

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Correspondence: Thierry Prazuck, MD, MPH, Service des maladies infectieuses et tropicales, CHR Orleans, 14 avenue de l'hopital, 45067 Orleans, France (thierry.prazuck@chr-orleans.fr).

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infection, and each specimen is tested for CT and NG using a nucleic acid amplification test (NAAT) [9]. NAATs for the detection of CT and NG are highly sensitive and are recommended by the United States Centers for Disease Control and Prevention (CDC) for genital and extragenital anatomic sites.

As NAATs are expensive, the cost of testing for CT and NG infections, particularly at multiple anatomic sites per individual, can be a barrier to screening. If rectal and pharyngeal samples are taken along with urogenital samples, the diagnostic cost could be unaffordable for most publicly funded sexual health services [10]. Over the last 3 years, a limited number of studies have aimed to evaluate the performance of pooled versus single-site testing to detect CT and NG using assays such as the Aptima Combo 2 Assay (Hologic Inc, San Diego, California), Xpert CT/NG (Cepheid, Sunnyvale, California), and Abbott RealTime CT/NG (Abbott, Chicago, Illinois) [11–19], showing a positive percentage agreement varying from 82.4% to 98.3% for NG and from 77.8% to 96.0% for CT [20].

To our knowledge, no study to date has evaluated the performances of pooled 3-anatomic-site testing for CT, NG, and MG single or multiple concomitant infections using a multiplex polymerase chain reaction (PCR) assay. The main objective of this study was to evaluate the sensitivity and specificity of pooled 3-anatomic-site (rectal, urine, and pharyngeal) versus single-site testing to detect NG, CT, and MG in MSM. The secondary objective of the study was to detect resistance to macrolides of MG in the studied population.

## MATERIALS AND METHODS

This multicenter prospective study took place in infectious disease departments and anonymous and free STI testing centers from 5 hospitals in France (Orléans, Poitiers, Niort, Quimper, and Tours) between 5 July 2018 and 18 May 2020. These 5 sexual health clinics provide >25 000 consultations per year. More than 800 patients with human immunodeficiency virus on preexposure prophylaxis are followed regularly in these centers, and MSM account for >95% of this population. As a standard of care, all MSM presenting for asymptomatic screening were offered triple-site testing for CT, NG, and MG using a self-collected anal swab, a clinician-collected pharyngeal swab, and a urine sample. Each sample was individually tested for CT, NG, and MG using local NAAT PCR kits.

As a standard of care, MSM with a positive result for CT, NG, or MG were recalled to the clinic for treatment and were asked to participate in this study. Eligible MSM were approached by 1 of 5 experienced research study nurses assigned to the study and were invited to participate. MSM were eligible if they were aged ≥18 years, returned to the clinic within 7 days of recall, had at least 1 positive result on study swabs on the day of enrollment, and had not received any antibiotics in the preceding 4 weeks.

After written consent was provided, study nurses collected new study swabs from the 3 sites as described below before any antibiotic treatment.

All participants were offered treatment immediately after the study swabs were taken. Antibiotics were given according to the bacteria, the clinical symptoms, and the international recommendations for STI treatment.

### Sample Collection

Each participant agreed to provide 1 self-collected anorectal swab, 1 pharyngeal swab collected by the research nurses, and a first-pass urine (FPU) sample collected in a sterile jar by the participant. Flocked swabs and medium designed to stabilize and preserve the nucleic acids (eNAT medium, Copan) were used. Research nurses instructed the participants on how to collect an FPU sample.

Pharyngeal swabs were taken by swabbing the tonsils or tonsillar crypts and the posterior pharynx by the study nurse. Anorectal swabs were self-taken by inserting a cotton swab 3 cm into the anus and gently rotating for 5–10 seconds to collect sample exudates inside the anal ring. For collection of FPU, participants were instructed to provide 20–30 mL of the initial urine stream or half a container in a sterile urine collection jar. Then, 3 mL was immediately transferred to eNAT medium for preservation of urine specimens. Then, the 3 eNAT-containing samples were stored at 4°C until shipping at ambient temperature to the microbiology laboratory of Poitiers University Hospital to perform the analysis on the pooled and separate samples. The 3 samples from each patient were used for both pooled and separate analyses, and separate testing was the reference standard for individual-site testing. After mixing by gentle vortexing, the pharyngeal and anorectal swabs were swirled and compressed against the inner wall of the eNAT tube and then removed. Two hundred microliters of each specimen, including urine, was taken to form the pooled specimen.

### Real-Time Multiplex PCR Assay

Testing was performed using the multiplex real-time PCR Allplex STI Essential Assay (Seegene, Seoul, Korea). This assay can simultaneously detect CT, NG, *Trichomonas vaginalis*, MG, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* in a single tube by using dual priming oligonucleotides (DPO) and multiple detection temperature (MuDT) technologies, providing individual cycle threshold (Ct) values for multiple pathogens in a single channel. The DPO system differs structurally and functionally from the conventional primer system by including a poly deoxyinosine (I) linker between the 2 segments of primer sequences. This poly (I) linker allows the DPO primer to be divided into 2 perfectly functional segments with different hybridization temperatures. The elongation will be conducted only when the 2 segments

both hybridize correctly, giving rise to a high specificity between similar or related sequences.

DNA extraction and PCR setup were performed using a STARMag Universal Cartridge kit (Seegene) in the Microlab Nimbus (Seegene) automated liquid handling workstation. Real-time PCR was performed in a CFX96 real-time thermocycler (Bio-Rad, Hercules, California) according to the manufacturer's instructions. The results of the analysis were performed using Seegene Viewer software. For this study, only the results for CT, NG, and MG were analyzed. For other pathogens and only for the urine sample, a positive result was given to the clinician for *T vaginalis* in all cases and for *U urealyticum* when the Ct was <35 with the following comment: "Treatment is recommended for *U urealyticum* only when clinical symptoms are present." Other bacteria (*M hominis* and *U parvum)* were not considered, according to national guidelines.

#### *Mycoplasma genitalium* Resistance Testing

Samples positive for MG were studied for mutations conferring resistance to macrolides using the S-DiaMG Res kit (Diagenode) following the manufacturer's instructions. This test allows us to confirm MG detection in the sample, screen for PCR inhibitors, and detect the 2 main mutation positions in the 23S ribosomal RNA (2071 and 2072, also numbered 2058 and 2059, respectively, following the *Escherichia coli* numbering), which are both associated with macrolide resistance. Mutations correspond to the replacement of adenine at position 2058 with cytosine, guanine, or thymine and the replacement of adenine at position 2059 with guanine or cytosine.

#### Statistical Analysis

We calculated that at least 32 men with NG, 32 with CT, and 32 with MG would provide 90% power ($P = .05$, 1-sided [paired]) to detect a difference in sensitivity of ≤5% with pooled-sample testing compared to individual-site testing. Positive individual-site testing was considered the reference sample to be compared with the pooled sample. To reduce the confidence interval (CI) of each proportion observed, we decided to recruit at least 40 positive men in each group, but their samples could be positive for more than a unique pathogen.

Sensitivity (and associated 95% CI) of pooled samples for CT, NG, and MG was calculated against an expanded gold standard where a positive result in either the pooled-sample test or individual-site test was assumed to represent a true infection. The Fisher exact test for matched pairs was used to test the statistical significance of the sensitivity difference between pooled-sample and individual-site testing.

Ethics approval was obtained from Comité de protection des Personnes (CPP) du Sud Ouest et Outre-Mer IV (Avis CPP18-037a/2018-A00749-46, 3 May 2018). The study is registered at ClinicalTrials.gov (NCT03568695).

## RESULTS

The median time between the screening and return to the clinic for treatment and providing the study samples was 3 days (interquartile range, 1–4 days). A total of 130 men with at least 1 positive CT, NG, or MG sample were included in the final analysis (CT = 58, NG = 67, MG = 39). The mean age of the participants was 37.5 years (range, 18–69 years). The anorectal anatomic site was the most often infected (n = 93 [71.5%]). CT was detected in 58 of 130 (44.6%) men on individual-site testing (11 pharyngeal, 12 urogenital, and 40 anorectal). NG was detected in 67 of 130 (51.5%) men on individual-site testing (36 pharyngeal, 14 urogenital, and 40 anorectal). MG was detected in 39 of 130 (30.0%) men on individual-site testing (3 pharyngeal, 15 urogenital, and 28 anorectal). Thirty-three of 130 (25.4%) men had a coinfection. Double or triple infections were mainly with CT and NG (14 of 33 mixed infections [42.4%], 10.8% of the patients), followed by NG plus MG (11 of 33 mixed infections [33.3%], 8.4% of the patients) and CT plus MG (7 of 33 mixed infections [21.2%], 5.3% of the patients). One patient of 33 (3.0%) had a triple coinfection (CT/NG/MG). Table 1 shows the characteristics of the population for each of the 3 pathogens.

Pooled-sample testing detected 55 of 58 CT infections (94.8% [95% CI, 89.1%–100%]). This sensitivity of pooled-sample testing was statistically similar to that of individual-site testing ($P = .24$). Pooled-sample testing detected 65 of 67 NG

**Table 1. Characteristics of the 130 Infected Patients, Site of Infection, and Multiple Infections**

| Variable | Total | CT | NG | MG |
|---|---|---|---|---|
| Overall | 130 (100) | 58 | 67 | 39 |
| Age, y | | | | |
| <35 | 60 (46.2) | 28 | 37 | 17 |
| 35–45 | 36 (27.7) | 16 | 17 | 8 |
| >45 | 34 (26.1) | 14 | 13 | 14 |
| Any symptom | | | | |
| No | 81 (79.4) | 31 (77.5) | 41 (75.9) | 27 (84.5) |
| Yes | 21 (20.6) | 9 (22.5) | 13 (24.1) | 5 (15.5) |
| Unknown | 28 | 18 | 13 | 7 |
| Infected sites | | | | |
| Pharyngeal | 48 (36.9) | … | … | … |
| Anal | 93 (71.5) | … | … | … |
| Urine | 38 (29.2) | … | … | … |
| No. of infected sites | | | | |
| 1 | 97 (74.6) | … | … | … |
| 2 | 32 (24.6) | … | … | … |
| 3 | 1 (0.8) | … | … | … |
| Mono- or mixed infections | | | | |
| Monoinfected | 97 | 36 | 41 | 20 |
| Mixed[a] | 33 | 22 | 26 | 19 |

Data are presented as No. (%).

Abbreviations: CT, *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; NG, *Neisseria gonorrhoeae.*

[a]CT/NG = 14; CT/MG = 7, NG/MG = 11, CT/NG/MG = 1.

infections (97.0% [95% CI, 92.9%–100%]). This sensitivity of pooled-sample testing was statistically similar to that of individual-site testing ($P = .49$). Pooled-sample testing detected 36 of 39 MG infections (92.3% [95% CI, 83.9%–100%]). This sensitivity of pooled-sample testing was statistically similar to that of individual-site testing ($P = .24$). The global agreement between pooled sample analysis and 1-site sample analysis was 93.8% (122/130). This agreement reached up to 96.2% considering only CT and NG. For the 3 pathogens, the specificity of pooled-sample analysis was 100% (Table 2). The positive predictive value was 100% for each pathogen, and the negative predictive values were 96.0%, 96.9%, and 96.8% for CT, NG, and MG, respectively.

Overall, pooling failed to detect a total of 8 infections in 7 different patients who were detected by individual-site testing. Table 3 shows the Ct level for each pathogen amplified, the result of the pooled sample, and the result of an additional pooled-sample analysis performed for each discordant result between the pooled-sample and individual anatomic site sample analysis. The pooled-sample analysis only failed to detect samples with a low bacterial load (Ct >35). For patient 2, pooling anal and pharyngeal samples without urine allowed us to detect CT and NG.

As an example, in France, the cost of individual-site testing for the 3 STI is 35 euros (3 samples + 3 PCRs), whereas the cost of pooled-sample testing is 23 euros (3 samples + time for pooling + 1 PCR), hence a reduction of 34%.

### Resistance of *M genitalium* to Macrolides

The resistance of MG to macrolides was interpretable for 34 patients from 40 different positive samples. Table 4 shows the 40 samples according to the anatomical site from the 34 patients. A mutation (A2058C/G/T or A2059G/C) was detected in 24 of 34 patients (70.6%), conferring resistance of MG to macrolides (10 mutations in position 2058 and 16 mutations in position 2059).

## DISCUSSION

In this prospective study of asymptomatic and symptomatic MSM being tested for CT, NG, and MG across 3 anatomical sites, we found a similar sensitivity with pooled-sample testing compared to the individual site–sample testing for all 3 pathogens using the Allplex STI Essential Assay. The sensitivity was 94.8%, 97.0%, and 92.3% for CT, NG, and MG, respectively, and the specificity was 100% for all pathogens.

To our knowledge, our study is the first to compare pooled-sample testing and individual site–sample testing for the 3 main bacteria-related STIs: CT, NG, and MG. This study also provides supportive information regarding discordant results between the 2 methods based on the threshold level.

In our study, for CT, NG, and MG detection, the positive percentage agreement of pooled testing with single-site testing was >93% and it was as high as 96.2% for CT and NG.

Overall, there have been 8 studies assessing pooling of genital and extragenital samples in MSM [11–19] and a recent meta-analysis of these studies and others involving women [20]. In this meta-analysis of these 8 studies, the positive percentage agreement of pooled testing with single-site testing was >93% using fixed-effects and random-effects models, and the negative percentage agreement was >99% for both infection types. For each pathogen, the combined positive percentage agreement for CT was 93.11% (95% CI, 91.51%–94.55%), and it was 93.80% (95% CI, 90.26%–96.61%) for NG. Sultan et al conducted the largest study of pooling to date in MSM in the United Kingdom using self-collected samples and the Aptima Combo 2 TMA assay [11]. Sultan and colleagues' pooled-sample sensitivity for detecting CT in MSM was lower than our figure (94.8% vs 89%, respectively), as was also the case for detecting NG (97.0% vs 82%). This difference could be related to the inclusion criteria in the Sultan et al study, which enrolled only asymptomatic patients, compared to 79.4% asymptomatic patients in our study. The absence of symptoms

**Table 2. Sensitivity and Specificity of Pooled-Sample Testing Compared to Single-Site Testing According to Each Pathogen**

| Organism and Single-Site Testing Result | No. With Pooled-Sample Testing Result Positive | Negative | Total | Pooling Performance No. of Infections Detected/Total | Sensitivity, % (95% CI) | Specificity, % | *P* Value |
|---|---|---|---|---|---|---|---|
| *Chlamydia trachomatis* | | | | | | | |
| Positive | 55 | 3 | 58 | 55/58 | 94.8 (89.1–100) | 100 | .24 |
| Negative | 0 | 72 | 72 | … | … | … | |
| Total | 55 | 75 | 130 | … | … | … | |
| *Neisseria gonorrhoeae* | | | | | | | |
| Positive | 65 | 2 | 67 | 65/67 | 97.0 (92.9–100) | 100 | .49 |
| Negative | 0 | 63 | 63 | … | … | … | |
| Total | 65 | 65 | 130 | … | … | … | |
| *Mycoplasma genitalium* | | | | | | | |
| Positive | 36 | 3 | 39 | 36/39 | 92.3 (83.9–100) | 100 | .24 |
| Negative | 0 | 91 | 91 | … | … | … | |
| Total | 38 | 93 | 130 | … | … | … | |

Abbreviations: CI, confidence interval; CT, Chlamydia trachomatis; MG, Mycoplasma genitalium; NG, Neisseria gonorrhoeae.

**Table 3. Characteristics of the 8 Discordant Results in 7 Different Patients, Single-Site Result and Cycle Threshold, Pooled-Samples Results, Additional Pooled-Samples Results, and Clinical Status**

| Patient No. | Pharyngeal | Ct | Anal | Ct | Urine | Ct | Pool Result | Pool Restart | Clinical Status |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **CT** | **37** | Neg | … | Neg | … | Neg | Neg | Unknown |
| 2 | NG | 34.4 | **CT** | **36.8** | MH | 33.4 | NG | Same result | Asymptomatic |
| | … | … | UU | 28.4 | … | … | UU | **Pooling only pharyngeal + anal: positive for both NG and CG** | … |
| | … | … | MH | 23.6 | … | … | MH | … | … |
| 3 | Neg | … | **MG** | **38.7** | Neg | … | UU | UU | Asymptomatic |
| | … | … | UU | 33.6 | … | … | … | … | … |
| 4 | Neg | … | **MG** | **37.4** | Neg | … | UU | UU | Asymptomatic |
| | … | … | UU | 36.4 | … | … | … | … | … |
| | … | … | MH | 37.1 | … | … | … | … | … |
| 5 | **NG** | 35.1 | MG | 21.7 | Neg | … | MG | **MG and NG** | Asymptomatic |
| 6 | MH | 38.2 | NG | 28.1 | MH | 33.3 | NG | NG | Unknown |
| | … | … | … | … | **CT** | **37.3** | MH | MG | … |
| 7 | … | … | **MG** | **38.4** | … | … | Neg | Neg | Asymptomatic |

Bold values/text indicate a non significant Ct value (Ct >36).

Abbreviations: Ct, cycle threshold; CT, *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; MH, *Mycoplasma hominis*; Neg, negative; NG, *Neisseria gonorrhoeae*; UU, *Ureaplasma urealyticum*.

can be associated with a lower bacterial load, closer to the detection limit of the assay. It could also be related to the sampling method (ie, urine volume).

In contrast, our results are close to those of Speers et al [13], which found a limited number of isolates that pooled pharyngeal swabs, rectal swabs, and urine samples from symptomatic and asymptomatic patients. The Cepheid GeneXpert CT/NG assay had 100% agreement for NG and 94% for CT compared with individual testing by the Roche Cobas 4800 CT/NG assay. In Speers and colleagues' study, 55 of 107 (51.4%) were symptomatic, which is higher than in our study population and could increase the percentage agreement but not reflect the general population coming for STI screening.

In addition, this study has explored, for the first time, the possibility of testing for MG in a pooled sample and showed a 92.3% sensitivity to detect this pathogen.

All of these previous studies used the Aptima Combo 2 (AC2) or the Cepheid assay, and this is the first study on pooled samples in STI that used the Allplex STI Essential Assay. The AC2 assay is considered to have high sensitivity for both CT and NG and it is able to detect very small amounts of bacteria (0.005 inclusion-forming units/mL for CT and 0.10 colony-forming units/mL for NG) [21]. One single study compared AC2 versus Allplex to detect CT and NG [22], showing the superiority of the AC2 assay compared to the Allplex assay. Our study was not designed to compare different assays. The Allplex assay appears to be adapted to analyze pooled samples for STI detection.

Very few studies have analyzed discordant results according to the Ct. We found that this analysis in the Speers et al study [13] focused only on CT in 3 participants. The Ct values were 35.4, 38.0, and 40.4, respectively. In our study, we were also able to investigate 8 discrepant result analyses in 7 different patients, including 3 CT, 3 MG, and 2 NG, which confirms the hypothesis of Speers et al suggesting that the CT organism burden was close to the lowest limit of detection. The same phenomena can now be suggested for NG and MG. Discordant results seem to occur only in patients with low bacterial loads or transient deposition, which appear to be less transmissible during sexual intercourse.

Macrolide resistance rates in this study are in accordance with recent data from France and Europe. Data from the French national STI reference center showed a macrolide resistance rate of 42%, ranging from 22% in women to 60% in men. Moreover, the resistance prevalence was significantly higher in men's anal samples (75.8%) versus other samples (54%) [23]. Recent studies in England and the Netherlands [24, 25] highlight a prevalence of resistance to macrolides of 69% and 66%, respectively, in patients similar to patients in our study.

This study has some strengths and some limitations. On the one hand, sample pooling was performed by the laboratory and not at the time of sampling by the patient or the clinician. Pooling 3 different samples, rectal, pharyngeal, and urine, must be performed by a biologist to ensure reproducibility, as the quantity of each sample must always be the same. After pooling in the laboratory, the biologist obtained 3 individual-site samples and 1 pooled sample. This will be particularly useful for CT infections, as rectal-individual swabs will be tested to diagnose a possible lymphogranuloma venereum (LGV) without resampling the patient. This is of particular relevance due to the worldwide emergence of the LGV 2b strain in MSM [26]. It could also be relevant to test the pharyngeal sample when the pooled sample is positive for NG; as the CDC has recently recommended, for persons with pharyngeal gonorrhea, a test-of-cure is needed, using culture or NAATs 7–14 days after initial treatment [27]. On the other hand, the study was focused

**Table 4. *Mycoplasma genitalium* Detection and Resistance Among the Different Samples**

| Sample | MG Detection in Pharyngeal Sample (Ct) | MG Detection in Anal Sample (Ct) | MG Detection in Urine Sample (Ct) | Macrolide Resistance Detection (Ct) |
|---|---|---|---|---|
| CN-002 | Neg | Neg | **Pos (25.2)** | **2058 muted (28.89)** |
| CO-007 | Neg | Neg | **Pos (32)** | Not detected |
| CN-008 | Neg | **Pos (34.5)** | **Pos (35)** | Not detected |
| CN-011 | **Pos (38.2)** | **Pos (32.6)** | **Pos (29.6)** | Not detected |
| CP-002 | Neg | Neg | **Pos (29.2)** | **2059 muted (36.65)** |
| CP-004 | Neg | **Pos (37.4)** | Neg | Not detected |
| CO-015 | Neg | **Pos (21.7)** | Neg | **2058 muted (30.7)** |
| SP-008 | Neg | **Pos (33.2)** | Neg | Not detected |
| SP-009 | **Pos (36.7)** | **Pos (32)** | Neg | Not detected |
| SO-007 | Neg | **Pos (26.6)** | Neg | **2058 muted (31.32)** |
| SO-010 | Neg | Neg | **Pos (34.3)** | Not detected |
| SP-022 | Neg | **Pos (31.6)** | Neg | **2059 muted (35.61)** |
| CT-001 | Neg | **Pos (32.9)** | Neg | Not detected |
| SP-025 | Neg | Neg | **Pos (25.2)** | **2058 muted (33.49)** |
| SO-016 | Neg | Neg | **Pos (32.7)** | **2059 muted (35.33)** |
| SP-028 | Neg | Neg | **Pos (21.9)** | **2059 muted (27.3)** |
| CO-030 | Neg | Neg | **Pos (31.5)** | **2058 muted (32.25)** |
| SP-035 | Neg | **Pos (28)** | Neg | **2058 muted (28.38)** |
| CT-009 | Neg | **Pos (23.4)** | Neg | **2058 muted (27.66)** |
| SP-040 | Neg | **Pos (30)** | Neg | **2058 muted (27.53)** |
| COP-037 | Neg | Neg | **Pos (31.6)** | **2059 muted (35.26)** |
| CO-038 | Neg | **Pos (34.2)** | **Pos (25.8)** | **2059 muted (35.63/30.24)**[a] |
| SP-041 | **Pos (31.6)** | Neg | Neg | **2059 muted (33.63)** |
| CO-040 | Neg | **Pos (33)** | Neg | **2058 muted (38.44)** |
| SP-042 | Neg | **Pos (36.9)** | Neg | **2058 muted (24.65)** |
| QU-003 | Neg | **Pos (35.4)** | Neg | **2059 muted (41.4)** |
| QU-006 | Neg | **Pos (33.5)** | Neg | **2059 muted (40.1)** |
| QU-008 | Neg | **Pos (28.0)** | Neg | **2059 muted (34.1)** |
| QU-009 | Neg | **Pos (30.5)** | Neg | **2059 muted (35.2)** |
| QU-010 | Neg | **Pos (32.5)** | **Pos (32.8)** | **2059 muted (38.1/39.2)** |
| QU-011 | Neg | **Pos (35.4)** | Neg | Not detected |
| QU-012 | Neg | **Pos (37.7)** | Neg | Not detected |
| SP-043 | Neg | **Pos (38.0)** | Neg | **2059 muted (40.2)** |
| QU-002 | Neg | … | **Pos (32.3)** | **2059 muted (37.2)** |

Positive results are shown in bold. For MG resistance, the mutated position is specified.

Abbreviations: Ct, cycle threshold; MG, *Mycoplasma genitalium*; Neg, negative; Pos, positive.

[a]For these patients, the first Ct value corresponds to the anal sample and the second Ct value is for the urine sample.

only on men, and the study was not designed to give figures with the Allplex assay when pooling pharyngeal, vaginal, and rectal swabs. Verougstraete et al [28] included 489 women and compared pooled versus single-site testing using the Abbott RealTime NG/CT assay on the m2000sp/rt system. From 42 patients positive on at least 1 nonpooled sample, 5 gave a negative result on the pooled sample, resulting in a sensitivity of 94% for CT and 82% for NG, suggesting that this approach needs to be validated on a larger number of infected women. Multiple-site testing in women, according to their sexual practices, is not routinely done in our STI centers, mainly for financial considerations. Testing only vaginal samples leads to missing 40% of CT infections and 60% of NG infections [28]. Extending the pooled-sample analysis approach from MSM to women could allow us to routinely perform multiple-site samples in women at the same cost.

Overall, we have shown that using pooled samples in MSM to detect CT, NG, and MG is as sensitive as individual-site testing for the 3 pathogens using the Allplex STI Essential Assay.

The main benefit is the significant cost savings that could be achieved using this method at the expense of a small sensitivity loss, particularly in health services with a high proportion of an MSM population that requires frequent triple-site testing, particularly as they are recommended to undergo screening every 3 months for STIs regardless of actual sexual risk. Our study was not designed as a cost-effectiveness study, but rather as a preliminary study to assess the overall performance of the pooling strategy. However, a cost reduction of at least a third is expected. Cost-effectiveness studies would be useful to assess the exact benefit of our strategy according to the population of interest. By reducing costs, more patients, including women and more extragenital samples, can be tested, resulting in public health benefits such as higher STI detection rates, interruption of transmission, and prevention of long-term complications.

## Notes

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