Successful Combination of Nucleic Acid Amplification Test Diagnostics and Targeted Deferred Neisseria gonorrhoeae Culture

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Nucleic acid amplification tests (NAATs) are recommended for the diagnosis of Neisseria gonorrhoeae because of their superrior sensitivity. Increasing NAAT use causes a decline in crucial antimicrobial resistance (AMR) surveillance data, which rely on culture. We analyzed the suitability of the ESresult system for NAAT diagnostics and deferred targeted Neisseria gonorrhoeae culture to allow selective and efficient culture based on NAAT results. We included patients visiting the STI Clinic Amsterdam, The Netherlands, in 2013. Patient characteristics and urogenital and rectal samples for direct Neisseria gonorrhoeae culture, standard NAAT, and ESresult were collected. Standard NAAT and NAAT on ESresult samples were performed using the Aptima Combo 2 assay for Neisseria gonorrhoeae and Chlamydia trachomatis. Two deferred Neisseria gonorrhoeae cultures were performed on NAAT-positive ESresult samples after storage at 4°C for 3 days. We included 2,452 samples from 1,893 patients. In the standard NAAT, 107 samples were Neisseria gonorrhoeae positive and 284 were Chlamydia trachomatis positive. The sensitivities of NAAT on ESresult samples were 83% (95% confidence interval [CI], 75 to 90%) and 87% (95% CI, 82 to 90%), respectively. ESresult samples were available for 98 of the gonorrhoea-positive samples. Of these, 82% were positive in direct culture and 69% and 56% were positive in the 1st and 2nd deferred cultures, respectively (median storage times, 27 and 48 h, respectively). Deferred culture was more often successful in urogenital samples than the patient had symptoms at the sampling site. Deferred Neisseria gonorrhoeae culture of stored ESresult samples is feasible and enables AMR surveillance. To limit the loss in NAAT sensitivity, we recommend obtaining separate samples for NAAT and deferred culture.

Antibiotic treatment for gonorrhoea has existed for around 80 years, and Neisseria gonorrhoeae has developed mechanisms of antimicrobial resistance (AMR) ever since. Resistance to the last-resort empirical monotherapy, extended-spectrum cephalosporins, has now emerged, and this has been recognized as a major public health problem (1). In response, the World Health Organization recommended actions to extend the existing surveillance and, where lacking, develop new AMR surveillance worldwide (2, 3).

In recent years, nucleic acid amplification tests (NAATs) have rapidly replaced the use of culture as a diagnostic test for gonorrhoea (4). While the use of highly sensitive NAATs results in the detection of more infections, it also results in fewer cultures (5). This compromises AMR surveillance, as molecular methods to detect AMR in NAAT samples are still suboptimal, and culture remains essential (6, 7).

Ideally, an NAAT to diagnose gonorrhoea would be combined with targeted deferred culture of positive samples for AMR surveillance. Using a single sample for NAAT and deferred culture requires special conditions for the collection medium, i.e., maintained diagnostic NAAT sensitivity and Neisseria gonorrhoeae survival until the NAAT result is available. The ESresult system (Copan Italia, Brescia, Italy) allows the prolonged survival of many bacterial species and might be an appropriate medium for deferred gonorrhoea culture (8–10).

We conducted a study to determine if (i) Neisseria gonorrhoeae can be successfully cultured after storing ESresult samples for 1 to 3 days, and (ii) the NAAT (Aptima Combo 2 assay [AC2], Hologic, San Diego, CA, USA) can be performed using the same ESresult sample stored for culture without compromising the diagnostic sensitivity to detect Neisseria gonorrhoeae and Chlamydia trachomatis. This approach would provide a highly sensitive gonorrhoea diagnosis and cultures for AMR determination and surveillance.

MATERIALS AND METHODS

Study population. In this cross-sectional study, we included all high-risk patients visiting the STI Outpatient Clinic Amsterdam, The Netherlands, from March through October 2013. The most important criteria for a high-risk profile were symptoms, notification by a sexual partner, or being a man who has sex with men (MSM) (11). Ethical approval for the study was obtained from the Academic Medical Center Amsterdam (W13_041 no. 13.17.0055); the committee declared that individual patient informed consent was not required.
Demographic and clinical characteristics. We extracted demographic and sexual characteristics (gender, age, sexual orientation, HIV status, and notification by a sexual partner) and clinical characteristics (symptoms and physical signs at the included sample site, anatomical site of infection with *N. gonorrhoeae*, and *C. trachomatis* coinfection status) from the electronic patient file.

Sample collection and testing. Routine diagnostic tests consisted of a direct *N. gonorrhoeae* culture and standard NAAT (AC2) for *N. gonorrhoeae* and *C. trachomatis*. From male patients, direct cultures were obtained from urethral exudate samples, and standard NAAT was performed on first-void urine samples. From MSM, we additionally obtained rectal samples. From female patients, we included cervical and rectal samples. Patients were allowed to provide samples from more than one anatomical site.

Direct *N. gonorrhoeae* culture. All direct culture samples were obtained by using a 10-μl disposable plastic loop to directly inoculate a GC agar plate (Becton Dickinson, Breda, The Netherlands), which was immediately incubated at 37°C in a 5% CO₂-enriched atmosphere for 48 to 72 h. *N. gonorrhoeae* species was verified using colony morphology, catalase and oxidase testing, Gram-stained smears, and DNA hybridization (Accu-Probe assay; Hologic, San Diego, CA, USA).

Standard NAAT samples. Standard NAAT samples were obtained using an Aptima swab and Aptima unisex swab specimen collection device, or by transferring urine into an Aptima urine specimen collection device, as instructed by the manufacturer (Hologic, San Diego, CA, USA).

ESwab samples. We obtained a single ESwab sample from each included anatomical site using a nylon-flocked swab placed in liquid Amies medium (480 CE; Copan Italia S.p.A., Brescia, Italy). The same ESwab sample was used for NAAT on ESwab and targeted deferred culture. The collection order of the swabs for standard NAAT and ESwab was reversed when half of the projected sample size was reached.

The ESwab samples were obtained from urine, as the use of urethral swabs is against clinic policy due to patient discomfort. First-void urine samples were collected in a 30-ml plastic container and immediately stored at 4°C. Within 30 min of sample collection, we dipped a flocked swab into the urine and placed it into an ESwab system. The urine samples were subsequently centrifuged to obtain urine sediment, in which a second flocked swab was dipped and placed into another ESwab system.

We conducted a pilot study of deferred gonorrhea culture in 35 ESwab samples from the rectum, cervix, or urine that were positive for *N. gonorrhoeae* using the AC2 assay. The results showed better culture yields after storage for 48 h at 4°C than after storage at room temperature (71% versus 37% positive cultures, respectively) (12). Therefore, the ESwab samples in this study were immediately stored at 4°C.

Standard NAAT. Standard NAAT for *N. gonorrhoeae* and *C. trachomatis* was performed using the AC2 assay on the Tigris DTS system (Hologic, San Diego, CA, USA). Positive or negative results and semiquantitative results consisting of relative light units (RLUs) were reported. Samples with equivocal results were retested using the Aptima GC single assay or Aptima CT single assay (Hologic).

Targeted deferred *N. gonorrhoeae* culture. We aimed to inoculate deferred cultures from ESwab samples within 3 days of sample collection. If the standard NAAT results were not available within this period, the sample was excluded from the analysis. When the standard NAAT results were *N. gonorrhoeae* positive, the corresponding ESwab sample was vortexed for 5 s to provide a homogeneous solution. We inoculated 200 μl of ESwab liquid medium onto GC agar plates and incubated at 37°C in a 5% CO₂-enriched atmosphere for 48 to 72 h (1st deferred culture). The following day, this procedure was repeated from the same ESwab sample (2nd deferred culture). Suspected colonies were further examined with catalase and oxidase testing and Gram-stained smears. Species identification was confirmed by an in-house PCR targeting the *ega* genes (13).

NAAT on ESwab samples. The day after collection, the ESwab samples were vortexed for 5 s, and 200 μl of ESwab liquid medium was transferred to an Aptima specimen transfer device (Hologic, San Diego, CA, USA). These transferred samples were stored at 4°C for a maximum of 2 weeks prior to testing using the AC2 assay. The AC2 results and RLUs were reported. Samples with equivocal results were retested using the Aptima GC single or CT single assay. Samples positive by NAAT on ESwab but negative with the standard NAAT were, after repeated transfer of ESwab medium, retested for confirmation.

Statistical analysis. (i) Sample size. We aimed to determine an overall success rate of deferred cultures of 80% compared to that with the standard NAAT, with 8% precision and a significance level of 0.05. This required 97 *N. gonorrhoeae*-positive samples with the standard NAAT (about 30 per anatomical site).

(ii) Analysis. We analyzed the proportion of *N. gonorrhoeae*-positive results of each test compared to the standard NAAT result. As coinfection with *C. trachomatis* is expected in many high-risk patients, we also analyzed the proportion of NAAT results that were positive for *C. trachomatis*. The deferred culture results of each ESwab sample were analyzed according to culture group (1st or 2nd) and with time since collection classified by 3 consecutive calendar days (day 1, ≤3 h; day 2, 35 to 58 h; day 3, >58 h). The agreement, using Cohen’s kappa, and the sensitivity (openEpi) of the test methods were calculated. To assess the predictors of a negative 1st deferred culture result, we used logistic regression models. A *P* value of ≤0.05 was considered statistically significant. All analyses were performed using SPSS Statistics version 21 (IBM, Armonk, NY, USA).

RESULTS

Included samples. We initially included 1,911 patients who provided 2,480 samples during 1,920 visits; 9 patients were included at two visits, and 560 patients provided two samples at the same visit. We excluded 28 samples due to invalid or incomplete reference test results (Fig. 1). Accordingly, 2,452 samples from 1,893 patients were included in the analyses.

ESwab samples from urine versus urine sediment. When analyzing the ESwab results from both whole urine and the accompanying urine sediment, we found no major differences in the positive deferred gonorrhea cultures (1st, 27 each; 2nd, 20 versus 21, respectively) or in NAAT on ESwab samples (*N. gonorrhoeae* positive, 32 versus 33, respectively; *C. trachomatis* positive, 81 versus 89, respectively). In the remainder of the analysis, we included the samples from whole urine only.

Standard NAAT and direct culture. In the standard NAAT, 107 samples were positive for *N. gonorrhoeae* (4%), and 284 samples were positive for *C. trachomatis* (12%). For 98 (92%) of the 107 gonorrhea-positive samples, an accompanying ESwab sample was available and used for testing and analyses. Direct *N. gonorrhoeae* culture was positive in 80 (82%) of these 98 samples; the positivity rate was significantly higher in the urogenital samples (94%) than that in rectal samples (70%) (*P* = 0.002) (Table 1).

When analyzing the collection order of swabs for the standard NAAT and ESwab samples, we did not find any significant differences in the test results by collection order or by anatomical location.

Targeted deferred culture from ESwab versus direct culture. The median time between sample collection and inoculation of deferred cultures was 27 h (interquartile range [IQR], 25 to 29 h) for the 1st and 48 h (IQR, 44 to 51 h) for the 2nd deferred culture. The 1st deferred culture was positive in 69% of the samples, and the 2nd deferred culture was positive in 56% of the samples (Table 1). In contrast to the direct cultures, the positivity rates of urogenital and rectal samples were not significantly different (for the 1st deferred culture, 77% versus 62%, respectively; *P* = 0.11). When all deferred cultures were classified according to calendar days.
since collection, the positivity rate for both urogenital and rectal samples decreased with each day (Fig. 2).

Eighty of the 98 NAAT gonorrhea-positive samples (with accompanying ESwab sample) were positive in direct culture, of which 67 were also positive in the 1st deferred culture. This resulted in an overall sensitivity of 84% (95% confidence interval [CI], 74 to 91%) and good agreement ($\kappa$, 0.62, 95% CI, 0.45 to 0.79) between the direct and 1st deferred cultures (Table 2). When the results were analyzed by anatomical site, we found a higher agreement for rectal samples ($\kappa$, 0.74, 95% CI, 0.54 to 0.94) than that for urogenital samples ($\kappa$, 0.37, 95% CI, 0.06 to 0.68), but the sensitivities were comparable (86% [95% CI, 70 to 95%] and 82% [95% CI, 68 to 92%], respectively) (Table 2).

In order to compare the culture results to the semiquantitative RLU results of the NAAT-positive samples, we selected all samples that were N. gonorrhoeae positive and C. trachomatis negative in standard NAAT (n = 78). The value of the AC2 positive control in our laboratory is approximately 850 to 1,100 RLU for N. gonorrhoeae monoinfections, and the cutoff value for positivity is 50 RLU. We therefore considered an RLU value of < 800 to be low positive. None of the samples positive in direct culture (n = 60) had a low RLU value in the standard NAAT (range, 1,064 to 1,428), while this was found in 6 samples (33%) with a negative direct culture (range, 216 to 1,297). These differences were similar when samples with a positive or negative 1st deferred culture were compared (data not shown).
TABLE 1 Included samples and results positive for N. gonorrhoeae from standard NAAT, direct culture, and targeted deferred culture from ESwab included from the STI Outpatient Clinic Amsterdam, The Netherlands, 2013\textsuperscript{a}

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No. of samples</th>
<th>No. with standard NAAT result</th>
<th>No. with ESwab available\textsuperscript{b}</th>
<th>No. (%) with positive direct culture result</th>
<th>1st deferred culture</th>
<th>2nd deferred culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. (%) positive\textsuperscript{c}</td>
<td>Median storage time (IQR) (h)</td>
</tr>
<tr>
<td>Rectum</td>
<td>562</td>
<td>55</td>
<td>50</td>
<td>35 (70)</td>
<td>31 (62)</td>
<td>27 (26–29)</td>
</tr>
<tr>
<td>Urethra\textsuperscript{d}</td>
<td>1,085</td>
<td>37</td>
<td>35</td>
<td>33 (94)</td>
<td>27 (77)</td>
<td>27 (25–30)</td>
</tr>
<tr>
<td>Cervix</td>
<td>805</td>
<td>15</td>
<td>13</td>
<td>12 (92)</td>
<td>10 (77)</td>
<td>25 (24–29)</td>
</tr>
<tr>
<td>Total</td>
<td>2,452</td>
<td>107</td>
<td>98</td>
<td>80 (82)</td>
<td>68 (69)</td>
<td>27 (25–29)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} NAAT, nucleic acid amplification test.
\textsuperscript{b} Samples for which standard NAAT was not available <3 days after collection (n = 2) and samples with missing ESwab (n = 7) were excluded.
\textsuperscript{c} The 1st targeted deferred culture from ESwab was inoculated on the day the standard NAAT result became available. The 2nd targeted deferred culture was inoculated 1 day later from the same ESwab tube as the 1st deferred culture.
\textsuperscript{d} Samples for standard NAAT and ESwab were obtained from urine, and direct culture was obtained from urethral exudate samples.

NAAT on ESwab samples versus standard NAAT. (i) NAAT for N. gonorrhoeae. Of the 107 samples positive by the standard NAAT, 89 were also positive in NAAT on the ESwab liquid medium (sensitivity, 83%; 95% CI, 75 to 90%). Although the sensitivity was reasonable and the agreement very good for all anatomical sites, the results were slightly better for urogenital samples than those for rectal samples (Table 3).

In a comparison of NAAT on ESwab samples from patients with symptoms and/or physical signs (n = 1,108) to those with neither symptoms nor signs (n = 1,344), we found significantly better results with samples from symptomatic patients (κ, 0.96 [95% CI, 0.92 to 1.00]; sensitivity, 92% [95% CI, 83 to 97%]) than with samples from asymptomatic patients (κ, 0.81 [95% CI, 0.71 to 0.91]; sensitivity, 68% [95% CI, 52 to 82%]) (P = 0.001).

(ii) NAAT for C. trachomatis. Of 284 samples that were positive with the standard NAAT, 246 were also positive with NAAT on ESwab (sensitivity, 87%; 95% CI, 82 to 90%). Six samples were positive with NAAT on ESwab while negative with the standard NAAT (Table 3). The sensitivity of NAAT on ESwab samples was significantly higher with cervical samples than that with rectal and urine samples (P = 0.001 and 0.002, respectively). As for N. gonorrhoeae, the sensitivity was significantly higher with samples from symptomatic patients than that for samples from asymptomatic patients (92% [95% CI, 87 to 96%] and κ, 0.94 [95% CI, 0.91 to 0.97] versus 77% [95% CI, 71 to 86%] and κ, 0.86 [95% CI, 0.81 to 0.91], respectively) (P = 0.003).

Determinants predictive of a negative targeted deferred N. gonorrhoeae culture. We analyzed the demographic and clinical determinants associated with a negative 1st targeted deferred culture among all samples positive for N. gonorrhoeae with the standard NAAT (n = 98, obtained from 92 individual patients [6 patients provided 2 samples]). A negative 1st deferred culture occurred in 30 samples (31%).

Being asymptomatic at the anatomical site of sampling was the strongest overall predictor of an unsuccessful deferred culture (OR, 4.80; 95% CI, 1.92 to 12.01; P = 0.001). Rectal samples more often yielded negative cultures than urethral samples (OR, 2.07; 95% CI, 1.29 to 3.27).

TABLE 2 Comparison of direct and 1st targeted deferred N. gonorrhoeae cultures of 98 samples positive in standard NAAT\textsuperscript{a}

<table>
<thead>
<tr>
<th>Sample type</th>
<th>1st targeted deferred culture result\textsuperscript{b}</th>
<th>Direct culture result \textsuperscript{a}</th>
<th>1st targeted deferred culture</th>
<th>2nd targeted deferred culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) positive</td>
<td>Median storage time (IQR) (h)</td>
<td>No. (%) positive</td>
<td>Median storage time (IQR) (h)</td>
</tr>
<tr>
<td>Urogenital\textsuperscript{c}</td>
<td>+</td>
<td>37 (0.06–0.68)</td>
<td>30 13 (82)</td>
<td>0.37 (0.06–0.68) 82 (68–92)</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>8 3 (11)</td>
<td>Total 45</td>
<td>48</td>
</tr>
<tr>
<td>Rectal</td>
<td>+</td>
<td>30 1 (76)</td>
<td>50 Total 35</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>5 14 (91)</td>
<td>30 Total 15</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>+</td>
<td>67 1 (84)</td>
<td>30 Total 17</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>13 17 (70)</td>
<td>Total 80</td>
<td>18</td>
</tr>
</tbody>
</table>

\textsuperscript{a} NAAT, nucleic acid amplification test.
\textsuperscript{b} The 1st targeted deferred culture from ESwab was inoculated on the day the standard NAAT result became available.
\textsuperscript{c} Samples for deferred culture were obtained from urine, and direct culture was obtained from urethral exudate samples.
TABLE 3 Results for *N. gonorrhoeae* and *C. trachomatis* of standard NAAT and NAAT on ESawb samples

<table>
<thead>
<tr>
<th>Sample type</th>
<th>NAAT on ESawb result$^a$</th>
<th>Total</th>
<th>Sensitivity (%) [95% CI]</th>
<th>NAAT on ESawb result$^a$</th>
<th>Total</th>
<th>Sensitivity (%) [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectum</td>
<td>+</td>
<td>44</td>
<td>0.88 (0.81–0.95)</td>
<td>80 (67–90)</td>
<td>51</td>
<td>0.85 (0.78–0.92)</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>11</td>
<td>0.71 (0.56–0.85)</td>
<td>56 (43–68)</td>
<td>14</td>
<td>0.71 (0.56–0.85)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>55</td>
<td>0.82 (0.77–0.87)</td>
<td>76 (69–83)</td>
<td>65</td>
<td>0.82 (0.77–0.87)</td>
</tr>
<tr>
<td>Urine</td>
<td>+</td>
<td>32</td>
<td>0.93 (0.87–0.99)</td>
<td>86 (71–95)</td>
<td>79</td>
<td>0.88 (0.83–0.93)</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>5</td>
<td>0.83 (0.70–0.94)</td>
<td>81 (71–91)</td>
<td>18</td>
<td>0.86 (0.78–0.93)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>37</td>
<td>0.89 (0.83–0.95)</td>
<td>83 (76–90)</td>
<td>97</td>
<td>0.89 (0.83–0.95)</td>
</tr>
<tr>
<td>Cervix</td>
<td>+</td>
<td>13</td>
<td>0.93 (0.83–1.00)</td>
<td>87 (60–98)</td>
<td>116</td>
<td>0.96 (0.93–0.99)</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>2</td>
<td>0.91 (0.79–1.00)</td>
<td>83 (63–93)</td>
<td>6</td>
<td>0.94 (0.85–1.00)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>15</td>
<td>0.92 (0.86–0.97)</td>
<td>85 (67–93)</td>
<td>122</td>
<td>0.94 (0.87–1.00)</td>
</tr>
<tr>
<td>Total</td>
<td>+</td>
<td>89</td>
<td>0.90 (0.86–0.94)</td>
<td>83 (75–90)</td>
<td>246</td>
<td>0.91 (0.88–0.94)</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>18</td>
<td>0.90 (0.83–0.97)</td>
<td>84 (70–92)</td>
<td>38</td>
<td>0.91 (0.87–0.96)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>107</td>
<td>0.90 (0.86–0.94)</td>
<td>84 (70–92)</td>
<td>284</td>
<td>0.91 (0.88–0.94)</td>
</tr>
</tbody>
</table>

$^a$ ESawb medium (200 μl) was transferred into an Aptima specimen transfer device prior to testing.

$^b$ One sample was repeatedly equivocal for *N. gonorrhoeae* in the NAAT on ESawb; this sample was counted as a positive result.

95% CI, 0.78 to 5.48; $P = 0.28$) (Table 4). The association between being asymptomatic and an unsuccessful deferred culture was stronger for urethral samples (OR, 26.0; 95% CI, 2.29 to 295.6; $P = 0.009$) than for rectal samples (OR, 2.44; 95% CI, 0.67 to 8.50; $P = 0.16$). In urethral samples from symptomatic patients, the overall success rate of the 1st deferred culture was 26/30 (87%), and for the 2nd deferred culture, this was 20/30 (67%).

A high correlation between various variables was noted: all heterosexual males who provided urine samples were symptomatic, all but one of the rectal samples were from MSM, and all but one of the HIV-positive participants were MSM. Therefore, we did not perform a multivariable analysis. In order to identify those with a high and those with a low likelihood of a negative deferred culture, we post hoc created four distinct subgroups: symptomatic HIV-negative patients, asymptomatic HIV-negative MSM, asymptomatic HIV-positive MSM, and an other group that included all remaining patients (Table 5). We found a large contrast between these groups, enabling good discrimination between the group with the highest likelihood of negative deferred culture (asymptomatic HIV-positive MSM, 56%) and the group with the lowest likelihood of a negative deferred culture (symptomatic HIV-negative patients, 13%).

**DISCUSSION**

Our study shows that a targeted deferred *N. gonorrhoeae* culture is possible from clinical samples stored up to 3 days in the ESawb medium. Prior to the current study, the performance of ESawb medium has been assessed only in reconstituted specimens containing cultured bacteria, and the results have shown a decrease in *N. gonorrhoeae* viability over time (8, 14–16). One study using other transport media found good culture results after 24 hr for endocervical specimens, but prolonged storage was not assessed (17).

The overall success rate of targeted deferred cultures in our study was 69% compared to that with NAAT. This is <100%, as is expected given the lower sensitivity of culture compared to that of NAAT, which is demonstrated by our direct culture positivity rate of 82% and in previous studies (4, 18–23). The lower success of deferred cultures compared to that of direct cultures can be explained by the decreased viability of *N. gonorrhoeae* over time. Another factor is that 51% of our deferred culture samples were of rectal origin, which are less successful in direct culture than urogenital samples (4, 18). Our results showed that if a direct culture of a rectal sample was successful, the deferred culture was likely to be positive as well. In contrast, for urogenital samples, we found that direct cultures were more often successful than deferred cultures. In addition, the majority of the urogenital samples were urethral samples. For these, direct cultures were obtained from urethral exudate samples, while the ESawb samples for deferred cultures were obtained from urine. While *N. gonorrhoeae* does not survive well in urine samples, in our study, dipping flocked swabs in urine within 30 min allowed the bacteria to survive in many cases (24, 25). However, the volume and bacterial load were likely lower than those in urethral exudate samples, resulting in fewer successful deferred cultures. The use of urethral ESawbs most likely improves the yield, but this was not tested in our study due to clinic policy against the use of intraurethral swabs. In addition, dipping ESawbs into urine within 30 min of collecting the urine could be challenging in many settings, particularly in settings with low numbers of gonorrhea cases or *N. gonorrhoeae* diagnostic tests.

An effect of bacterial load on culture outcome was also suggested when the semiquantitative RLU values of the NAAT samples were compared to the culture results. Compared to positive cultures, we found that negative direct and deferred cultures were more often from low-positive samples, that is, samples with an RLU value of <800.

As in previous research, we noted the highest success rate (87%) of deferred cultures in samples from symptomatic patients and in samples of urogenital origin; these patients are the best candidates for AMR surveillance using targeted deferred culture (18). The samples from asymptomatic HIV-positive MSM were
were undiluted (30 min diluted into specimen tubes, whereas standard NAAT samples were performed on only 200 μl of ESwab liquid medium is possible for N. gonorrhoeae culture. Given the difference in the success rate of ESwab compared to urethral samples. Using larger volumes, urethral exudate, or eluting the flocked ESwab into Aptima tubes might increase the chance of N. gonorrhoeae isolation. As opposed to direct urine samples, this might explain the significant difference in the C. trachomatis results of cervical compared to urethral samples. Using larger volumes, urethral exudate, or eluting the flocked ESwab into Aptima tubes might increase the sensitivity of NAAT on ESwab samples (9).

Notably, six samples were positive for C. trachomatis with NAAT on ESwab samples but negative with the standard NAAT. Unfortunately, negative routine samples were not stored, and the standard NAAT sample could not be retested. Whether these samples represent contamination or false-negative standard NAAT results, possibly due to borderline low loads or incorrect sampling, remains unclear.

Our study has additional limitations. First, the prevalence of gonorrhea among women in our clinic was low, resulting in few positive cervical and rectal samples from women. Second, the correlation between determinants was high; a multivariable logistic regression analysis was therefore not possible. Third, we did not examine pharyngeal specimens. Finally, this study was performed at a single center with good laboratory facilities, and our results might not be generalizable to other settings, including those using other NAAT procedures or transport media.

In conclusion, using NAAT with targeted deferred N. gonorrhoeae culture in these patients, irrespective of HIV serostatus (26–28).

Our study also showed that the performance of NAAT on ESwab liquid medium is possible for N. gonorrhoeae and C. trachomatis. The agreement between standard NAAT and NAAT on ESwab samples was relatively good, but we found a loss of sensitivity in NAAT on ESwab samples. As in previous research, NAAT on ESwab samples was performed on only 200 μl of ESwab medium diluted into specimen tubes, whereas standard NAAT samples were undiluted (29). Especially in samples with a low bacterial load, this might have caused false-negative results. Also, in ESwab samples used to diagnose urethral gonorrhea, the swabs were dipped in urine only, causing an additional dilution of the sample as opposed to direct urine samples. This might explain the significant difference in the C. trachomatis results of cervical compared to urethral samples. Using larger volumes, urethral exudate, or eluting the flocked ESwab into Aptima tubes might increase the sensitivity of NAAT on ESwab samples (9).

Notably, six samples were positive for C. trachomatis with NAAT on ESwab samples but negative with the standard NAAT. Unfortunately, negative routine samples were not stored, and the standard NAAT sample could not be retested. Whether these samples represent contamination or false-negative standard NAAT results, possibly due to borderline low loads or incorrect sampling of the standard NAAT in these patients, remains unclear.

Our study has additional limitations. First, the prevalence of gonorrhea among women in our clinic was low, resulting in few positive cervical and rectal samples from women. Second, the correlation between determinants was high; a multivariable logistic regression analysis was therefore not possible. Third, we did not examine pharyngeal specimens. Finally, this study was performed at a single center with good laboratory facilities, and our results might not be generalizable to other settings, including those using other NAAT procedures or transport media.

In conclusion, using NAAT with targeted deferred N. gonorrhoeae culture is an efficient method to preserve or establish sufficient AMR surveillance in settings where direct culture is not possible or gonorrhea prevalence is low. The ESwab system allows prolonged transport time, preselection with NAAT, and subsequent N. gonorrhoeae culture. Given the difference in the success...
rates of direct and deferred cultures, we recommend obtaining a direct culture for patients failing empirical therapy. Based on the methods and results of our study, we recommend obtaining two separate samples to prevent a loss in sensitivity with NAAT: one sample for NAAT, as recommended by the NAAT manufacturer, and one ESwab sample to be used for deferred culture. Ideally, the deferred cultures should be inoculated within 3 days of collection.

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