Short report

Confirmatory testing of *Neisseria gonorrhoeae* in a sexual health clinic: implications for epidemiology and treatment policy

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► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/sextrans-2020-054525).

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Received 9 April 2020
Revised 25 January 2021
Accepted 30 January 2021
Published Online First 25 February 2021

To cite: Tielemans M, van Westreenen M, Klaassen C, *et al*. *Sex Transm Infect* 2022;**98**:121–124.

ABSTRACT

Objectives European guidelines advise the use of dual nucleic acid amplification tests (NAAT) in order to minimise the inappropriate diagnosis of *Neisseria gonorrhoeae* (Ng) in urogenital samples from low prevalence areas and in extragenital specimens. In this cross-sectional study, we investigated the effect of confirmatory testing and confirmation policy on the Ng-positivity in a population visiting the sexual health clinic in Rotterdam, the Netherlands.

Methods Apart from urogenital testing, extragenital (oropharyngeal/anorectal) testing was performed for men who have sex with men (MSM) and according to sexual exposure for women and heterosexual men. Ng detection using NAAT was performed using BD Viper and for confirmatory testing BD MAX. Sexual transmitted infection consultation data were merged with diagnostic data from August 2015 through May 2016.

Results In women (n=4175), oral testing was performed in 84% and 22% were tested anally. In MSM (n=1828), these percentages were 97% and 96%, respectively. Heterosexual men (n=3089) were tested urogenitally. After confirmatory testing, oropharyngeal positivity rates decreased from 7.3% (95% CI 6.5 to 8.2) to 1.5% (95% CI 1.1 to 1.8) in women and from 13.9% (95% CI 12.3 to 15.5) to 5.4% (95% CI 4.3 to 6.4) in MSM. Anorectal positivity rates decreased from 2.6% (95% CI 1.6 to 3.7) to 1.8% (95% CI 0.9 to 2.6) in women and from 9.3% (95% CI 7.9 to 10.7) to 7.2% (95% CI 6.0 to 8.5) in MSM. Urogenital Ng-positivity rate ranged between 3.0% and 4.4% and after confirmation between 2.3% and 3.9%. When confirming oropharyngeal samples, Ng-positivity was 3.8% in women, 3.0% in heterosexual men and 12.5% in MSM. Additional confirmation of urogenital and anorectal samples led to 3.0% Ng positivity in women, 2.7% in heterosexual men and 11.4% in MSM.

Conclusions Confirmation of urogenital and anorectal samples reduced the Ng-positivity rates, especially for women. However, as there is no gold standard for the confirmation of Ng infection, the dilemma within public health settings is to choose between two evils: missing diagnoses or overtreatment. In view of the large decrease in oropharyngeal positivity, confirmation Ng-positivity in oropharyngeal samples remains essential to avoid unnecessary treatment.

INTRODUCTION

Diagnostic testing of *Neisseria gonorrhoeae* (Ng) is performed using nucleic acid amplification tests (NAATs), as their high sensitivity makes them particularly suitable for screening applications. However, in areas with a low prevalence of Ng, the positive predictive values (PPV) of these NAATs can be unacceptably low.[1 2] In this respect, confirmatory testing with a NAAT that detects a different Ng target sequence is advised when PPVs are less than 90% and when testing extragenital samples.[3 4]

The sexual health clinic (SHC) of the greater Rotterdam-Rijnmond region (The Netherlands) serves a population at high risk for sexual transmitted infection (STI). Incidentally, we observed that the confirmatory second NAAT was negative, whereas the sample was Ng-culture positive or that Ng was cultured at another body side. A more detailed evaluation of the 2015 data revealed four of these cases, which was the reason to perform this research. Therefore, we investigated the impact of confirmatory testing on Ng-positive urogenital (urine or vaginal) and extragenital (oropharyngeal and anorectal) samples as well as on patient Ng-infection status positivity in clients of the Rotterdam-Rijnmond SHC.

METHODS

Study population and setting

This retrospective study of cross-sectional design was performed on data from clients attending the SHC of the Public Health Service Rotterdam-Rijnmond (The Netherlands) whose specimens were analysed at the Department of Medical Microbiology and Infectious Diseases at the Erasmus University Medical Centre Rotterdam. Reported sexual exposure determined which body locations were sampled. All clients agreed to the use of their anonymous data for research.

Nucleic acid amplification tests

Two NAATs were used for the detection of Ng in clinical samples. The first NAAT was the BD Viper XTR system (Becton Dickinson, New Jersey, USA), with a reported sensitivity ranging from 96.9% to 100% and a specificity from 98.9% to 100%.[5] The second NAAT, amplifying a different target, was the BD MAX (Becton Dickinson, New Jersey, USA), with an estimated sensitivity between 95.5% and 99.1% and a specificity >98.6% when compared with several NAAT platforms and Ng culture.[6]

Testing algorithm

Between 1 August 2015 and 19 May 2016, all samples were tested in the BD Viper. All extragenital

and urogenital BD Viper-positive samples were confirmed using the BD MAX. For each participant, the patient Ng-infection status was assessed by combining the positive results of the BD Viper with the positive BD MAX results from urogenital and extragenital tests, and the influence of confirmatory testing policy on the Ng-positivity rate was evaluated in our population.

Additionally, samples from the consultations between January until July 2015—in which no large differences had been found—were used to compare the reported baseline characteristics and NAAT results per anatomical location (online supplemental table 1). Unfortunately, the patient infection status for Ng could not could be compared, as BD Viper positive urogenital samples were not confirmed by BD MAX during the January until July 2015 period.

RESULTS

Baseline characteristics

The study period included 9092 consultations: 4175 women (45.9%), 3089 heterosexual men (34.0%) and 1828 MSM (20.1%). One or more STIs were found in 25% of MSM, 20% of heterosexual men and 21% of women. In all groups, *Chlamydia trachomatis* was the STI most frequently diagnosed, followed by Ng. Urogenital testing was performed during all consultations. Oropharyngeal testing was performed in 85% of women and 98% of MSM. For anorectal testing, these percentages were 22% and 96%. Extragenital testing was performed in ≤1.5% of heterosexual men.

NAAT results per anatomical location

Oropharyngeal samples tested positive with BD Viper in 7.3% of women, as compared with 13.9% of MSM. The majority of these samples were not confirmed as positive by BD MAX—confirmation rates were 19.8% (95% CI 12.5 to 27.2) in women and 38.7% (95% CI 29.6 to 47.9) in MSM(online supplemental table 1).

Anorectal samples tested positive with BD Viper in 2.6% of women, as compared with 9.3% of MSM. The rate of confirmation with BD MAX was 66.7% (95% CI 38.2 to 95.1) in women and 77.9% (95% CI 68.3 to 87.5) in MSM.

Urogenital samples tested positive with BD Viper in 3.0% of women and heterosexual men and in 4.4% of MSM. Confirmatory testing of urogenital samples yielded results of 75.4% (95% CI 64.0 to 86.8) in women, 88.0% (95% CI 78.0 to 98.1) in heterosexual men and 88.9% (95% CI 78.3 to 99.2 in MSM.

The percentage of oropharyngeal samples that were confirmed Ng-positive was much lower in clients where only oropharyngeal samples were initially tested Ng-positive, as compared with clients where multiple anatomical locations were initially found Ng-positive. More specifically, 26 out of 219 women with Ng-positive oropharyngeal samples only were confirmed Ng-positive with BD MAX (11.9% (95% CI 7.6 to 16.2)). This compared with 25 out of 38 women with multiple anatomical locations initially Ng-positive (65.8% (95% CI 50.7 to 80.9)). Slightly higher Ng-positive confirmation rates were observed in oropharyngeal-only positive samples from MSM as compared with multiple Ng-positive body locations: 43 out of 169 MSM (25.4% (95% CI 18.9 to 32.0)) vs 53 out of 79 MSM (67.1% (95% CI 56.7 to 77.5)).

Patient Ng-infection status by different policies for confirmatory testing

The patient infection status for Ng was assessed for various combinations of confirmatory testing policy, with Ng-positivity rates being most highly influenced after confirmatory testing of oropharyngeal samples (figure 1). Without confirmatory testing, the Ng-infection rate was 8.4% in women and 19.4% in MSM.

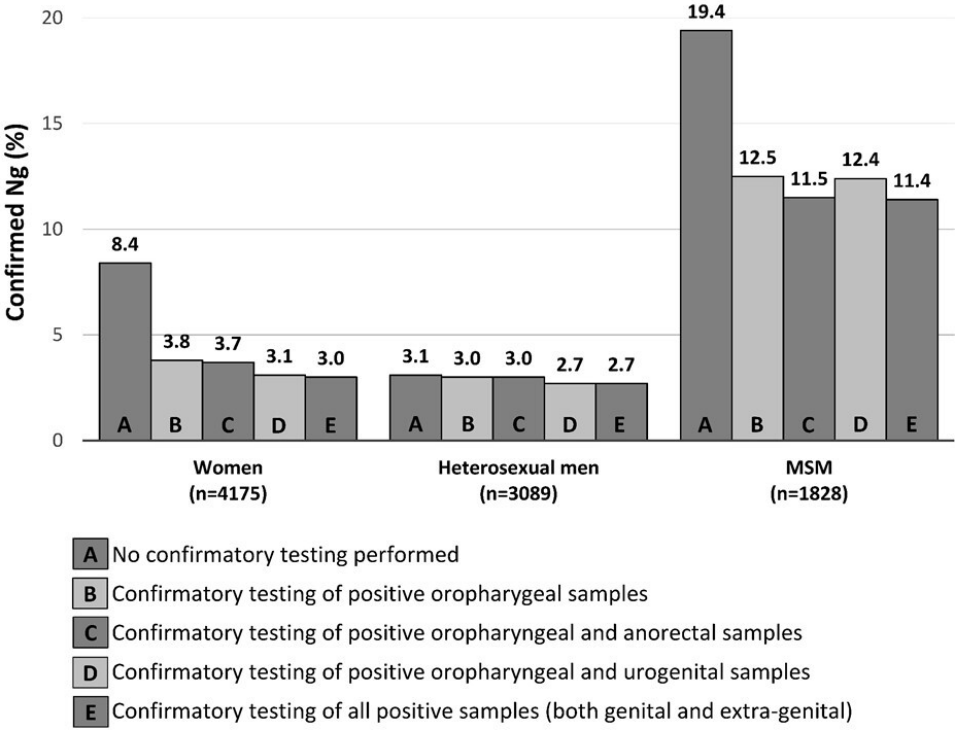

Figure 1 Ng infection status of patients using different policies for confirmatory testing. Ng infection was determined as a percentage of confirmed Ng-positive results (BD MAX) after initial positive result testing (BD Viper). Ng, *Neisseria gonorrhoeae*.

Confirming oropharyngeal Ng led to a Ng-infection rate of 3.8% in women and 12.5% in MSM. The addition of anorectal confirmatory testing led to a slight decrease in Ng-infection rate to 3.7% in women and 11.5% in MSM. Confirmatory testing at all sites led to a further decrease in women to 3.0%, but remained similar in MSM.

DISCUSSION

Our results indicated major differences in Ng-positivity rates when using a single NAAT testing policy (BD Viper) compared with a confirmatory testing policy (BD Viper combined with BD MAX). These major differences were observed between: (i) women, MSM and heterosexual men and (ii) between urogenital and extragenital locations. Additionally, we found that the Ng-positive confirmation rates in oropharyngeal samples were higher when Ng was also detected at additional anatomical locations. The lower confirmation rates in oropharyngeal samples are most likely due to false-positive results due cross reactivity of Ng with other commensal *Neisseria species* in the oral flora.[3]

The higher Ng-infection rates found after confirmatory testing in MSM (as compared with women) could possibly be explained by differences in Ng prevalence within these patient groups, as the PPV of true positive results increases with increasing prevalence of a disease. No differences in bacterial Ng load are being expected between genders.[7]

Our research distinguished from previous studies by sampling extensively at different anatomical locations based on type of sexual contact in a large population. This allowed us to gain insight into the effect of different confirmatory testing policies on patient Ng-infection status.

While this study confirmed the need for extragenital sampling to detect Ng infections, it also showed the difficulty of determining the true Ng-infection status of a client, especially when individuals are asymptomatic. Asymptomatic sites of infection may play a major role in the persistence of gonorrhoeae at a population level.[8 9] Obviously confirmatory testing of oropharyngeal samples is necessary, even in our high-risk population when using BD Viper. Also, BD Viper may have lower Ng-confirmation rates than other platforms.[10] In particular, isolated oropharyngeal NAAT results need to be interpreted with care.

In the absence of a gold standard to detect Ng (culture is not sensitive enough) and the knowledge that our confirmatory test may have missed Ng infections, we chose the following strategy in our high-risk population at our SHC: (i) to use the Ng-confirmed results in oropharyngeal samples, (ii) to use the initial Ng-results in anorectal and urogenital samples and (iii) in case of doubts about the Ng-infection status, especially in isolated positive body locations, we use the confirmatory test results of anorectal and urogenital samples for the definitive interpretation. Evidently, cases in which a positive Ng-culture is available are treated. Our research implicates that extragenital testing is essential for the patient Ng-infection status and that confirmatory policies of Ng greatly influences Ng-positivity rates. With respect to confirmatory policies and validation of test-platforms collaboration of laboratory and clinic is conditional.

A major strength of this study is the stratification of results by sexual contact, therefore differentiating between MSM and heterosexual men, which is essential when considering the large difference in Ng-positivity between these groups. Published research in this area tends to be limited. Additionally, our inclusion of extensive extragenital testing in women and MSM is a strength of the study, allowing differentiation of Ng-positivity rates per confirmatory policy.

There are also limitations to this study: the BD Viper-negative samples were not tested in the BD MAX, and consequently, sensitivity and specificity could not be determined. Also, discordant results (BD Viper positive/BD MAX negative) were not tested further using a third platform, which could have provided more information on the 'true' test result.

To conclude, confirmation of oropharyngeal testing is needed to avoid unnecessary treatment. The decision whether to use additional confirmatory testing of samples to confirm Ng-positivity is a dilemma in public health settings and forces to choose between two evils: missing Ng diagnoses or overtreatment of Ng. Ng-confirmatory testing policies should be adapted based on Ng prevalence, which NAATs are used for initial and confirmatory testing, and all relevant anatomical locations should be sampled.

Key messages

- This study investigated the effect of various confirmation policies on *Neisseria gonorrhoeae* (Ng)-positivity (BD Viper/BD MAX) on apparent Ng-positive urogenital and extragenital samples obtained from clients attending a Sexual Health Center.
- On confirmation of oropharyngeal samples, Ng-positivity decreased from 8.4% to 3.8% in women, 3.1% to 3.0% in heterosexual men and 19.4% to 12.5% in MSM.
- Additional Ng-confirmatory testing of urogenital and anorectal samples led to 3.0% Ng positivity in women, 2.7% in heterosexual men and 11.4% in MSM.
- As there is no gold standard for confirmation of Ng infection, the dilemma faced by public health settings is to choose between two evils: missing diagnoses or overtreatment.

Handling editor Claudia S Estcourt

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Acknowledgements We acknowledge Bram Meima for the management of laboratory and surveillance data. The authors gratefully acknowledge the contributions of staff of the Rotterdam SHC for collecting the data.

Contributors HMG, MvW and CK designed the study. MT and HMG collected the data and performed the statistical analyses. Data interpretation was performed by MvW, HMG, CK and MT. All authors contributed to drafting and revising the text and all authors approved the final manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The Medical Ethics Committee of the Erasmus University Medical Centre Rotterdam (MEC-2015–306) approved the anonymous use of the specimen results.

Provenance and peer review Not commissioned; externally peer reviewed.

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