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Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* by PCR in a sample pool (urine, rectum and pharynx) in asymptomatic patients at risk of sexually transmitted infections



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ABSTRACT

Introduction: Detection of *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) is periodically indicated in asymptomatic patients with risky sexual practices. The objective of this study was to assess the efficacy of employing a rapid polymerase chain reaction (PCR) test in a combined pool of three samples from the same patient and compare it with the standard PCR performed in the three different samples separately.

Methods: Samples were collected from asymptomatic patients at risk of sexually transmitted infections (STI). Urine samples, two pharyngeal swabs, and two rectal swabs were collected from each patient. Two PCR techniques were performed: standard PCR (Allplex CT/NG/MG/TV[®], Seegene) in each of the three samples separately, and rapid PCR (Xpert CT/NG[®], Cepheid) in a pool of three samples.

Results: A total of 429 pooled samples from 403 patients were included in the study, and 426 urine samples, 412 rectal swabs and 426 pharyngeal swabs were also processed. Concordances between pooled and individual PCRs for both CT and NG identification were 94.17%, with 77 (17.9%) positive samples. The concordance for CT was 98.60%, with 38 positive samples (8.88%), being 95.33% for NG, with 51 positive samples (11.88%).

Conclusion: The use of a pool of three samples (urine, rectum and pharynx) for the detection of NG and CT using rapid PCR can be a cost-effective alternative to performing conventional PCR in the three samples separately in asymptomatic patients at risk of developing STI.

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Detección de *Neisseria gonorrhoeae* y *Chlamydia trachomatis* mediante PCR en una agrupación de muestras (orina, recto y faringe) en pacientes asintomáticos con riesgo de infecciones de transmisión sexual

RESUMEN

Introducción: La detección de *Neisseria gonorrhoeae* (NG) y *Chlamydia trachomatis* (CT) está indicada periódicamente en pacientes asintomáticos con prácticas sexuales de riesgo. El objetivo de este estudio fue evaluar la eficacia de emplear una prueba rápida de reacción en cadena de la polimerasa (PCR) en una agrupación de tres muestras del mismo paciente y compararla con la PCR estándar realizada en las 3 muestras diferentes por separado.

Palabras clave:

Neisseria gonorrhoeae

Chlamydia trachomatis

PCR

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Orina
Recto
Faringe
Agrupación de muestras

Métodos: Se recolectaron muestras de pacientes asintomáticos con riesgo de padecer infecciones de transmisión sexual (ITS). De cada paciente se recogieron muestras de orina, 2 hisopos faríngeos y 2 hisopos rectales. Se realizaron dos técnicas de PCR: PCR estándar (Allplex CT/NG/MG/TV[®], Seegene) en cada una de las 3 muestras por separado, y PCR rápida (Xpert CT/NG[®], Cepheid) en la agrupación de las 3 muestras. **Resultados:** Se incluyeron en el estudio un total de 429 muestras agrupadas de 403 pacientes y también se procesaron 426 muestras de orina, 412 hisopos rectales y 426 hisopos faríngeos. La concordancia entre las PCR combinadas e individuales para la identificación de CT y NG fue del 94,17%, con 77 (17,9%) muestras positivas. La concordancia para CT fue del 98,60%, con 38 muestras positivas (8,88%), siendo del 95,33% para NG, con 51 muestras positivas (11,88%).

Conclusión: El uso de una agrupación de 3 muestras (orina, recto y faringe) para la detección de NG y CT mediante una PCR rápida puede ser una alternativa coste/efectiva a la realización de una PCR convencional en las 3 muestras por separado en pacientes asintomáticos con riesgo de desarrollar ITS.

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Introduction

There has been a troubling increase in the prevalence of sexually transmitted infections (STI) worldwide, particularly among younger, sexually active people. According to the World Health Organization (WHO), over a million people contract STI daily. The most common causative bacterial pathogens are *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). In 2020, 129 million cases of CT and 82 million cases of NG were reported.¹ In Spain, men of all age groups are more frequently affected by NG than CT, while CT is more prevalent among women of 15–24 years of age.²

Molecular diagnostic methods have proven to be highly effective in the detection of STI. However, their widespread adoption remains limited in many countries due to the associated high costs. Periodic STI screening is crucial, particularly among at-risk populations. NG and CT often manifest with nonspecific symptoms, underscoring the importance of routine screening as a vital public health strategy to disrupt the transmission chain while minimizing costs and response times.

The objective of this study was to assess the efficacy of employing a rapid PCR test in a combined pool of samples (urine, rectum, and pharynx) from the same patient and compare it with the standard PCR performed in the three samples separately for the detection of NG and CT in asymptomatic patients engaged in risky sexual practices, with the goal of implementing rapid PCR as a point of care.

Methods

Study population

This was a prospective study conducted from June 2021 to January 2022, including samples collected for the diagnosis of STI in asymptomatic patients engaging in risky sexual practices. Inclusion criteria encompassed men of all sexual orientations who sought consultation at the STI clinic at our hospital and provided informed consent. Exclusion criteria included refusal to participate, failure to provide informed consent, antibiotic usage within the last month due to a recent STI diagnosis, and the presence of symptoms suggestive of a STI. Participants were also questioned about various aspects of their sexual life, including the number of partners in the last three months, condom usage, previous STI, and personal behaviours, such as HIV pre-exposure prophylaxis, the use of recreational substances during sexual activity (Chemsex use) and participation in group sex, among others. The study was approved by the Clinical Research Ethics Committee of our hospital.

Sample collection

Urine from the initial urination stream (10–20 mL) was obtained in a sterile container (Deltalab[®]), utilizing an automatic tube filling system (BD Vacutainer[®]). In total, two tubes were collected for each urine sample. For pharyngeal samples, two swabs were taken and placed in Amies liquid transport medium (Cliniswab[®]). Similarly, after cleaning the anal area, two rectal swabs were collected and placed in Amies liquid transport medium. Trained healthcare professionals conducted the sampling process. Samples received at the laboratory during the weekend were stored at between 2 and 4 °C until they could be processed during regular hours. The maximum time elapsed between sample collection and processing was 72 h.

Microbiological techniques

Two PCR techniques were employed:

- 1) Standard PCR: the Allplex CT/NG/MG/TV[®] by Seegene was used for the detection of CT, NG, *Mycoplasma genitalium* and *Trichomonas vaginalis*. Each of the three samples was processed separately, following the manufacturer's instructions. Microlab Starlet IVD Hamilton was used for extraction and preparation and Bio-Rad C1000 Thermal Cycler was used for amplification. A minimum of 1 mL of urine and 0.5 mL of Amies liquid medium from each rectal and pharyngeal swab were necessary for the assay. Any remaining sample was aliquoted and stored at –20 °C for potential repetitions. Subsequently, the Seegene Viewer facilitated automated data analysis.
- 2) Rapid PCR: the Xpert CT/NG[®] assay by Cepheid was performed for the detection of CT and NG using the GX-IV and GX-VI module instruments with a laptop software version 4.3. A tube was used, containing 1 mL of urine and 0.5 mL of the Amies transport medium of each pharyngeal and rectal swab. These samples were vortexed before processing. A total of 1 mL of the pooled sample was used in the GeneXpert cartridge. Any remaining sample was aliquoted and stored at –20 °C for potential repetitions.

Analysis

We employed the “Clopper-Pearson” formula for sample size calculation, with a minimum sample size of 380 individuals being considered necessary. Assay concordance was tested using Cohen's kappa coefficient. For invalid tests or discordant results between the two platforms, the tests were repeated using an aliquot of the

Table 1
Characteristics of the 403 patients: number (N) and percentage of the total responses issued (%).

	N	%
<i>Place of birth</i>		
Spain	219	54.6
Rest of Europe	37	9.2
America	139	34.7
Asia and Oceania	4	1.0
Africa	2	0.5
Unknown	2	
<i>Chemsex use</i>		
Yes	221	56.2
No	172	43.8
Unknown	10	
<i>HIV Pre-exposure Prophylaxis (PreP) use</i>		
Yes	331	83.0
No	68	17.0
Unknown	4	
<i>Previous sexually transmitted infections</i>		
Yes	270	68.4
No	125	31.6
Unknown	8	
<i>Sexual activity</i>		
Sex with men	379	96.7
Sex with women	8	2.0
Sex with men and women	5	1.3
Unknown	11	
<i>Attitude towards anal sexual activity</i>		
Active: insertive anal sex	47	18.4
Passive: receptive anal sex	28	10.9
Active and passive: both	181	70.7
Unknown	147	
<i>Condom usage</i>		
Yes	120	46.9
No	136	53.1
Unknown	147	
<i>Group sex</i>		
Yes	134	34.9
No	250	65.1
Unknown	19	
<i>Number of sexual partners in the last three months</i>		
0	6	1.7
1–5	138	38.5
6–10	93	26.0
11–20	73	20.4
21–40	29	8.1
41–60	16	4.5
Over 60	3	0.8
Unknown	45	

original sample. Samples that consistently yielded the same discordant result upon repetition were interpreted as discrepant.

Results

A total of 429 pooled samples from 403 patients were included in the study. Among the 403 patients, 375 (93%) were men who have sex with men (MSM), 23 (5.7%) men who have sex with women (MSW) and 5 (1.2%) were identified as transgender women. The median age of the participants was 35 years (range 20–67). Characteristics of the 403 patients are described in Table 1.

Among individual samples, seven were not received at the laboratory (one urine, five rectal swabs and one pharyngeal swab) and another 16 samples were excluded for repeated invalid results (two urines, 12 rectal swabs and two pharyngeal swabs) (Fig. 1).

The number of positive samples concordant between pooled and individual PCR was 77 (17.9%) for both CT and/or NG; 38 (8.8%) for CT and 51 (11.8%) for NG. The concordance between pooled and

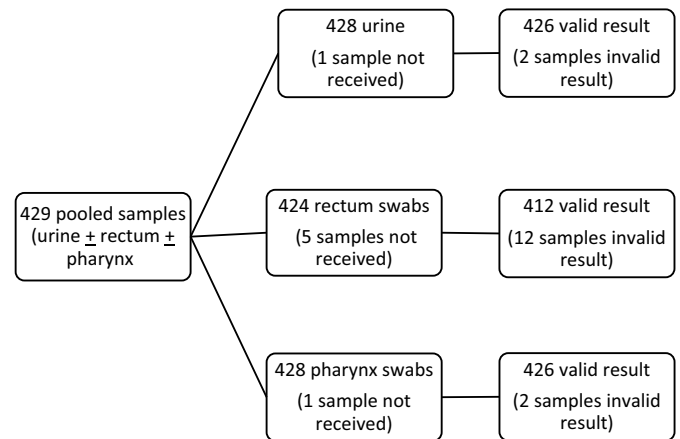


Fig. 1. Samples analyzed in the study.

Table 2
Concordance between pooled PCR (urine sample + rectal swab + pharyngeal swab) and standard PCR (three separate samples) for the detection of *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT).

	Standard PCR NG and/or CT positive	Standard PCR NG and CT negative
Pooled PCR NG and/or CT positive	77	7
Pooled PCR NG and CT negative	18	327

Table 3
Concordance between pooled PCR (urine sample + rectal swab + pharyngeal swab) and standard PCR (three separate samples) for the detection of *Chlamydia trachomatis* (CT).

	Standard PCR CT positive	Standard PCR CT negative
Pooled PCR CT positive	38	2
Pooled PCR CT negative	4 (4 pharynx)	385

Table 4
Concordance between pooled PCR (urine sample + rectal swab + pharyngeal swab) and standard PCR (three separate samples) for the detection of *Neisseria gonorrhoeae* (NG).

	Standard PCR NG positive	Standard PCR NG negative
Pooled PCR NG positive	51	3
Pooled PCR NG negative	17 (16 pharynx 1 rectum)	358

individual PCR for both CT and/or NG was 94.17% (Cohen’s kappa of 0.82, 95% confidence intervals (CI) 0.72–0.91) (Table 2). For CT, the concordance was 98.60% (kappa of 0.91, 95% CI 0.82–1.00) (Table 3), being 95.33% (kappa of 0.80, 95% CI 0.71–0.90) for NG (Table 4). The most frequent discrepancies were observed in pharyngeal swabs for NG: 16/426 (3.75%). Positive samples for NG and CT in standard PCR that were concordant with pooled PCR are described in Table 5.

M. genitalium was detected in 44 of 429 cases (any of the three samples analyzed individually) by standard PCR (10.25%): six in urine, 33 in rectal swabs, four in pharyngeal swabs and one in urine plus pharynx swab. Thirteen of these samples (seven pharyngeal and six rectal swabs) were positive for CT (eight samples) and/or NG (six samples). No *T. vaginalis* was detected.

Table 5Positive samples for *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) in standard PCR concordant with pooled PCR.

	Urine standard PCR positive	Rectum standard PCR positive	Pharynx standard PCR positive	Total pooled PCR positive
NG	1	30	35	51
CT	4	35	11	38

Discussion

The findings of this study provide a comprehensive look at the prevalence of CT and NG infections among asymptomatic, high-risk men, underscoring the significance of regular STI screening in this population. Our data indicate a prevalence of 17.9% for CT and/or NG using both pooled and individual PCR methods, aligning with previous studies that report similar prevalence rates for these infections in high-risk populations.³ Such findings support the need for accessible, cost-effective screening methods to aid in the detection and early treatment of STIs, ultimately helping to curb transmission.

The high concordance rate observed between rapid PCR testing on pooled samples and standard PCR testing on individual samples (94.17% for both CT and NG) underscores the potential of pooled testing as a viable alternative to individual sample testing, as tested before.^{4–9} The concordance for CT was notably high at 98.6%, while NG concordance was slightly lower at 95.33%, with most discrepancies occurring in pharyngeal samples. These discrepancies may be due to lower bacterial inoculum in the pharynx¹⁰ or potential cross-reactions with other *Neisseria* species.¹¹

Although *M. genitalium* was detected in 10% of the cases (any of the three samples analyzed individually by standard PCR) in the present study, there is consensus that its detection and treatment are not indicated in asymptomatic patients.¹²

Some limitations in this study should be acknowledged. The inability to verify discrepant pharyngeal samples with a third PCR test is a limitation, particularly given the noted discrepancies in NG detection at this site. Additionally, this study focused exclusively on male patients, which limits the generalizability of findings to other populations. Despite these limitations, the results indicate that pooled sample testing could provide an efficient, cost-saving alternative for large-scale screening, particularly in asymptomatic populations. The 90-min turnaround time of Xpert for CT and NG detection at the point of care may enable prompt treatment.¹³ Future research could explore the efficacy of pooled testing across broader demographics, including females and transgender individuals, to validate these results.

In conclusion, this study demonstrates that rapid PCR testing on pooled samples of urine, rectal, and pharyngeal specimens is a highly specific and sensitive method for CT and NG detection, with potential for point-of-care use. The rapid 90-min turnaround time of the Xpert CT/NG assay makes it especially suited for clinical environments where timely diagnosis and treatment are critical. This pooled approach could serve as an efficient, cost-effective strategy for large-scale STI screening, particularly in asymptomatic high-risk groups. Nevertheless, individual sample testing remains the preferred method when infection site-specific information is necessary or for symptomatic patients.

Incorporating pooled testing into routine clinical practice may enable broader STI surveillance, reduce transmission rates, and enhance resource allocation in STI clinics, especially those serving high-risk populations.

Conflict of interest

The authors declare that they have no conflict of interest.

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