

## BACKGROUND

Sexually transmitted infections (STIs) are a major public health concern worldwide, affecting quality of life and causing serious morbidity and mortality.

*Neisseria gonorrhoeae* is challenging to diagnose by culture due to its fastidious nature. A rapid, highly sensitive PCR assay is urgently needed for routine *N. gonorrhoeae* detection to prevent widespread infection and reproductive complications.

The aim of this study was to evaluate the sensitivity of an in-house PCR assay specifically for the detection of *N. gonorrhoeae*.

## METHODS

For the design of the in-house PCR, the 16S rRNA of a *N. gonorrhoeae* isolate was accessed from GenBank (AJ247239.2). Using a primer design tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>), the primers were selected and used in a qPCR SYBR green assay.

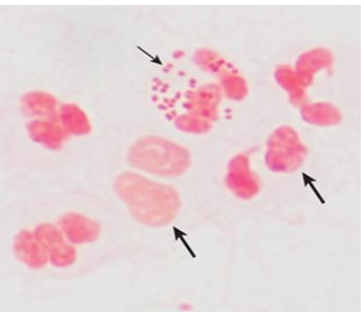
The in-house 16S rRNA assay was compared to the commercially available TaqMan assay which uses primers and probes specific for *N. gonorrhoeae*.

The 16SrRNA primers were tested for cross reactivity towards six Neisseria strains. The DNA from the following Neisseria isolates: *N. elongate*, *N. cinera*, *N. weaver* and *N. sicca* were tested.

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CATGCAAGTCGGACGGCAGCAGACAGGGAAGCTTGCTTCTCGGGTGGCGAGTGGCGAACGGGTGAGTAACAT
ATCGGAACGTACCGGTAGCGGGGATAAAGTATCGAAAGATCAGCTAATACCGCAATACGTTTGAGAGG
GAAAGCAGGGGACCTTCGGGCTTGCCTATCCGAGCGGCCGAATATCTGATTAGCTGGTTGGCGGGTAA
AGGCCACCAAGCGCAGCATCAGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGC
CCAGACTCTACGGGAGGCAGCAGTGGGGAATTTGGACAATGGCGCAAGCCTGATCCAGCCATGCCCGC
GTGCTGAAGAAGGCTTCGGGTTGTAAGGACTTTTTCAGGGAAGAAAAGGCCGTTGCCAATATCGGC
GGCCGATGACGGTACCTGAAGAATAAGCACCCGGCTAA
    
```

Figure 1: >AJ247239.2 *Neisseria gonorrhoeae* 16S rRNA gene, partial sequence accessed from Genbank. The yellow highlights shows the position of the selected primers.



Source: Karen C. Carroll, Stephen A. Morse, Timothy Mietzner, Steve Miller, Jawetz, Melnick, & Adelberg's Medical Microbiology, 27th Edition. www.accessmedicine.com. Copyright © McGraw-Hill Education. All rights reserved.

## RESULTS

The analysis showed successful 16S rRNA amplification in all clinical *N. gonorrhoeae* isolates.

Positive amplification matched in-house and TaqMan results, displaying strong correlation.

Cross-reactivity analysis confirmed assay specificity for *N. gonorrhoeae* detection, excluding non-gonococcal Neisseria strains.

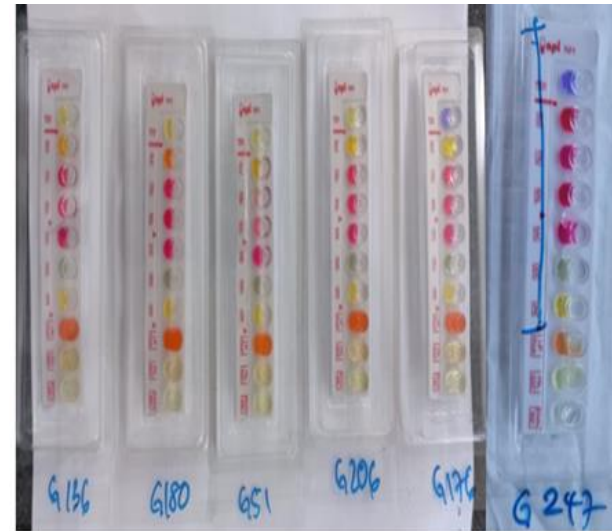


Figure 1. API test strip used to detect *N. gonorrhoeae* cultures.

## CONCLUSION

This study showed that the in-house PCR had successfully detected *N. gonorrhoeae*. The in-house assay showed a good correlation with a commercially available assay.

This assay needs to be validated using different clinical samples to show its promise as a diagnostic test.

Sample Name	Target Name	Ct Mean	Amp Status
WHO Y	16S	11.275	Amp
<i>N. elongata</i>	16S	Undetermined	No Amp
<i>N. cinera</i>	16S	38.266	No Amp
<i>N. weaveri</i>	16S	39.596	No Amp
<i>N. sicca</i>	16S	37.523	No Amp
Negative control	16S	Undetermined	No Amp

Figure 2: Cross reactivity of the 16S rRNA assay against *N. elongata*, *N. cinera*, *N. weaver* and *N. sicca*



## REFERENCES

1. Procop, G. W., Church, D. L., Hall, G. S., & Janda, W. M. (2020). *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*: Jones & Bartlett Learning
2. Murray, P., Rosenthal, K., & Pfaller, M. (2020). *Medical Microbiology* (9th ed.)