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Short report

2025 Review of Public Health Laboratory Network Australia *Neisseria gonorrhoeae* National Nucleic Acid Amplification Testing Guidelines

David M Whiley, Sebastiaan van Hal, Todd M Pryce, Monica M Lahra, on behalf of the National Neisseria Network and the Public Health Laboratory Network

Abstract

Since the introduction of *Neisseria gonorrhoeae* nucleic acid amplification tests (NG-NAATs) into routine clinical use, false-positive results caused by cross-reaction with non-gonococcal *Neisseria* species have been an issue, particularly in specimens from the pharynx. Therefore, since 2005 in Australia, a confirmatory assay has been recommended, with a positive result issued only when both assays are concordant.

At the request of the Public Health Laboratory Network (PHLN) Australia, the National Neisseria Network (NNN) met to review the 2015 PHLN NG-NAATs Guidelines in October 2024, in the context that some later generation *N. gonorrhoeae* NAATs have claims for testing pharyngeal samples without the need for supplemental testing for confirmation. Adequacy of performance in this context was considered by the NNN as a positive predictive value of 95% in line with World Health Organization guidance.

Based on the 2024 review, it is recommended that:

1. Supplementary testing continue to be performed for all non-urogenital (pharyngeal and rectal) samples.
2. Supplementary testing be at the discretion of individual laboratories, based on local validation data demonstrating adequate performance based on WHO recommendations, for urogenital samples. Additional testing should continue to be considered when testing low-risk populations.

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Nucleic acid amplification tests (NAATs) for *Neisseria gonorrhoeae* are the primary method for diagnosing gonorrhoea. In 2024, NAATs accounted for 86% of diagnosis in Australia.[1] Whilst bacterial culture-based diagnostic methods are encouraged in the context of escalating antimicrobial resistance (AMR), the proportion diagnosed by culture remains relatively unchanged in Australia.[1]

It is important that NG diagnostic techniques exhibit a reliability consistent with World Health Organization (WHO) guidance on testing performance.[2] Since the introduction of *N. gonorrhoeae* NAATs (NG-NAATs) into routine clinical use, false-positive results caused by cross-reaction with non-gonococcal *Neisseria* species have been detected, particularly in pharyngeal samples.[3–5] This problem arises because of frequent genetic exchange between *N. gonorrhoeae* and commensal *Neisseria* species colonising human urogenital and pharyngeal sites.[6–7]

Early generation NG-NAATs demonstrated false-positive *N. gonorrhoeae* rates as high as 94–95% for pharyngeal samples.[8] This led to the publication of the Australian Public Health Laboratory Network's '*Guidelines for the use and interpretation of nucleic acid detection tests for Neisseria gonorrhoeae in Australia*' in 2005.[9] Based on local assay performance data, the 2005 Guidelines recommended that all NG-NAAT positive results (urogenital and extragenital samples) should also test positive on a reliable supplemental assay before a *N. gonorrhoeae*-detected result is reported. As a result, this strategy was adopted by clinical diagnostic laboratories in Australia, despite the increased burden of testing and rebate to support the supplementary testing.

The 2005 guidelines were reviewed in consultation with the National Neisseria Network (NNN), endorsed by the PHLN, and published in 2015.[3] The review focused on two key points. Firstly, the substantial improvement in the specificity of commercial NG-NAATs since 2005, raising questions about whether supplemental testing remained warranted, particularly for urogenital sites that are less likely to be colonised with non-gonococcal *Neisseria* strains. Secondly, negative results from supplemental assays may represent false negatives. This can occur due to variability in the sequence of supplemental targets or when samples contain low levels of *N. gonorrhoeae* DNA, which may fail to amplify in the supplemental assay.[3] The consensus opinion continued to recommend supplementary testing for NG-NAAT testing, even for urogenital samples.[3] In the setting of discordant results, adding an appropriate comment (highlighting possible false negative supplementary results) was recommended.

In 2024, these guidelines were again reviewed by the NNN, with a focus on the performance of newer assays testing specimens at different anatomical sites. It was noted that manufacturer-led evaluations of newer commercial assays include performance claims for non-urogenital samples,[10] challenging the need for supplemental testing.

Jurisdictional stakeholders discussed paired screening and supplemental NG-NAAT test results, where available. Similar to previous reviews, the highest concordance for screening and supplementary testing continued to be observed for urogenital samples, whilst rectal and pharyngeal samples showed the lowest rates of agreement.

The NNN and PHLN acknowledge:

1. Ongoing challenges with NG-NAATs:

   The high rate of intraspecies genetic exchange within the *Neisseria* genus continues to pose challenges for NG-NAAT testing. As a result, decisions regarding the use of supplementary NG-NAATs should take into account assay performance, the risk profile of the tested population, and the anatomical site of the sample.

2. Lack of a gold standard for NG detection by NAAT:

   There is no universally accepted gold standard NG detection by NAAT. This makes it difficult to resolve discordant results between the screening and supplemental assays. Discrepancies may arise due to cross-reactivity in the screening assay (leading to false positives) or reduced sensitivity in the supplemental assay (leading to false negatives).

Based on the 2024 NNN review:

1. Supplemental testing for NG-NAAT is recommended for non-urogenital samples.
2. The need for supplementary NG-NAAT for urogenital samples is at the discretion of individual laboratories. Decisions should be supported by local validation data demonstrating adequate performance (based on WHO recommendations for urogenital samples). Additional testing should continue to be considered when testing low-risk populations.

Further review of the guidelines is scheduled for 2028.

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- Tiffany Hogan (New South Wales)
- Sebastiaan van Hal (New South Wales).

Author details

David M Whiley,[1,2]

Sebastiaan van Hal,[3,4]

Todd M Pryce,[5]

Monica M Lahra,[6,7]

on behalf of the National Neisseria Network and the Public Health Laboratory Network.

1. UQ Centre for Clinical Research, Faculty of Medicine, The University of Queensland, Brisbane, Queensland, Australia
2. Pathology Queensland, Queensland Health, Brisbane, Queensland, Australia
3. New South Wales Health Pathology, Microbiology, Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia
4. School of Medicine, University of Sydney, Sydney, New South Wales, Australia
5. PathWest Laboratory Medicine WA, Department of Clinical Microbiology, Fiona Stanley Hospital, Murdoch, Western Australia, Australia
6. World Health Organization Collaborating Centre for STI and AMR, New South Wales Health Pathology Microbiology, The Prince of Wales Hospital, Randwick, New South Wales, Australia
7. Faculty of Medicine, The University of New South Wales, Sydney, New South Wales, Australia

Corresponding author

David M Whiley

UQ Centre for Clinical Research, Faculty of Medicine, The University of Queensland, Brisbane, Queensland, Australia

Email: d.whiley@uq.edu.au

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GPO Box 9848, Canberra ACT 2601

Website: **cdc.gov.au/cdi**

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