

Short report

REVIEW OF 2005 PUBLIC HEALTH LABORATORY NETWORK *NEISSERIA GONORRHOEAE* NUCLEIC ACID AMPLIFICATION TESTS GUIDELINES

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Abstract

At the request of the Public Health Laboratory Network (PHLN), the National Neisseria Network (NNN) met to discuss the 2009 PHLN *Neisseria gonorrhoeae* nucleic acid amplification test (NAAT) guidelines and the need for supplementary testing. A central point of discussion at this NNN meeting, which took place in May 2013, was the potential for *N. gonorrhoeae* supplementary testing to lead to false-negative results. Data were presented at the meeting that questioned the sensitivity of commonly used in-house supplementary methods as compared with later generation commercial NAAT systems. It was the opinion of the NNN that supplementary testing remains best practice, but that caution should be used when reporting negative results. The NNN recommends that urogenital samples providing a positive result in a screening method and a negative result by a supplemental method should not be reported as negative for *N. gonorrhoeae* without an appropriate explanatory comment indicating that gonococcal infection cannot be excluded. *Commun Dis Intell* 2015;39(1):E42–E45.

Keywords: gonorrhoea, nucleic acid amplification tests, NAAT, supplementary

Nucleic Acid Amplification Tests (NAATs) for diagnosis of gonococcal infection are increasingly utilised in laboratories but their use has been complicated by specificity problems since their introduction. This is mostly due to frequent exchange of genetic material between commensal *Neisseria* species and *Neisseria gonorrhoeae*^{1–3} and cross-reactivity with commensal *Neisseria* strains has been observed with most *N. gonorrhoeae* NAAT methods.³ Given the potential medical, legal, social or psychological implications that may arise from an incorrect gonorrhoea diagnosis, laboratories have a duty to avoid issuing false positive results. This has prompted the development of the Australian Public Health Laboratory Network's (PHLN) *Guidelines for the use and interpretation of nucleic acid detection tests for Neisseria gonorrhoeae in Australia* in 2005.⁴ Briefly, the 2005 Guidelines

state that all *N. gonorrhoeae* NAAT positive results should also test positive on a reliable supplemental assay before a positive result is reported. In response, most clinical laboratories in Australia implemented supplemental NAAT methods (typically in-house polymerase chain reaction (PCR) methodology) for this purpose.

Whilst the implementation of supplemental testing has largely resolved the specificity problems associated with *N. gonorrhoeae* NAAT-based testing, new concerns have been raised about the overall sensitivity of the NAAT algorithm (i.e. false-negative results). At the National Neisseria Network (NNN) annual meeting in Canberra on 30–31 May 2013, three key data sets were presented as follows:

1. Data showing that sequence target variability may impact upon the sensitivity of in-house real-time polymerase chain reaction methods

In recent years there have been several reports of genetic mutations in gonococcal strains that have led to false-negative results in some in-house real-time PCR methods. Such problems have been observed in Australia for assays targeting the *porA* pseudogene, the *opa* genes and *cppB* gene.^{5–7} Data kindly provided by the Royal College of Pathologists Quality Assurance Programs (RCPAQAP) indicate that *porA* and *opa*-based PCR methods are widely used by Australian laboratories for supplementary testing, and so there is potential for *porA* or *opa* variant strains to cause false-negative results in testing algorithms. However, a recent nationwide analysis of Australian gonococci (n = 2,455 isolates) conducted by the NNN showed that the prevalence of *porA*, *opa* and *cppB* variant strains is low (0.12%, 0.04% and 1.14 % of gonococci respectively) and not widespread throughout Australia at this point in time.⁷ Hence, the overall impact of such variants may in fact be minimal. Ongoing monitoring of strains for genetic variation in sequences targeted by NAAT assays is critical.

2. Data showing that supplementary testing may lead to false-negative results for 'low load' samples

Data kindly provided by the RCPAQAP indicate that it is not uncommon for laboratories to correctly detect *N. gonorrhoeae* nucleic acid in a quality assurance program sample by a screening NAAT method, but then fail to detect *N. gonorrhoeae* in the same sample by a supplementary NAAT method. Such discrepancies are typically observed for samples that provide the highest cycle threshold (Ct) values in the screening methods (where such data are available), suggesting that the issue relates to low DNA loads. It should be noted that RCPAQAP does occasionally deliberately select gonococcal strains that are known to lack certain sequence targets (e.g. *porA* pseudogene variants) to use in their panels, and that this does explain some of the RCPAQAP discrepancies. However, for this point we are primarily concerned with RCPAQAP samples that are known to contain gonococcal nucleic acids, for which there are no known sequence target issues, yet provide positive results in screening methods and negative results in supplementary methods. For example, for one 2013 sample there were 11 laboratories that obtained a positive result by a commercial screening method and that also separately reported the indi-

vidual results for their supplementary methods; of these, 6 laboratories obtained negative results in the supplementary tests.

Unpublished data from NNN laboratories also show that up to 5% of urogenital samples and 20% of pharyngeal samples positive by a later generation NAAT, are not detected by a supplementary assay. Examples of these data are provided in Tables 1 and 2; samples are from The Canberra Hospital (n = 369) and The Prince of Wales Hospital (n = 1,174) where in-house real-time PCR (targeting the gonococcal *opa* genes and/or *porA* pseudogene) were used to confirm samples testing positive by the Roche 4800 NG PCR Assay. Further data were shown indicating that the majority of samples that are 'screen positive/supplementary negative' typically provide the highest Ct values in the screening methods, again suggesting low DNA loads are involved. For example, a subset of samples (n = 427) from The Prince of Wales Hospital sample set showed that 'screen positive/supplementary negative' samples (n = 98) provided an average Ct value of 38.3 cycles by the Roche 4800 NG PCR, whereas those that were positive by supplementary PCR (n = 329 samples) provided an average Ct value of 32.2 cycles.

Table 1: Results of supplementary testing of samples providing positive results by the Roche 4800 NG PCR Assay, The Canberra Hospital, 2011 to 2013

Sample site	Roche 4800 <i>Neisseria gonorrhoeae</i> positive	Supplementary PCR (<i>porA</i> pseudogene)		
		Positive	Negative	Confirmation rate (%)
Urogenital	152	146	6	96.1
Rectal	106	95	11	89.6
Throat	110	88	22	80.0
Eye	1	1	0	100.0

PCR Polymerase chain reaction

Table 2: Results of supplementary testing of samples providing positive results by the Roche 4800 NG PCR Assay, The Prince of Wales Hospital, 2011 to 2013

Sample site	Roche 4800 <i>Neisseria gonorrhoeae</i> positive	Supplementary PCR (<i>opa</i> genes and <i>porA</i> pseudogene)		
		Positive	Negative	Confirmation rate (%)
Urogenital	245	234	11	95.5
Rectal	601	562	39	93.5
Throat	325	276	49	84.9
Eye	3	2	1	66.6

PCR Polymerase chain reaction

3. Data showing later generation commercial *Neisseria gonorrhoeae* nucleic acid amplification tests methods are more specific

Published data by Tabrizi et al.⁸ and others indicate that later generation *N. gonorrhoeae* NAAT methods have substantially less cross-reactivity with non-gonococcal *Neisseria* species compared with earlier generation methods. It was however noted that cross-reactions were still possible, as evidenced by recent studies.^{9,10}

The above data are highly suggestive that true gonococcal infections (particularly those with low bacterial load) are providing positive results in a screening method but negative results upon supplemental testing. Whilst sampling issues at low load leading to 'hit and miss' results are a well-recognised limitation of NAAT technology, this is not of primary concern. The key issue here is how such results are being interpreted and reported. The NNN discussions revealed that different laboratories handle such results in different ways; including:

- a.) issuing the results as negative i.e. *gonococcal infection not detected*;
- b.) issuing the results as *equivocal* or *indeterminate*;
- c.) reporting both the screening and supplementary results; or
- d.) a, b or c but with a comment discussing the discrepancy.

At the Canberra NNN meeting there was considerable discussion over how these issues should be addressed. These discussions included debate over whether the 2005 *N. gonorrhoeae* NAAT PHLN guidelines remain relevant, particularly in light of recent improvements in the specificity of the commercial systems. It was also highlighted that the Australian guidelines are amongst the most stringent in the world and that other regions (e.g. *United Kingdom National Guideline for Gonorrhoea Testing 2012*¹¹), only recommend the use of supplementary testing for extra-genital samples (frequented by commensal *Neisseria* species, being the key source of *N. gonorrhoeae* NAAT cross-reaction) and not urogenital samples. The consensus opinion of the NNN was that (1) the PHLN guidelines remain best practice for *N. gonorrhoeae* NAAT testing, and that (2) the requirement for supplementary testing should not be relaxed, even for urogenital samples.

It was also the opinion of the NNN that gonococcal infection cannot be excluded for urogenital samples that provide positive results in a later generation *N. gonorrhoeae* NAAT method, but negative results upon supplementary testing. In

the light of escalating rates of gonorrhoea infection in Australia and elsewhere, combined with concerns over emerging antimicrobial resistance, it is the opinion of the NNN that laboratories should err on the side of caution when issuing such results for urogenital samples. In such instances a laboratory should not issue a negative result in the absence of an appropriate explanatory comment. While the precise wording of the comment may be determined by the respective laboratory, at a minimum the comment should indicate that *N. gonorrhoeae* infection cannot be excluded and that re-collection should be considered where warranted. At the NNN meeting there was no consensus as to whether discrepant results should be issued as 'negative' or 'indeterminate'; however the US Centers for Disease Control and Prevention¹² suggests that an interpretation of 'inconclusive', 'equivocal', or 'indeterminate' would be most appropriate. Again, this may depend on local requirements.

Furthermore, the above also highlights that some laboratories may need to change their supplementary NAAT methods so as to improve assay performance. A review of recent results in *N. gonorrhoeae* quality assurance panels would help ascertain if individual laboratories have a potential problem with assay performance.

In summary, the NNN advocates ongoing adherence to the guidelines laid out in the 2005 PHLN *N. gonorrhoeae* NAAT document; however it recommends that appropriate explanatory comments are provided with results for urogenital samples so as to negate any potential negative impacts that may arise through the use of supplementary testing.

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