

***Neisseria gonorrhoeae* NAA Testing – new Australian recommendations**

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GC NAA testing

Advantages

- Improved sensitivity compared to bacterial culture
- Suitable for screening
- Do not require viable organism (less stringent transport conditions)
- Can be used on non-invasive specimens (urine and self-collected specimens)
- Simultaneous detection of CT / GC

Limitations

Non-sequence-related:

- NAAT inhibition
 - (i) Inhibitory substances
 - (ii) Competitive inhibition
- Cost
- Laborious
- Quality control
- Antibiotic resistance data

Sequence-related:

- False-positive results
- False-negative results

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Sequence-related:

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GC NAA: False-positive results

Commercial nucleic acid amplification tests:

Roche Cobas Amplicor assay –

Targets: cytosine DNA methyltransferase gene

Cross-reactions: *N.cinerea*, *N.flavescens*, *N.lactamica*,
N.Sicca & *N.subflava*

(Farrell J Clin Microbiol 1999;37:386-390 & Palmer et al. J Clin Microbiol 2003;41:835-837)

Becton Dickinson BDProbeTec –

Targets: pilin gene

Cross-reactions: *N.cinerea*, *N.flavescens*, *N.lactamica*,
& *N.subflava*

(Palmer et al. J Clin Microbiol 2003;41:835-837)

GC NAA: False-positive results

In-house PCR tests:

***cppB* PCR –**

Cross-reactions: *N. cinerea, N. meningitidis, N. subflava*

***gyrA* PCR -**

Cross-reactions: *N. sicca, N. mucosa, N. subflava*

***altA* PCR -**

Cross-reactions: *N. subflava*

(Palmer et al. J Clin Microbiol 2003;41:835-837; Farrell J Clin Microbiol. 1999;37:386-90 & Whiley and Sloots, unpublished data)

GC NAA: False-positive results

<i>GC NAAT</i>	<i>PPV range</i>
Roche Amplicor	31.3 - 100%
ProbeTec SDA	54.8 - 100%
Abbott LCx	59.3 - 100%
Gen-probe APTIMA	88.1 – 97.4%

GC NAA: False-negative results

GC subtypes and sequence variation

- multiple subtypes
- only a few may be circulating in any one population at any one time
- subtypes not randomly distributed - associated with particular patient groups *(Tapsall ASM 2004. Abstract no. 0129)*

GC NAA: False-negative results

Eg. cppB gene based GC PCR

- Popular supplementary test for Roche Amplicor
Farrell J Clin Microbiol. 1999 Feb;37(2):386-90.
Whiley et al. Diagn Microbiol Infect Dis. 2002 Feb;42(2):85-9.
Tabrizi et al. Sex Transm Infect. 2004 Feb;80(1):68-71.
- Some *N.gonorrhoeae* strains lack the *cppB* gene
- major problem in NT
(Tapsall et al. 15th ISSTDR Congress. Abstract no. 0129 &
Palmer et al. J Clin Microbiol 2003;41:835-837)
- Multi-centre evaluation - The Netherlands
"...we consider the cppB gene to be an unsuitable target."
(Bruisten et al. J Clin Microbiol. 2004; 42:4332-4.)

Public Health Laboratory Network (PHLN) workshop – Melbourne, 23 March 2005

- Australian experts in gonococcal NAA
- *“identify laboratory issues of relevance and suggest guidelines for use of nucleic acid detection tests (NADT) for diagnosis of gonorrhoea in Australia”*
- **made 11 recommendations**
- Extended upon CDC 2002 guidelines
- proceedings of the meeting were endorsed by PHLN and Communicable Diseases Network of Australia.

Smith DW, Tapsall JW, Lum G.

Guidelines for the use and interpretation of nucleic acid detection tests for Neisseria gonorrhoeae in Australia: a position paper on behalf of the Public Health Laboratory Network.

Commun Dis Intell. 2005;29(4):358-65.

Recommendation 1: *cppB* target not to be used

1. *“Assays using detection of the *cppB* gene should not be used for either screening or supplemental assays.”*

Recommendations 2 - 4: supplementary testing to be used

2. *“All in-house screening assays that are positive should also be positive on a reliable supplemental assay before a positive result is reported.”*
3. *“All commercial screening assays that are positive should also be positive on a reliable supplemental assay before a positive result is reported.”*
4. *“If a sample is positive in a screening assay but a suitable supplemental assay is negative, then the result should be reported as negative.”*

Recommendations 5 & 6: PPV & validation

5. *“Laboratories should ensure that the test combination they use would yield a **positive predictive value** of at least 90 per cent in a population with a prevalence of 1 per cent.”*

6. *“For the purposes of test evaluation, as distinct from diagnostic testing, **true positives** be defined by meeting one or more of the following criteria:*
 - 1) ***culture positive** using contemporary isolation and identification techniques;*

 - 2) *positive result on NADTs directed to targets on **three separate genes** that are known to have discriminatory capacity;*

 - 3) ***sequencing** of a gene known to separate gonococcal from non-gonococcal species.”*

Recommendation 7: NAA inhibition control

7. "Inhibitor controls should be routinely included in all NADT."

Recommendation 8: extra-genital sites

8. *“Cultures are the preferred test for samples from non-genital sites. If however it is necessary to perform a NADT, then more stringent criteria should be applied, and positive samples should meet the ‘test evaluation’ criteria for a ‘true positive’.”*

Recommendation 9: Quality assurance

9. *"In order to properly assess the routine diagnostic system in Australia, the following quality assurance samples should be distributed in addition to the routine samples currently used:*

1. *cppB negative N. gonorrhoeae;*

2. *Non-gonococcal species known to cause false positive reactions: these should be dispatched both as a single species, as well as mixture with N. gonorrhoeae. In the latter circumstance, the non-gonococcal species should be present in 10-fold excess;*

3. *Urine samples: preferably a single patient sample, otherwise a spiked sample.*

4. *Validation panels should be made that include samples that are culture positive but PCR negative. True positive samples should also be made available.*

Recommendations 10 & 11: Antimicrobial resistance

10. *“Strategies should be put in place to ensure that sufficient numbers of gonococcal isolates are obtained to allow reliable monitoring of antimicrobial resistance.”*
11. *“Public health practitioners need to define the relevant populations that need to be targeted and identify any that require enhanced surveillance.”*

Public Health Laboratory Network (PHLN) workshop – Melbourne, 23 March 2005

List of attendees:

David Smith, Perth: Public Health Laboratory Network; PathWest Laboratory Medicine WA

John Tapsall, Sydney: National Neisseria Network; Public Health Laboratory Network

Gary Lum, Darwin: National Neisseria Network; Public Health Laboratory Network

Sue Alderson, Sydney: Institute of Clinical Pathology and Medical Research

Miles Beaman, Perth: Western Diagnostic Pathology

Sue Garland, Melbourne: Women's and Children's Hospital

Rod Givney, Adelaide: Communicable Diseases Network Australia

Gerry Harnett, Perth: PathWest Laboratory Medicine WA

Geoff Higgins, Adelaide: IMVS, Public Health Laboratory Network

Geoff Hogg, Melbourne: Microbiological Diagnostic Unit, Public Health Laboratory Network

David Leslie, Melbourne: Victorian Infectious Diseases Reference Laboratory

Theo Sloots, Brisbane: Sir Albert Sakzewski Virus Research Centre

Sepehr Tabrizi, Melbourne: Women's and Children's Hospital

David Whiley, Brisbane: Sir Albert Sakzewski Virus Research Centre

**A real-time PCR assay targeting the
N. gonorrhoeae porA pseudogene**

GC porA pseudogene PCR

Why?

- Replace *cppB*-based PCR assay
- Wanted a more specific assay
 - extragenital specimens etc.
 - Sensitivity
 - uniform testing

GC porA pseudogene PCR

Development:

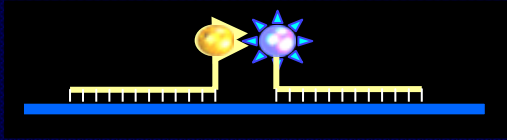
- *porA* gene PCR used successfully *Neisseria meningitidis*
 - good specificity
- *porA* sequence not identified in non-pathogenic *Neisseria* spp.
- Benefits of being a pseudogene?? – minimal variation

However -

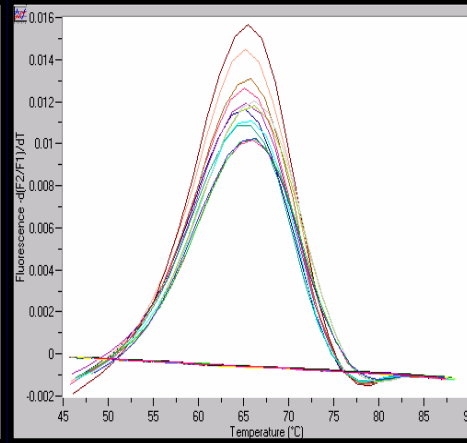
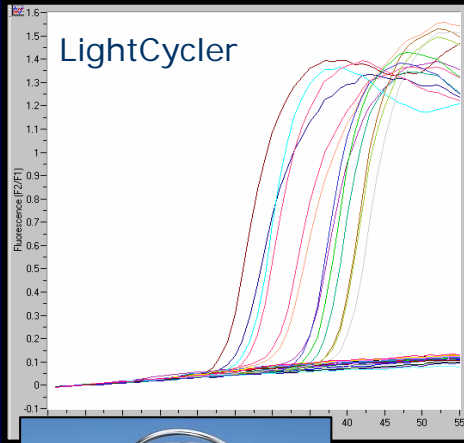
High homology with *N. meningitidis* *porA* gene

- found *gono* specific targets.

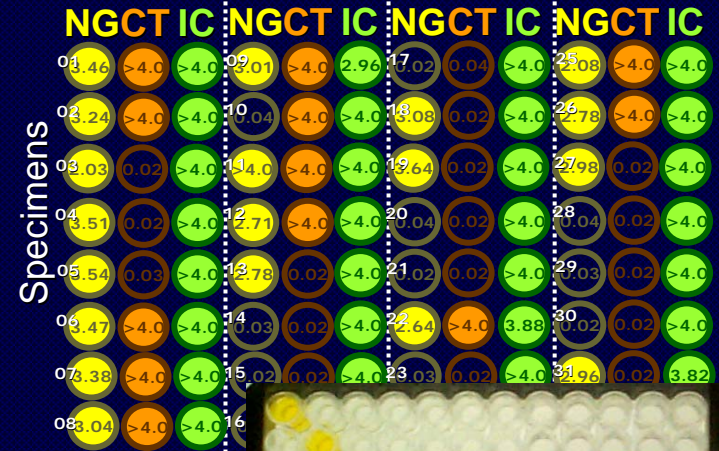
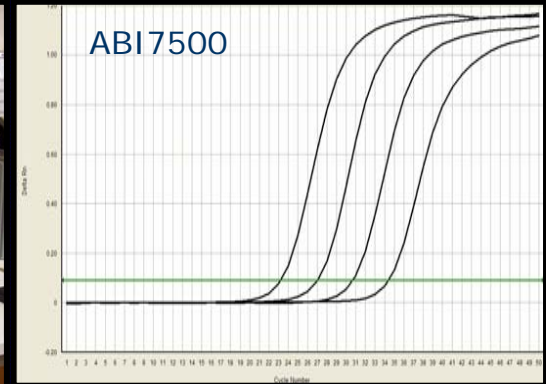
porA-PCR platforms:



Hyb-probe
(LightCycler)



TaqMan
(ABI)



Conventional
PCR
(ELAHA)



GC porA pseudogene PCR

Evaluations:

1. Bacterial panel
2. porA PCR vs Cobas/cppB-LC algorithm
3. porA PCR vs bacterial culture
3. Multi-centre

1. Bacterial panel:
Common pathogens & normal flora (86 isolates)
- *all provided negative results*

<i>Acinetobacter aranitratus</i>	<i>Bacillus subtilis</i>	<i>Flavobacterium multivoram</i>	<i>Staphylococcus epidermidis</i>
<i>Acinetobacter baumannii</i>	<i>Bacillus thuringiensis</i>	<i>Haemophilis influenzae</i>	<i>Staphylococcus haemolyticus</i>
<i>Acinetobacter haemolyticus</i>	<i>Bacteroides distasonis</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus hominus</i>
<i>Acinetobacter johnsonii</i>	<i>Bacteroides gingivalis</i>	<i>Listeria monocytogenes</i>	<i>Staphylococcus intermedius</i>
<i>Acinetobacter junii</i>	<i>Bacteroides vulgatus</i>	<i>Micrococcus luteus</i>	<i>Staphylococcus lugdenensis</i>
<i>Acinetobacter lwoffii</i>	<i>Bordetella bronchiseptica</i>	<i>Proteus mirabilis</i>	<i>Staphylococcus scuri</i>
<i>Aeromonas hydrophilia</i>	<i>Bordetella parapertussis</i>	<i>Proteus vulgaris</i>	<i>Staphylococcus warneri</i>
<i>Alcaligenes faecalis</i>	<i>Burkholduria cepacia</i>	<i>Providencia stuartii</i>	<i>Staphylococcus xylosus</i>
<i>Bacillus amyloliquifaciens</i>	<i>Campylobacter jejuni</i>	<i>Pseudomonas aeruginosa</i>	<i>Stenotrophomonas maltophilia</i>
<i>Bacillus brevis</i>	<i>Candida albicans</i>	<i>Pseudomonas vesicularis</i>	<i>Streptococcus agalactiae</i>
<i>Bacillus cereus</i>	<i>Candida krusei</i>	<i>Saccharomyces cerevisiae</i>	<i>Streptococcus bovis</i>
<i>Bacillus circulans</i>	<i>Candida tropicalis</i>	<i>Salmonella typhimurium</i>	<i>Streptococcus equi</i>
<i>Bacillus coagulans</i>	<i>Citrobacter freundii</i>	<i>Serratia marcescens</i>	<i>Streptococcus equisimilis</i>
<i>Bacillus firmus</i>	<i>Corynebacterium diphtheriae</i>	<i>Serratia oderifera</i>	<i>Streptococcus (group B)</i>
<i>Bacillus laterosporus</i>	<i>Enterobacter aerogenes</i>	<i>Shigella flexneri</i>	<i>Streptococcus (group F)</i>
<i>Bacillus licheniformis</i>	<i>Enterobacter cloacae</i>	<i>Shigella sonnei</i>	<i>Streptococcus (group G)</i>
<i>Bacillus macerans</i>	<i>Enterococcus durans</i>	<i>Staphylococcus simulans</i>	<i>Streptococcus mutans</i>
<i>Bacillus megaterium</i>	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>
<i>Bacillus mycoides</i>	<i>Enterococcus faecium</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>
<i>Bacillus polymyxa</i>	<i>Erysipelothrix rhusiopathiae</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus salivarius</i>
<i>Bacillus pumulis</i>	<i>Esherichia coli</i>	<i>Staphylococcus capitis</i>	<i>Vibrio alginolyticus</i>
<i>Bacillus sphaericus</i>	<i>Flavobacterium indologenes</i>	<i>Staphylococcus cohnii</i>	<i>Vibrio parahaemolyticus</i>
			<i>Yarrowia lipolytica</i>
			<i>Yersinia enterocolitica</i>

1. Bacterial panel:

Neisseria species - only gonos provided positive results

Species	Number tested	Number positive porA-PCR
<i>N. gonorrhoeae</i>	231	231
<i>M. catarrhalis</i>	9	0
<i>N. cinerea</i>	4	0
<i>N. elongata</i>	3	0
<i>N. flavescens</i>	1	0
<i>N. lactamica</i>	8	0
<i>N. meningitidis</i>	42	0
<i>N. mucosa</i>	4	0
<i>N. sicca</i>	8	0
<i>N. subflava</i>	23	0
Total	333	231

2. porA-PCR vs Cobas/cppB-LC algorithm:

Specimen	Number tested	<i>Number of positive specimens:</i>		Discrepant results
		porA-PCR	Cobas / cppB-LC	
Urine	224	59	59	2*
Genital swabs	58	21	21	0
Total	282	80	80	2*

* one specimen was positive by cppB-LC only whereas another specimen was positive by NGpAp-PCR only.

(Whiley et al. Eur J Clin Microbiol Infect Dis. 2004 Sep; 23(9): 705-10.)

3. porA-PCR vs Bacterial culture:

Specimen site	Number tested	<i>Number of positive specimens:</i>		Discrepant results
		porA-PCR	Bacterial culture	
Cervix	216	1	1	0
Urethra	185	9	9	0
Throat	196	5	2	3*
Rectum	39	8	7	1*
Total	636	23	19	4*

* NGpAp-PCR results confirmed by three additional PCR assays.

(Whiley et al. Diagn Microbiol Infect Dis. 2005 May; 52(1): 1-5.)

4. Multi-centre:

- Adelaide
- Darwin
- Perth
- Sydney
- New Caledonia
- New Zealand

Question:

- Are the *porA* pseudogene PCR targets conserved across a diverse range of *N. gonorrhoeae* isolates?
- *Unemo et al.* - consideration should be given to the risk of deletion of the *porA* pseudogene from the gonococcal genome.
(*Unemo et al. APMIS 2005; 113:410-419.*)

4. Multi-centre:

- 240 gonococcal isolates from Australia (various states), China, Indonesia, Japan, Korea, Mongolia, New Caledonia and New Zealand
- All detected by porA PCR assay.

Conclusions

porA-PCR assay

- Data suggests target sequences are conserved and specific to *N. gonorrhoeae*
- **However –**
 - we emphasize the need for assay validation; &
 - highly recommend the use of supplementary testing

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