Confirmation of problematic *Neisseria gonorrhoeae* in a reference laboratory using conventional and commercial identification systems

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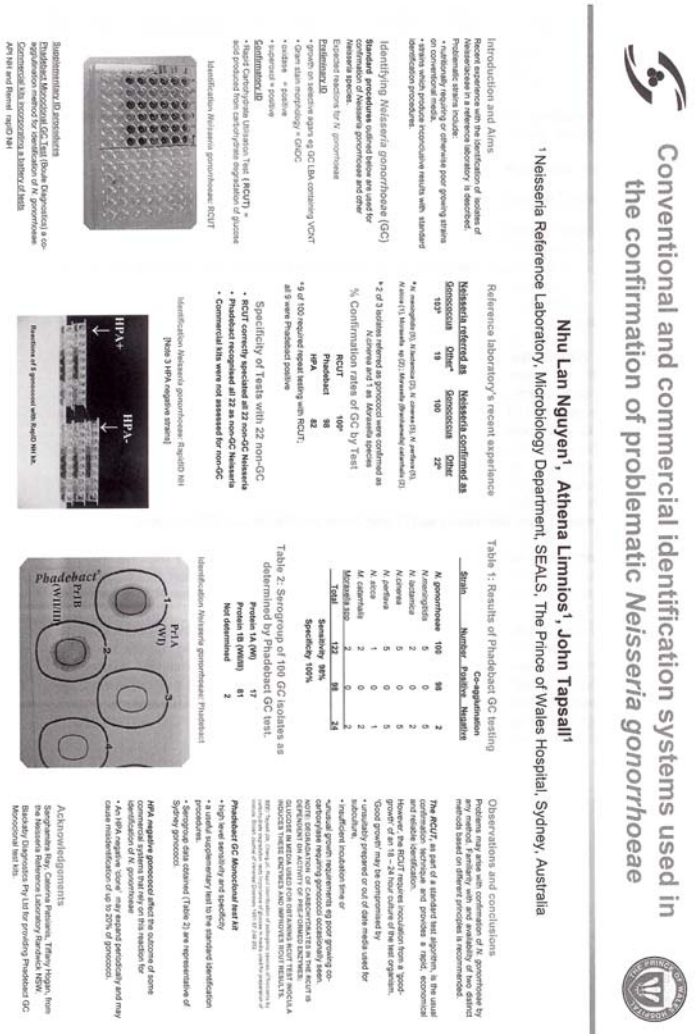
Abstract: Recent experience with the identification of 122 isolates of *Neisseriaceae* in a reference laboratory is described. Organisms examined were 63 sequential strains referred as *N. gonorrhoeae* (GC), 18 strains of GC which failed to produce hydroxy-proline amino-peptidase (HPA) in API NH and Remel tests systems, 22 randomly selected stored GC and 19 non–gonococcal Neisseria.

60 of 63 isolates referred as GC were confirmed by the standard procedures of growth on selective agars, Gram stain morphology, positive oxidase and superoxol reactions and acid production from glucose in a rapid carbohydrate utilization test (RCUT); two were *N. cinerea* and one a *Moraxella* sp. Nine of the 60 GC were initially glucose negative on RCUT but all were Phadebact GC positive. Repeat RCUT testing of these 9 isolates using fresh or enriched media was positive. All 18 HPA negative and 22 selected GC were RCUT and Phadebact GC positive. 19 other *Neisseriaceae* were identified as *N. meningitidis* (5), *N. lactamica* (2), *N. cinerea* (5), *N. perflava* (5), *N. sicca* (1), *Moraxella catarrhalis* (2) or *Moraxella* sp (2) and were all Phadebact GC negative.

Problems may arise with confirmation of *N. gonorrhoeae* by any method. RCUT, as part of a standard test algorithm, is the usual confirmation technique and provides a rapid, economical and reliable identification. However RCUT requires inoculation with material from well formed colonies from an 18 – 24h culture, and out-of-date media, insufficient incubation time or unusual growth requirements may compromise this growth. In this series, poor-growing co-carboxylase requiring GC were occasionally seen. A cluster of HPA negative GC expanded and represented about 20% of GC for a period. These were not confirmed by commercial tests relying on this reaction. The Phadebact GC was a useful supplementary test for all isolates, showing 98% sensitivity for GC and no cross reactions with other *Neisseria*.



Conventional and commercial identification systems used in the confirmation of problematic *Neisseria gonorrhoeae*

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Introduction and Aims

Recent experience with the identification of isolates of *Neisseriaceae* in a reference laboratory is described. Problems encountered with:

- nutritionally requiring or otherwise poor growing strains on conventional media,
- strains which produce inconclusive results with standard identification procedures.

Identifying *Neisseria gonorrhoeae* (GC)

Rapid Confirmation of Isolates cultured from suspected *Neisseria gonorrhoeae* and other *Neisseria* species.

Expected reactions for *N. gonorrhoeae*

Preliminary ID

- Gram stain morphology + GNDC
- oxidase + positive
- superoxol + positive

Confirmation ID

- Rapid Carbohydrate Utilization Test (RCUT) + acid produced from carbohydrates degradation of glucose

Identification *Neisseria gonorrhoeae*: RCUT

Supplementary ID procedures

Phadebact Monoclonal GC Test (Boule Diagnostics) a co-agglutination test for identification of *N. gonorrhoeae*.

Gonochek-II (Biotec Laboratories Ltd) a latex test for API NH and Remel - rapID NH.

Reference laboratory's recent experience

Neisseria referred as	Neisseria confirmed as	
Gonococcus	Gonococcus	Other
102*	100	22*
19		

*ie: meningitidis (5), N.lactamica (2), N. cinerea (5), N. perflava (5), N. sicca (1), Moraxella (Branhamella) catarrhalis (2).

- 2 of 3 isolates referred as gonococci were confirmed as *N. cinerea* and 1 as *Moraxella* species.

% Confirmation rates of GC by Test

	RCUT	100*
	Phadebact	98
	HPA	82

*9 of 100 required repeat testing with RCUT; all 9 were Phadebact positive

Specificity of Tests with 22 non-GC

- **RCUT correctly identified all 22 as non-GC Neisseria**
- **Phadebact correctly identified all 22 as non-GC Neisseria**
- **Commercial kits were not assessed for non-GC**

Identification *Neisseria gonorrhoeae*: RapID NH [Note 3 HPA negative strains]

Reactions of 6 gonococci with RapID NH kit.

Table 1: Results of Phadebact GC testing

Strain	Number	Co-agglutination Positive	Negative
N. gonorrhoeae	100	98	2
N.meningitidis	5	0	5
N. lactamica	2	0	2
N. cinerea	5	0	5
N. perflava	5	0	5
N. sicca	1	0	1
M. catarrhalis	2	0	2
Moraxella spp.	2	0	2
Total	122	98	24
Sensitivity 98%			
Specificity 100%			

Table 2: Serogroup of 100 GC isolates as determined by Phadebact GC test.

Protein 1A (W)	17
Protein 1B (W/III)	81
Not determined	2

Identification *Neisseria gonorrhoeae*: Phadebact

Observations and conclusions

Problems may arise with confirmation of *N. gonorrhoeae* by any method. Repeat testing of isolates using different methods based on different principles is recommended.

The RCUT, as part of a standard test algorithm, is the usual confirmation technique and provides a rapid, economical and reliable identification.

However, the RCUT requires inoculation from a 'good growth' of an 18 – 24 hour culture of the test organism. 'Good growth' may be compromised by

- unsuitably prepared or out of date media used for subculture,
- insufficient incubation time or
- poor growing co-carboxylase requiring gonococci occasionally seen.

NOTE: DEGRADATION OF CARBOHYDRATES IN THE RCUT IS ACHIEVED BY ENZYMES PRESENT IN GC. ENZYMES ARE ALSO PRESENT IN MOST OTHER NEISSERIA BUT THESE ENZYMES ARE NOT USUALLY INDUCED UNDER THESE CONDITIONS AND IMPROVES RCUT RESULTS.

Phadebact GC Monoclonal test kit

- high level sensitivity and specificity
- a useful supplementary test to the standard identification procedures.
- Serogroup data obtained (Table 2) are representative of Sydney gonococci.

HPA negative gonococci affect the outcome of some commercial systems that rely on this reaction for identification of *N. gonorrhoeae*.

- Such isolates are likely to be encountered periodically and may cause misidentification of up to 20% of gonococci.

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