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August 2006

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Neisseria spp identification kits: A review of evaluation literature

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Background and Scope of Review

This review has been undertaken to assess current knowledge on identification kits for *Neisseria* species other than *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Diagnosis of the two main *Neisseria* pathogens is well established and therefore the focus of this review is to analyse evaluation results for kits used to identify other opportunistic *Neisseria spp*. The biochemical differences between *Neisseria spp* are small and therefore the identification of this pathogen can be problematic. For accurate diagnosis it is important to know the sensitivity and specificity of available kits.

There are nine *Neisseria spp* associated with human infections, *N. gonorrhoeae*, *N. meningitidis*, *N. lactamaica*, *N. sicca*, *N. subflava*, *N. canis*, *N. cinerea*, *N. elongata subspecies nitroreducens*, and *N. mucosa*. Of these *N. gonorrhoea* and *N. meningitidis* are the species most commonly associated with human infection. *N. gonorrhoeae* is one of the most commonly occurring sexually transmitted pathogens. *N. meningitidis* is the causative agent of meningitis and its isolation is normally indicative of that condition. The other seven *Neisseria spp* have not been studied to the same extent, having low virulence and only causing infection opportunistically or in patients who are immunocompromised.

Neisseria species are Gram-negative cocci, 0.6-1.0 µm in diameter, occurring singly but more frequently in pairs with adjacent sides flattened. The current 'gold standard' for the diagnosis of *Neisseria* species is culture from a selective plate followed by further identification using carbohydrate metabolism, immunoassay or preformed enzyme substrate. GC agar base is the preferred selective media and is supplemented with lysed or chocolatised horse blood with or without the addition of VitoX or IsoVitaleX, all available from Oxoid. Antibiotic cocktails used for selection contain vancomycin or lincomycin, colistin, trimethoprim and nystatin or amphotericin. GC selective agar should be incubated for up to 48 hours in 5-10% CO_2 at 35-37°C. For maximum isolation rates culture plates should be incubated for 72 hours.

This review covers 12 peer-reviewed papers published between 1983 and 2005. It includes reference to evaluation of biochemical, immunological and enzymatic kits only and does not consider molecular methods. The papers were retrieved from PubMed using the keywords; *Neisseria*, kit, evaluation, identification.

Findings

On examining the papers it is clear that there is no agreed 'gold standard' for the identification of *Neisseria spp* other than for *N. gonorrhoeae* and *N meningitidis*. From the search it was apparent that most research has concentrated on the ability of the kits to accurately identify *N. gonorrhoeae* and *N. menigitidis* and that little attention has been given to the other pathogenic species within the family. It was also apparent that the closeness of the species makes accurate identification difficult. Insufficient analysis of *Neisseria spp* has been carried out and this makes it hard to design accurate identification kits. *Neisseria spp* other than *N. gonorrhoeae* and *N meningitides* have been considered of little clinical significance and it is only due to the increase in patients who are immunocompromised that the need to correctly identify them has become a matter of importance (1;2).

The fastidious nature of these organisms also poses problems (3). The only species that can be correctly identified with ease are *N. gonorrhoeae, N. meningitidis* and *N. lactamica*. *N. lactamica* is identified due to the presence of a ß galactosidase enzyme (4;5). *N. cinera* is the hardest to identify because it most closely resembles *N. gonorrhoea* and can be easily misidentified (1;6). *N. cinera* is normally identified by establishing that it is not *N. gonorrhoeae*. However an evaluation of API NH (BioMerieux) showed that with an appropriate level of care and additional tests it is possible to correctly identify this and other *Neisseria spp* (7). Little consideration has been given to factors such as identification of isolates from selective and non selective media. Most kits are designed to identify isolates grown from selective media (8) and as a pure culture (9). However this fails to take into consideration isolates grown on non-selective plates from unusual sites from which the other *Neisseria spp* are likely to be isolated. There is insufficient data in almost all the studies to calculate the specificities and sensitivities of the kits evaluated.

The most comprehensive evaluation carried out showed that API NH (BioMerieux) was the most accurate method of identification as long as additional confirmatory tests were carried out (7). In this study the author concluded that automatic methods of identification for *Neisseria spp* were not accurate. However the study was carried out in 1999 and the paper used to make this decision was published in 1992 (10), this being the most up-to-date review on the subject available. This paper therefore does not take into consideration advances in technology and new automatic means of identification such as the Phoenix machine (BD). An evaluation of the Phoenix machine for identifying *Neisseria spp* has yet to be carried out. An evaluation involving the Vitek NHI card stated that automated identification was useful for *Neisseria spp* that react slowly to chromogenic tests (11) but the authors agreed that the machine had difficulty identifying species that did not display a typical biochemical profile.

All the papers concluded that kits were an improvement on CTA sugar testing (1;4;9) when trying to identify these species, but were not accurate when

identifying *Neisseria spp* other than the main two. In all cases it was felt that additional confirmatory tests should be carried out, that features such as colony morphology have a role to play (7) and that all results should be treated with caution. In some studies the evaluators left the test colour to develop beyond the time frame defined in the kit instructions for use. This means that the validity of certain studies must be questioned (12). Research into the effectiveness of kits must be carried out according to manufacturers' instructions otherwise the findings are not valid for a clinical environment. It is known that some isolates are slower than others in producing colour (12). There are also a number of differences related to the quality of the isolate used and how the laboratory scientist manipulates the sample. Care is required to correctly homogenise the isolate and prepare the correct McFarland concentration (2;7;11). This can impact on the quality of the results obtained within the time frame specified for a test.

Conclusions

Our conclusions and recommendations are as follows:

- the *Neisseria spp* evaluations undertaken to date are small and include many variables
- most evaluations were undertaken in the early 1990s which does not reflect the current importance of these pathogens among the increasing number of patients that are immunocompromised
- most Neisseria evaluation studies concentrate on identification of *N. gonorrhoeae* and *N. meningitidis*. Other species of clinical significance have been incompletely assessed
- it is currently difficult to make performance comparisons for *Neisseria spp* kits
- a well designed evaluation of diagnostic *Neisseria spp* kits / systems needs to be carried out against an agreed test algorithm / 'gold standard'
- currently there is not a definitive test(s) or 'gold standard' recommended for the identification of *Neisseria* species
- it is possible that in time a molecular method may prove to be the definitive test but this has not yet been established
- several of the studies were carried out in the United States and are not necessarily transferable to the UK where strain types may be different. Some kits available in the United States are not available in the UK and Europe.

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Appendix

The following tables provide a summary of findings as described in the papers reviewed. They are shown in the order that they appear in the references.

Dillon *et al*, 1988

Isolates	Percentage of species correctly identified					
	CTA sugars	Gonobio-Test	Gonochek II	Minitek	Neisseria -Kwik	RIM-N
N. gonorrhoeae	97.1	100	98.1	100	100	99.0
N meningitides	100	93.8	95.8	100	100	100
N. lactamica	100	66.7	100.0	100	100	100
B. catarrhalis	100	100	66.7	100	100	100
N. sicca	100	100	0*	100	100	0
N. cinerea	100*	100*	0*	100*	100*	0*
N. subflava	100	40	0*	80*	100*	40
Other	100*	100*	0*	100*	100*	100*

Note: Isolates were identified on the basis of testing by CTA sugars (Difco)
Kits evaluated: Gonobio-Test (I.A.F Production Inc), Gonochek II (Du Point Co), Minitek (BBL Microbiology systems), Neisseria-Kwik test, (Micro Bio Logics), RIM-N (Austin Biological Laboratories)
* Manufacturers make no claims to identify these species.

Lairscey *et al*, 1985

Isolates	Number tested	Number correctly identified by RIM-N kit
N. gonorrhoeae	97	97
N meningitides	23	22
N. lactamica	20	19
N. sicca	19	17
N. subflava	22	19
N. mucosa	16	14
B. catarrhalis	21	21

Note: Isolates were identified on the basis of testing by CTA sugars (BBL Microbiology systems)
Kit evaluated: RIM-N kit (Austin Biological Laboratories)

Hughes *et al*, 1987

Isolates	Number tested	Number correctly identified		
		IDN 22°C	IDN 35°C	NHI
N. gonorrhoeae	118	106	118	118
N meningitides	18	17	17	18
N. lactamica	7	7	7	7
B. catarrhalis	14	14	14	14

Note: From the paper, it is not clear if the original identification of the isolates was based on using CTA sugars. However they were all identified using 4h Minitek system (BBL Microbiology Systems)
Kit evaluated: Identicult-Neisseria (IDN Scott Laboratories), Neisseria/Haemophilus Identification test kit, NHI (Vitek systems)

Janda *et al*, 2002

Isolates	Number tested	Number correctly identified by Bacticard
N. gonorrhoeae	254	254
N meningitides	125	125
N. lactamica	54	53
M. catarrhalis	125	123
N. subflava	9	0
Non-catarrhalis Moraxella spp	7	6
Kingella denitrificans	3	0
Kingella kingae	1	0

Note: Isolates were identified on the basis of testing by CTA sugars and QuadFerm+ (BioMerieux)
Kit evaluated: BactiCard Neisseria (Remel)

Barbe *et al* 1994

Isolates	Number tested	Number correctly identified by API NH
M. catarrhalis	22	22
N. gonorrhoeae	27	27
N meningitides	33	33
N. lactamica	5	5
N. cinerea	7	7*
N. subflava (various biovars)	25	25*
N. mucosa	8	8*
N. sicca	11	11*
N. polysaccharea	3	3*
H. parainfluenzae	28	24 (3)*
H. influenza	117	114 (3)*
H. aphrophilus	13	13*
H. paraphrophilus	6	6*

Note: From the paper, it is not clear if the original identification of the isolates was based on using CTA sugars. However Neisseria were all identified initially using Neisseria 4H system (Diagnostic Pasteur) and the Haemophilis using API 20E (bioMerieux) strips.
* Specimens identified correctly after extra tests with API NH
Kit evaluated: API NH (bioMerieux)

Philip *et al*, 1985

Isolates	Percentage of species correctly identified				
	API NeIdent	Gonochek II	Minitek	Phadebact GC	RapID NH
N. gonorrhoeae	82.1	100.0	74.3	82.1	100.0
N meningitides	54.5	95.4	81.8	0	90.9
N. lactamica	50.0	100.0	100.0	41.6	91.6
B. catarrhalis	94.1	100.0	88.2	0	11.8

Note: Isolates were identified on the basis of testing with CTA sugars
Kits evaluated: API NeIdent (Analytab Products), Gonochek II (E-Y laboratories), Minitek (BBL Microbiology systems), Phadebact GC (Pharmacia Diagnostics), RapID NH (Innovative Diagnositcs)

Robinson *et al*, 1983

Isolates	Number tested	Number correctly identified		
		RapID NH	CPPA sugars 24hr incubation	CPPA sugars 48hr incubation
N. gonorrhoeae	162	162	156	4
N meningitides	28	28	23	3
N. lactamica	5	5	5	0
N. sicca	7	4	0	7
N. subflava	10	8	0	10
N. mucosa	5	5	0	5
B. catarrhalis	23	23	0	23

Note: Isolates were identified on the basis of testing with CTA sugars
Kit evaluated: RapID NH system (Innovative diagnostics Systems); CPPA sugars (Prepared media laboratory)

Janda *et al*, 1987

Isolates	Number tested	Number correctly identified by Vitek ID Card
N. gonorrhoeae	110	109
N meningitides	68	63
N. lactamica	20	20
N. cinerea	10	10
N. subflava	11	1
N. mucosa	4	0
N. sicca	1	0
B. catarrhalis	20	0

Note: Isolates were identified on the basis of testing with CTA sugars
Kit evaluated: Vitek Neisseria-Haemophilus ID card (Vitek systems Inc)

Germer *et al*, 1985

Isolates	Number tested	Number correctly identified by RIM-N
N. gonorrhoeae	36	36
N meningitides	9	9
N. lactamica	4	4
N. mucosa	3	3
N. sicca / N. subflava	13	13
B. catarrhalis	22	22

Note: Isolates were identified on the basis of testing by CTA sugars
Kit evaluated: RIM-N kit (Austin Biological Laboratories); after an incubation period of one hour using the RIM-N kit (not according to manufacturer instructions) the results agreed with those obtained after 72 hours using conventional systems