ORIGINAL ARTICLE

Laboratory confirmation of meningococcal disease in Scotland, 1993–9

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Aims: To describe the laboratory confirmation of meningococcal disease, using culture and non-culture based techniques, between 1993 and 1999 as part of a national service in Scotland.
Methods: Samples from patients with suspected meningococcal disease in Scotland were analysed by culture and non-culture based techniques to gain a laboratory confirmation of disease. Data were analysed to establish the number of disease cases, the serogroups of the organisms involved, and the importance of the techniques used.
Results: Between 1993 and 1999, there was a total of 1749 notified cases of meningococcal disease in Scotland. Culture based methods provided a laboratory confirmation of 788 cases whereas non-culture techniques confirmed 461 cases.
Conclusions: Non-culture techniques were a useful addition to culture based techniques in Scotland and improved the dataset required for public health management, disease surveillance, and vaccine policy.

Meningococcal disease is an important cause of morbidity and mortality in the UK and elsewhere.[1] The incidence of disease has increased in many parts of Europe[2] and, in Scotland, much of this increase results from the emergence of serogroup C strains after the decline in group B disease in the early 1990s.[3 4] Many of the serogroup C strains that occur in Scotland belong to the ET-37 strain complex,[4 5] which is often associated with clusters or outbreaks,[6] although the incidence of serogroup C disease has decreased in individuals from those age groups that have received immunisation with serogroup C meningococcal conjugate (MenC) vaccine.[7 8] Since the introduction of the MenC vaccines, laboratory confirmation and serogroup determination of the infecting organism has become more important.[9–12]

Funding for the Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL) was made available from 1993 to provide a national service for specialist services relating to the laboratory confirmation of meningococcal and pneumococcal disease. The laboratory also supplies epidemiological data to the Scottish Centre for Infection and Environmental Health (SCIEH). Annual notifications of meningococcal disease are based on clinical notifications and laboratory confirmations. Both culture and non-culture based methods are used for the laboratory confirmation of meningococcal disease. Since 1995, the annual number of cases of meningococcal disease in Scotland has increased during a time of corresponding awareness of the disease among the medical profession and the public.[4 10–12] Although this has probably led to improved patient outcome, the laboratory confirmation of the disease by culture has become less straightforward, mainly because of the administration of antibiotics before hospital admission.[13] This has important implications because, traditionally, isolation of the infecting organism provides the most typing information. Non-culture based diagnosis has become correspondingly more important. Techniques including latex agglutination, co-agglutination, the polymerase chain reaction (PCR), and antibody detection have been used to allow non-culture confirmation of meningococcal disease.[14] These methods provide less strain type data on a case than culture confirmation, although recent improvements in technology and methodology may bring improvements in the future. Here, we describe the laboratory confirmation of meningococcal disease between 1993 and 1999 as part of a national service for the laboratory confirmation of meningococcal disease in Scotland.

METHODS

Patients and samples

Samples were taken from patients with suspected meningococcal disease in Scotland and sent by area diagnostic microbiology laboratories to the SMPRL in Glasgow. Organisms were sent for characterisation whereas body fluids were sent for agglutination, PCR, and antibody testing. All samples sent for meningococcal investigations were included for analysis in our study.

Meningococcal disease notification database

The notified meningococcal disease database is held in Scotland by the SMPRL. Information is gained through the strategic notification scheme between the SMPRL, Information and Statistics Division, Public Health Consultants, and SCIEH.[10] The database uses a system of classification for each notification depending on whether the case is a clinical or laboratory notification (fig 1).

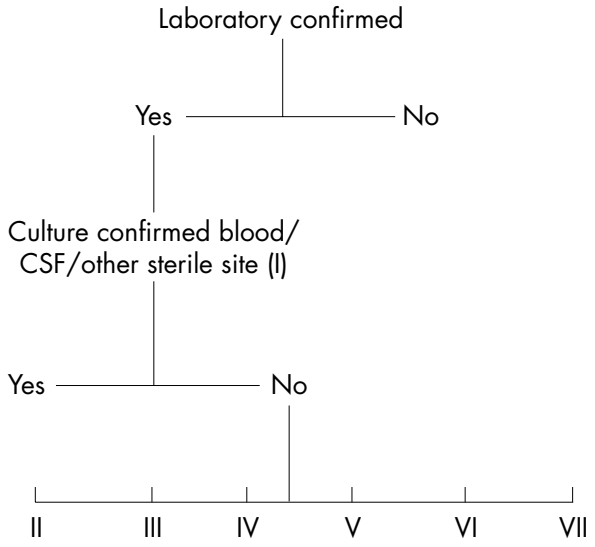
Culture confirmation of meningococcal disease from blood, CSF, eye, or other normally sterile site

Culture confirmation of meningococcal disease was performed on presumed isolates of *Neisseria meningitidis* using standard procedures for the biochemical identification, serogrouping, and serotyping/subtyping of meningococci.[15–17] Culture confirmation was assigned to diagnostic category I as shown in fig 1.

Microscopy of cerebrospinal fluid (CSF)

Microscopy of CSF was performed in area laboratories. The presence of Gram negative diplococci in a CSF sample with

Abbreviations: CSF, cerebrospinal fluid; MenC, serogroup C meningococcal conjugate; OMP, outer membrane protein; PBS, phosphate buffered saline; PCR, polymerase chain reaction; SCIEH, Scottish Centre for Infection and Environmental Health; SMPRL, Scottish Meningococcus and Pneumococcus Reference Laboratory



Diagnostic categories

I	Culture confirmed from blood, CSF or other sterile site
II	Gram negative diplococci in CSF in clinical meningitis
III	Gram negative diplococci in skin aspirate in clinical septicaemia
IV	Clinical meningitis: PCR positive CSF or blood
V	Clinical meningitis: antigen positive CSF
VI	Clinical meningitis or septicaemia: positive throat swab
VII	Clinical meningitis or septicaemia: positive serology

Figure 1 Diagnostic categories for the confirmation of meningococcal disease in Scotland. CSF, cerebrospinal fluid; PCR, polymerase chain reaction.

other laboratory findings consistent with bacterial infection, and in a patient with symptoms suggestive of meningococcal meningitis, was recorded as a laboratory confirmed case of clinical meningitis under diagnostic category II.

Microscopy of skin aspirate/scrapings

Microscopy was performed in local laboratories on skin aspiration/scrapings taken from patients with a rash and other symptoms suggestive of meningococcal septicaemia. When Gram negative diplococci were present the case was recorded as a laboratory confirmed case of clinical septicaemia under diagnostic category III.

PCR testing of CSF, blood, or other normally sterile fluid

PCR was introduced in 1995 and is performed primarily on CSF, serum, and plasma to detect the insertion element IS1106, as described previously.[18 19] Serum and plasma samples were diluted 1/2 in sterile water and boiled for five minutes. Undiluted samples of CSF samples were boiled for five minutes. The thermocyling conditions were 27 cycles of 95°C for 25 seconds, 61°C for 40 seconds, and 72°C for 60 seconds, followed by extension for 60 seconds at 72°C. PCR products were run on a 1.5% agarose gel stained with ethidium bromide and visualised under an ultraviolet transilluminator. IS1106 positive samples were serogrouped by amplification and restriction fragment length polymorphism analysis of the siaD gene when disease was caused by serogroups B or C.[16] A positive PCR result was recorded as a laboratory confirmed case of meningococcal septicaemia and/or meningitis under diagnostic category IV.

Latex agglutination and co-agglutination on CSF

Latex agglutination was performed on CSF using the commercially available Wellcogen kit (Bio-stat Diagnostic Systems, Stockport, UK) for serogroups A, B, C, Y, and W135. Co-agglutination was performed as described previously using in house reagents for serogroups A, B, C, Y, W135, X, and Z_1.[15] A positive result was recorded as a laboratory confirmed case of meningococcal septicaemia and/or meningitis under diagnostic category V.

Throat swab culture

Throat swabs were taken from patients with suspected meningococcal disease. The isolation of *N meningitidis* from the throat was recorded as a laboratory confirmed case of meningococcal septicaemia and/or meningitis under diagnostic category VI.

Antibody confirmation

An indirect enzyme linked immunosorbent assay method was performed on serum to detect antibodies directed against the outer membrane protein (OMP) of the infecting meningococcus.[20 21] Briefly, OMPs were purified from common meningococcal strains circulating in the UK and pooled as the antigen source. The antigen was coated on to the wells of a flat bottomed 96 well microtitre plate by drying overnight. The plate was then washed with phosphate buffered saline (PBS) and blocking was performed by the addition of 200 µl of 3% bovine serum albumin in PBS and incubation for one hour at 37°C. The plate was washed again with PBS and 100 µl of patient's serum or positive/negative control diluted 1/1000 was added to the appropriate wells. The plate was then incubated at 37°C for 1.5 hours. Aliquots of 100 µl of antihuman immunoglobulin–enzyme IgM and IgG conjugates (Sigma, Poole, Dorset, UK) were diluted 1/1000, added to the appropriate wells, and incubated for one hour at 37°C. The plate was washed again with PBS, 100 µl of OPD substrate was added to all wells, followed by incubation at room temperature for 0.5 hours. The reaction was stopped with 50 µl H_2SO_4 and the aborbance read at 492 nm. Positive and negative cut off values were calculated by (negative/(positive − negative)) and the result for each test was calculated by ((test − negative)/positive − negative)) × 100. Raised titres of IgM and/or IgG were recorded as a laboratory confirmed case of meningococcal septicaemia under diagnostic category VII after

Table 1 Notified cases of meningococcal disease 1993–9

	Year							
	1993	1994	1995	1996	1997	1998	1999	Total
Culture confirmed cases	127 (58%)	114 (56%)	90 (47%)	108 (51%)	96 (36%)	122 (39%)	131 (38%)	788 (45%)
Non-culture confirmed cases	3 (1%)	25 (12%)	79 (41%)	82 (39%)	62 (23%)	87 (28%)	123 (35%)	461 (26%)
Total number of cases	217	202	193	210	265	313	349	1749

Table 2 Serogroups of culture confirmed cases of meningococcal disease 1993–9

Serogroup	1993	1994	1995	1996	1997	1998	1999	Total
A	–	–	2	–	–	–	–	2
B	89	77	54	63	49	47	61	440
C	35	33	28	42	42	62	59	301
Y	–	2	1	2	2	3	2	12
W135	3	–	1	–	2	2	2	10
X	–	–	–	–	1	2	3	6
Z	–	–	1	–	–	–	–	1
29E	–	–	–	1	–	–	–	1
NG	–	2	3	–	–	6	4	15

NG, non-serogroupable.

appropriate interpretation depending on the date of onset and dates of sera taken for testing.

Data analysis

Data from each test were analysed for each year and tabulated or presented in graphical form. Data comparisons were made between the culture confirmed and non-culture confirmed cases using the χ^2 test to show the significance of using non-culture based methods.

RESULTS

Cases of meningococcal disease 1993–1999

Clinical and laboratory information originating from outside the SMPRL was recorded on the database. Approximately 5000 samples were received by the SMPRL between 1993 and 1999 from patients with suspected meningococcal disease. SMPRL data were combined with the data already on the database, resulting in 1749 notified cases between 1993 and 1999, with an increase in notification each year, apart from 1995 (table 1).

Culture confirmation of meningococcal disease from blood, CSF, eye, or other normally sterile sites

Between 1993 and 1999, 788 isolates of *N meningitidis* were received by the SMPRL from blood, CSF, eye, or other normally sterile sites. These were serogroup A (two), serogroup B (440), serogroup C (301), serogroup Y (12), serogroup W135 (10), serogroup X (six), serogroup Z (one), serogroup 29E (one), and non-serogroupable (15) (table 2).

Microscopy of CSF

The microscopy of CSF resulted in a total of 43 confirmed cases of clinical meningitis (table 3). The number of cases confirmed by CSF microscopy each year did not follow a trend but was highest in 1994 and 1997. No cases were confirmed by this method in 1998.

Microscopy of skin aspirate/scrapings

Microscopy of skin aspirations or scrapings resulted in a total of 16 confirmed cases of clinical septicaemia (table 3). The numbers of cases confirmed by skin aspirate microscopy were similar each year, although no cases were confirmed in 1993 by this method.

PCR testing of CSF, blood, or other normally sterile fluid

Approximately 1469 PCR tests were performed for the detection of the insertion element IS1106. These resulted in the laboratory confirmation of 186 cases, 48 of which were from serum, five from plasma, and 132 from CSF (table 3). The siaD PCR method provided a serogroup in 98 (49%), of which 55 were serogroup B and 43 serogroup C. After the introduction of the PCR tests, the number of requests received each year increased from 47 tests in 1995 to 762 tests in 2000.

Latex agglutination and co-agglutination on CSF

These tests were used alongside PCR to detect meningococcal antigen in CSF. A total of 16 confirmed cases of meningococcal septicaemia and/or meningitis were diagnosed by this method, nine of which were in 1996. Overall, however, very few cases were confirmed; in 1993, 1994, and 1997 there were no confirmed cases by this method.

Throat swab culture

The isolation of *N meningitidis* from throat swabs resulted in the confirmation of 68 cases meningococcal septicaemia and/or meningitis. Although no cases were confirmed by this method in 1993 and only one in 1994, the method was useful in gaining a probable confirmation of disease in other years.

Antibody confirmation

Antibody confirmation of meningococcal disease was gained in 118 cases (fig 2). Although the method confirmed no cases in 1993, it accounted for 7.2% of all laboratory confirmations

Table 3 Non-culture confirmation of meningococcal disease 1993–9

	II	III	IV							V					VI	VII
Year	GNdC in CSF	GNdC in skin	Positive	B	C	Serum	CSF	Plasma	Other	B	C	Y	W135	NK	Throat swab	Antibody titre
1993	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
1994	12	2	–	–	–	–	–	–	–	–	–	–	–	–	1	10
1995	6	5	45	5	5	7	37	1	–	–	–	1	–	–	6	16
1996	6	2	32	8	4	8	22	2	–	3	2	1	–	3	16	17
1997	14	1	29	8	10	5	24	–	–	–	–	–	–	–	6	12
1998	–	2	43	16	15	17	26	–	–	–	3	–	–	–	18	21
1999	2	4	37	18	9	11	23	2	1	–	3	–	–	–	21	42

Diagnostic categories: I, culture confirmed from blood, CSF or other sterile site; II, Gram negative diplococci in CSF in clinical meningitis; III, Gram negative diplococci in skin aspirate in clinical septicaemia; IV, clinical meningitis: PCR positive CSF or blood; V, clinical meningitis: antigen positive CSF; VI, clinical meningitis or septicaemia: positive throat swab; VII, clinical meningitis or septicaemia: positive serology.
GndC, Gram negative diplococci; CSF, cerebrospinal fluid; B, C, Y and W135 relate to meningococcal capsule serogroups; NK, not known.

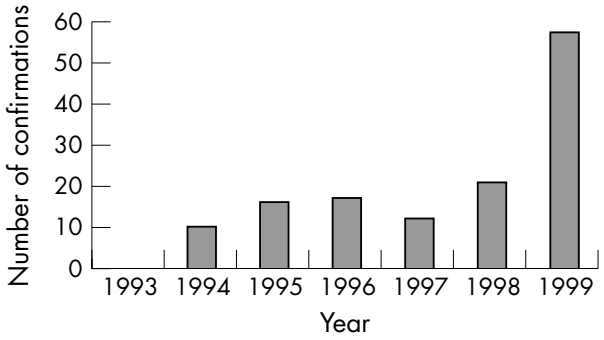


Figure 2 Non-culture confirmation of meningococcal disease by outer membrane protein antibody testing.

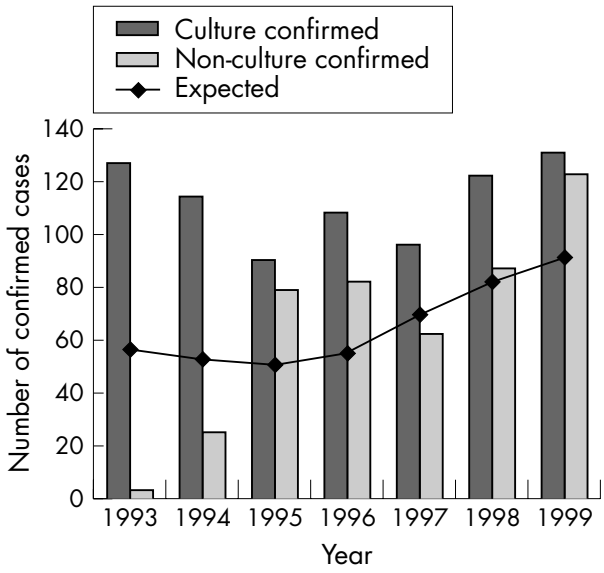


Figure 3 Laboratory confirmation of meningococcal disease in Scotland 1993–9 showing the expected annual number of non-culture confirmed cases.

in 1994, 9.5% in 1995, 8.9% in 1996, 7.6% in 1997, 10% in 1998, and 16.5% in 1999. Antibody testing could be performed up to four weeks after the onset of symptoms and still provide laboratory confirmation.

Overall, non-culture confirmation of meningococcal disease was achieved in 461 cases (table 1). Of these, 65 cases were also confirmed by culture methods (29 in 1995, 10 in 1996, two in 1997, 10 in 1998, and 14 in 1999). With culture confirmed cases this provided laboratory confirmed cases totalling 130 in 1993, 139 in 1994, 169 in 1995, 190 in 1996, 158 in 1997, 209 in 1998, and 254 in 1999 (table 1). We found that non-culture techniques made a significant contribution to the laboratory confirmation of meningococcal disease (fig 3). Apart from in 1993, 1994, and 1997, a greater number of cases of meningococcal disease were confirmed by non-culture methods than had been expected and in 1999 it vastly exceeded our expectations.

DISCUSSION

The awareness of meningococcal disease has increased as a result of better education of the medical profession and the public and, at the same time, new methods have been made available for the laboratory confirmation of this disease. In our study, culture methods provided a confirmation of meningococcal disease in 44% of notified cases between 1993 and 1999, and a serogroup was obtained in 98%. This shows that culture confirmation is very useful for the laboratory confirmation of meningococcal disease and for providing a serogroup. However, 56% of notified cases could not be confirmed by culture, which would leave a large gap in the confirmation of disease and serogroup determination if we relied on culture methods alone. Since the SMPRL introduced non-culture based techniques, the number of laboratory confirmed cases of meningococcal disease has increased each year. PCR and serological testing have been particularly useful and have greatly increased the percentage of laboratory confirmed cases since 1998,[4 22] and made a considerable difference to case confirmation. PCR provided a further 186 confirmations of meningococcal disease between 1995 and 1999 and a serogroup was obtained in 98 cases (53%). Methods used in diagnostic laboratories, such as CSF microscopy, also aid in the non-culture confirmation of meningococcal disease according to the criteria used in Scotland.[4 14] The overall laboratory confirmation rate of meningococcal disease improved from 58%, when culture methods alone were available, to 67% in 1999, when both culture and non-culture methods were available. However, the overall rate was as high as 77% (in 1996) and this degree of confirmation was again reached in 1999.[22]

Take home messages

- Non-culture techniques were a useful addition to culture based techniques
- Non-culture techniques can help improve public health management, disease surveillance, and vaccine policy
- Non-culture methods will probably play a major part in the future of the laboratory confirmation of meningococcal disease

> "Without non-culture methods, the information available for public health management would be inadequate and our understanding of the epidemiology of meningococcal disease would be reduced"

Non-culture diagnostic techniques provide most of the information required for public health management. Methods such as DNA sequencing can now provide as much or even more information as culture based methods, and will play an important part in the future of the laboratory confirmation of meningococcal disease. Although non-culture methods are already important, the methods are not without their shortcomings. Testing for the presence of antibodies to meningococcal OMPs has led to an increase in laboratory confirmed cases but the interpretation is difficult when low titres are detected. The SMPRL hopes to introduce capsular antibody testing, which will aid in the interpretation of OMP antibody testing and also provide a serogroup of the infecting meningococcus. The isolation of a meningococcus from the throat does not necessarily confirm the disease because approximately 10% of the population carry the organism.[23 24] In addition, concerns have been raised in relation to the use of IS1106 as a PCR target for the non-culture confirmation of meningococcal disease.[25] However, in Scotland, PCR false positives are probably very few and most PCR positive cases are confirmed by antibody testing. Improved PCR techniques now allow more sensitive and specific methods to be used.[26]

Without non-culture methods, the information available for public health management would be inadequate and our understanding of the epidemiology of meningococcal disease would be reduced. The benefits of gaining laboratory data for confirming meningococcal disease are well acknowledged,[27] and the recent introduction of MenC vaccines[7 8] also increases the importance of confirming meningococcal disease, particularly by non-culture methods. The potential for capsule

switching cannot be ignored,[11 12] and has been described in isolated cases.[28 29] Therefore, the SMPRL is sure that non-culture methods will play a major part in the future of the laboratory confirmation of meningococcal disease in Scotland.

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REFERENCES

1 **Achtman M**. Global epidemiology of meningococcal disease. In: Cartwright K, ed. *Meningococcal disease*, Vol. 7. Chichester: Wiley & Sons, 1995:159–75.
2 **Connolly M**, Noah H. Is serogroup C disease increasing in Europe? A report of surveillance of meningococcal infection in Europe 1993–6. European Meningitis Surveillance Group. *Epidemiol Infect* 1999;**122**:41–9.
3 **Smart LE**, Christie P, Clarke SC, *et al*. Meningococcal disease in Scotland 1997. *SCIEH Weekly Rep* 1999;**33**:2–6.
4 **Clarke SC**, Christie P, Reid JA, *et al*. Meningococcal disease in Scotland 1998. *SCIEH Weekly Report* 1999;**33**:286–8.
5 **Wang J-F**, Caugant DA, Morelli G, *et al*. Antigenic and epidemiological properties of the ET-37 complex of Neisseria meningitidis. *J Infect Dis* 1993;**167**:1320–9.
6 **Vogel U**, Claus H, Frosch M, *et al*. Molecular basis for detection of the ET-15 clone within the ET-37 complex of Neisseria meningitidis. *J Clin Microbiol* 2000;**38**:941–2.
7 **Chief Medical Officer**. *Introduction of immunisation against group C meningococcal infection*. Edinburgh: Health Department, Scotland; 1999, PAL51979.
8 **Chief Medical Officer**. *Start of the new meningococcal C conjugate vaccine immunisation programme*. London: Department of Health, England and Wales; PL/CMO/99/4.
9 **Anon**. Enhanced surveillance of meningococcal disease. *Commun Dis Rep CDR Wkly* 1998;**8**:1.
10 **Clarke SC**, Christie P, Edwards GFS, *et al*. Enhanced surveillance and diagnosis of meningococcal disease in Scotland. *SCIEH Weekly Report* 1999;**33**:235–6.
11 **Maiden MCJ**, Spratt BG. Meningococcal conjugate vaccines: new opportunities and new challenges. *Lancet* 1999;**354**:615–16.
12 **Clarke SC**, Edwards GFS. Serotype implications for the diagnosis of meningococcal disease. *Scott Med J* 2000;**45**:67.
13 **Cartwright K**, Reilly S, White D, *et al*. Early treatment with parenteral penicillin in meningococcal disease. *BMJ* 1992;**305**:484.
14 **Clarke SC**, Edwards GFS. Guidance for requests and interpretation of services provided by the Scottish Meningococcus and Pneumococcus Reference Laboratory. *SCIEH Weekly Report* 2000;**34**:228–32.
15 **Eldridge J**, Sutcliffe EM, Abbott JD, *et al*. Serological grouping of meningococci and detection of antigen in cerebrospinal fluid by coagglutination. *Med Lab Sci* 1978;**35**:63–6.
16 **Borrow R**, Claus H, Guiver M, *et al*. Non-culture diagnosis and serogroup determination of meningococcal B and C infection by a sialyltransferase (SiaD) PCR ELISA. *Epidemiol Infect* 1997;**118**:111–17.
17 **Frasch CE**, Zollinger WD, Poolman JT. Serotype antigens of Neisseria meningitidis and a proposed scheme for designation of serotypes. *Rev Infect Dis* 1985;**7**:504–9.
18 **Ni H**, Knight AI, Cartwright K, *et al*. Polymerase chain reaction for diagnosis of meningococcal meningitis. *Lancet* 1992;**340**:1432–4.
19 **Newcombe J**, Cartwright K, Palmer WH, *et al*. PCR of peripheral blood for diagnosis of meningococcal disease. *J Clin Microbiol* 1996;**34**:1637–40.
20 **Kristiansen B**, Flaegstad T, Falk ES, *et al*. ELISA test for antimeningococcal IgG and IgM antibodies: application to epidemiology and diagnosis. *Scand J Infect Dis* 1992;**24**:47–55.
21 **Saunders NB**, Shoemaker DR, Brandt BL, *et al*. Confirmation of suspicious cases of meningococcal meningitis by PCR and enzyme-linked immunosorbent assay. *J Clin Microbiol* 1997;**35**:3215–19.
22 **Clarke SC**, Brownlie S, Denham B, *et al*. Meningococcal disease in Scotland 1999. *SCIEH Weekly Report* 2001;**35**:130–3.
23 **Broome CV**. The carrier state: Neisseria meningitidis. *J Antimicrob Chemother* 1986;**18**(suppl A):25–34.
24 **Neal KR**, Nguyen-Van-Tam JS, Jeffrey N, *et al*. Changing carriage rate of Neisseria meningitidis among university students during the first week of term: cross sectional study. *BMJ* 2000;**320**:846–9.
25 **Borrow R**, Guiver M, Sadler F, *et al*. False positive diagnosis of meningococcal infection by the IS1106 PCR ELISA. *FEMS Microbiol Lett* 1998;**162**:215–18.
26 **Guiver M**, Borrow R, Marsh J, *et al*. Evaluation of the Applied Biosystems automated Taqman polymerase chain reaction system for the detection of meningococcal DNA. *FEMS Immunol Med Microbiol* 2000;**28**:173–9.
27 **Ragunathan L**, Ramsay M, Borrow R, *et al*. Clinical features, laboratory findings and management of meningococcal meningitis in England and Wales: report of a 1997 survey. *J Infect* 2000;**40**:74–9.
28 **Swartley JS**, Marfin AA, Edupganti S, *et al*. Capsule switching of Neisseria meningitidis. *Proc Natl Acad Sci U S A* 1997;**94**:271–6.
29 **Vogel U and Claus H**. Rapid serogroup switching in Neisseria meningitidis. *N Engl J Med* 2000;**342**:219–20.

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