Introduction of an automated service for the laboratory confirmation of meningococcal disease in Scotland

S C Clarke, M A Diggle, J A Reid, L Thom, G F S Edwards

Abstract

The Scottish Meningococcus and Pneumococcus Reference Laboratory provides a national service for the laboratory confirmation of meningococcal and pneumococcal disease in Scotland. The main tests used for the laboratory confirmation of meningococcal disease are culture, the polymerase chain reaction (PCR), antibody testing, and more recently DNA sequencing. This paper describes the automation of PCR for the laboratory confirmation of meningococcal disease and the typing of meningococcal isolates using DNA sequencing. Both methods have been automated using a robotic liquid handler and automated DNA sequencer. These methods, along with standard culture phenotyping and antibody testing, provide Scotland with an excellent service for the confirmation of meningococcal disease.

Keywords: automation; Neisseria meningitidis; meningococci; meningitis

Meningococcal disease receives a high level of medical, public, and media attention because of its rapid onset and high level of morbidity and mortality. The Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL) provides several tests for the laboratory confirmation of meningococcal disease including a polymerase chain reaction (PCR) test, antibody test, and full isolate characterisation.1 In recent years, the number of test requests has increased substantially so that the workload has put considerable strain on the department. However, laboratory automation has become affordable and therefore its use in microbiology is growing. Such automation often allows tasks to be completed within shorter time scales, with little user intervention and greater reproducibility. Molecular biology lends itself particularly well to automation because many methods are repetitive and involve the transfer of liquids.2 Time can then be spent analysing the resulting data.

A PCR test for the laboratory confirmation of meningococcal disease was introduced as a national service in Scotland in 1995. However, since its introduction, the awareness of meningococcal disease has increased such that requests rose from 200/year in 1995 to 1000/year in 1999 (fig 1). Therefore, automation was needed to process this number of samples so that labour time could be saved and test reproducibility retained. Multilocus sequence typing (MLST) was recently introduced as a national service for the genotypic characterisation of Neisseria meningitidis strains to complement the phenotypic characterisation service that has been available for some time. The standard MLST method for characterising meningococci involves the sequencing of seven genes to provide a digital, and therefore portable, DNA genotype.3,4 Although MLST provides data of high quality, the procedures involved in setting up and performing the method are very labour intensive. In addition, large quantities of DNA sequence data require editing and analysis. Automation of the procedures involved in performing the methods appeared attractive so that personnel time could be used more efficiently. Our report describes the introduction of automation for the confirmation of meningococcal disease in Scotland using PCR and MLST.

Figure 1 Meningococcal PCR requests from 1995 to 1999.

Keywords: Neisseria meningitidis; meningococci; meningitis

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laboratory confirmation of meningococcal disease was performed as described previously.7
Reddy-mix PCR reagent (ABgene, Epsom, UK) was used to reduce the time involved in
making master mix reagent and to retain reproducibility. Furthermore, the master mix
contained a loading dye, which reduced robot set up time. MLST was performed as de-
scribed previously3 with appropriate modification for the reagent requirements of the Licor
DNA sequencer.

The Roboamp 4200 was programmed ac-

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The Roboamp 4200 liquid handling robot was used to automate all the procedures required
for the execution of PCR amplification and labelling of subsequent PCR products. Again,
the NCC microtitre plates were used during PCR amplification to stop cross contamination
between samples; this was important for the execution of PCR amplification and
labelling of subsequent PCR products. Again, the NCC microtitre plates were used during
PCR amplification to stop cross contamination between samples; this was important for the
characterisation of clinical isolates. We pro-

Table 1  The eight genes used for multilocus sequence typing

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>abcZ</td>
<td>adk</td>
</tr>
<tr>
<td>aroE</td>
<td>fumC</td>
</tr>
<tr>
<td>gdh</td>
<td>pdrC</td>
</tr>
<tr>
<td>pgm</td>
<td>porA</td>
</tr>
</tbody>
</table>

After PCR amplification, internal fragments
of each gene were sequenced on the Licor
L4200-L2. The results were analysed and edited
accordingly, and the data from the first seven
genes were downloaded on to the MLST
website (http://mlst.zoo.ox.ac.uk). This website
accesses a database that contains the allelic pro-
files and associated epidemiological data for more
than 300 meningococcal isolates. The sequence
data were compared with the database and
a seven digit allelic profile determined. In
addition, the edited porA sequence was entered
into the porA website database (http://
mlst.zoo.ox.ac.uk/porA-VR/VR_index.htm) to
provide a bacterial serotype.

Results and discussion

The SMPRL has set up, to our knowledge, the world's first automated, national PCR and
MLST service. The laboratory is now able to

3 Maiden MCJ, Bygraves JA, Feil E, et al. Multi-locus sequence typing: a portable approach to the identification of clones within populations of pathogenic micro-
6 Saunders NB, Zollinger WD, Rao VB. A rapid and sensitive strategy employed for amplification and sequencing of

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