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Short reports

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

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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.

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Keywords: automation; *Neisseria meningitidis*; meningococci; meningitis

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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.

Methods

AUTOMATED PCR

A liquid handling robot, the Roboamp 4200, and automated DNA sequencer, the Licor L4200-L2 (MWG Biotech, Milton Keynes, UK), were used to automate the procedures involved in setting up PCR reactions, sequence labelling reactions, agarose gel loading, and DNA gel analysis. The PCR test used for the

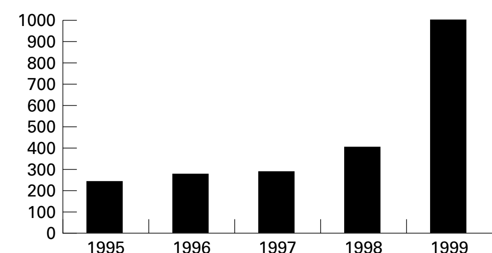

Figure 1 Meningococcal PCR requests from 1995 to 1999.

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Table 1 The eight genes used for multilocus sequence typing

abcZ	adk
aroE	fumC
gdh	pdhC
pgm	porA

laboratory confirmation of meningococcal disease was performed as described previously.[5] 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 described previously[3] with appropriate modification for the reagent requirements of the Licor DNA sequencer.

The Roboamp 4200 was programmed according to the manufacturer's instructions to provide full automation of the procedures involved in a diagnostic PCR test, apart from reading of the results on the agarose gel. Throughout the procedure, cross contamination was avoided by the use of 96 well non-cross contamination (NCC) microtitre plates (MWG Biotech, Milton Keynes, UK). In addition, all reagents were maintained at 4°C in refrigerated racks within the Roboamp 4200 platform. PCR reactions were automatically transferred to and from the integrated 96 well thermocycler by the Roboamp 4200. After thermocycling, the samples were loaded on to a ready poured agarose gel. The electrophoresis tank was then connected to a power supply, followed by manual analysis of the gel on a standard ultraviolet transilluminator for the presence or absence of a 596 base pair DNA product.

AUTOMATED MLST

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 characterisation of clinical isolates. We programmed the system to amplify and sequence eight genes from 12 different meningococcal isolates at one time, thus providing four 96 well result plates. We chose to sequence the standard seven housekeeping genes[3] and one outer membrane protein antigen gene, porA (table 1).[6] The system allows full automation on three isolates at one time to coincide with throughput capacity on the automated DNA sequencer.

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 profiles 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 provide a high throughput service for PCR testing so that the continuing increase in workload can be accommodated. Meningococcal isolates can be characterised genotypically in a timely manner from clusters of cases so that public health management can be used effectively. In addition, the long term epidemiology of meningococcal disease can be monitored on a national basis. Although the SMPRL has been performing the IS1106 PCR method since 1995, the recent increase in requests has justified the automation of the method, as described, and will enable the laboratory to use other gene targets for PCR in the future. The automation of high throughput PCR and DNA sequencing, particularly MLST, is essential so that time can be spent by trained personnel in analysing the resultant data. The provision of these data, in a timely manner, to agencies such as the Scottish Centre for Infection and Environmental Health (SCIEH) is essential for determining long term disease trends and for analysing the effects of introducing the MenC conjugate vaccine.

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