Semi-automation of the polymerase chain reaction for laboratory confirmation of meningococcal disease

M.A. DIGGLE and S.C. CLARKE

Scottish Meningococcus and Pneumococcus Reference Laboratory, House on the Hill, Stobhill Hospital, North Glasgow University Hospitals NHS Trust, Glasgow G21 3UW, UK.

Accepted: 20 March 2002

ABSTRACT

Demand for accurate high-throughput detection and characterisation of medically important bacteria has increased dramatically within research and clinical laboratories. Liquid-handling robots have been developed to achieve high levels of accuracy and reproducibility. Assay automation can play a key role in the modern diagnostic laboratory and the data presented here shows that automated PCR is comparable with manual methods. Importantly, automation is preferred when high-quality results cannot be guaranteed using manual methods. This is particularly important when results are required quickly for public health management.

KEY WORDS: Automation. Meningococcus. Neisseria meningitidis. Polymerase chain reaction.

Introduction

Advances in techniques used to detect and characterise diseases have resulted in a rapid increase in demand within research and clinical laboratories.[1-5] In some circumstances, liquid-handling robotics can be used to perform menial tasks that are repetitive and easily executed on such a platform.[6] Initially, only basic liquid distribution from one vessel to another was achievable. To improve on this, and achieve a high level of accuracy and reproducibility, it became apparent that a more complex and integrated system was needed that would eliminate fundamental problems such as contamination.[7 8]

Over the past few years, automation has proved that it can play a key part in the effective and reliable performance of appropriate assays for the detection and characterisation of disease-causing organisms.[9-11] Automation of high-throughput or rapid assays is attractive because of its reproducibility, reliability and long-term cost-effectiveness major factors that any research or clinical laboratory must consider.

Laboratory automation and new technologies have considerable potential to improve disease detection and characterisation through protocols that reduce liquid-handling errors and cross contamination. It is within such areas that the greatest advances have been made. Development of non-cross contamination (NCC) technology for reagent and sample vessels, together with disposable filter tips and washable tips with integrated tip cleaning facilities, have eliminated major drawbacks such as contamination and allow automated protocols to compete favourably against traditional manual preparations.

Use of automated robotic components is relatively easy and does not require complex information or specialist training. Thus, successful operation requires only an average level of laboratory competence. Another advantage is the broad range of complex liquid-handling procedures that can be performed with relative ease. Moreover, an array of equipment (DNA extraction systems, PCR preparation areas, thermocyclers and refrigerated sample and reagent units) can be integrated onto robot platforms, permitting a much more flexible approach to assay design.

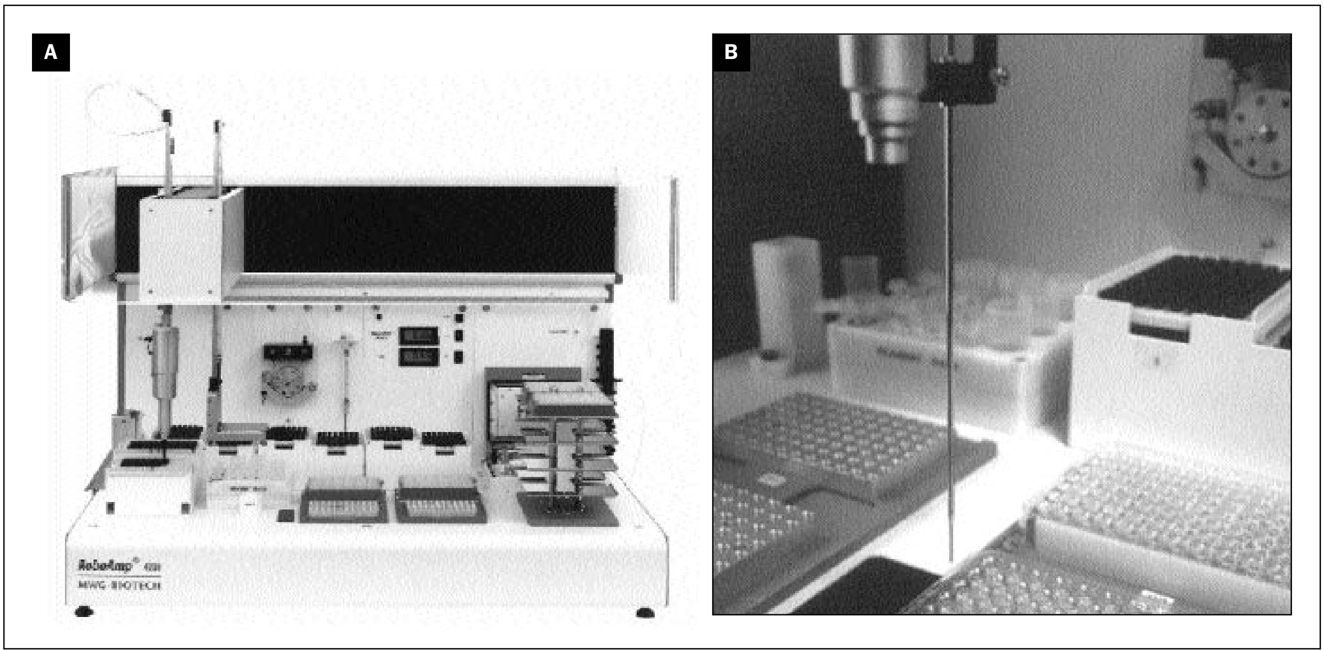
Nucleotide amplification assays have been available for over a decade now, and the polymerase chain reaction (PCR) has had a profound effect on the development of various techniques for the laboratory confirmation of many infectious diseases.[12] Various technical advances in computer technology and robotic liquid-handling systems have given rise to the possible automation of common laboratory procedures, and fully automated DNA detection systems could be used both in high-throughput research facilities and in the routine clinical diagnostic laboratory.

Here, we describe the use of automation for PCR diagnosis of meningococcal disease (MD) in a national reference laboratory. MD is commonly diagnosed using PCR if no culture has been isolated due to prior antibiotic therapy.[13] Rapid and accurate confirmation is essential to distinguish outbreaks of genuine disease.[14] Although manual PCR assays are performed, often they prove inadequate and inaccurate for this purpose, especially for high reproducibility on a large number of clinical samples. Continual rise in PCR requests over the past few years has resulted in the procedures becoming labour-intensive, with the possibility of errors associated with liquid handling and contamination.

The national reference laboratory started to use a liquid-handling robot as a means to automate the technique (preparation of PCR reactions, integrated thermocycler and a gel tray for loading and electrophoresis of PCR products)

Correspondence to: Dr. S.C. Clarke
Email: stuart.clarke@northglasgow.scot.nhs.uk

Fig. 1. Roboamp-4200 robotic liquid-handling system showing A) the robot platform and B) the washable tip system.



used for the laboratory confirmation of MD. PCR is a proven method for the confirmation of meningococci [13,15-18] and automation can be used for the high-throughput detection of MD.

Using *Neisseria meningitidis*, evaluation of the methodology provides a good example in which automation is necessary to maintain high standards as requests increase. Therefore, we developed an automated PCR method using the MWG-Biotech Roboamp-4200 (MWG Biotech, Milton Keynes, UK), a 96-well format liquid-handling system. As a consequence, MD confirmation by automated PCR is now a routine national service and the methodology required is described here.

Materials and methods

A collection of 100 blood and CSF samples, sent to the Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL) between 1996 and 2000, were studied. All were taken at the time of admission from patients with suspected MD in hospitals across Scotland, and originally were positive by *IS1106* PCR using manual methodology. A random collection of 20 bacterial strains was used as positive controls of *N. meningitidis*, referred to SMPRL by microbiology laboratories from January 1996. Sterile distilled water negative controls also were included.

CSF samples were suspended in sterile saline and boiled for 1 min. DNA was extracted from blood samples (200 µL), using a NucleoSpin blood DNA extraction kit (ABgene, Surrey, UK), and the method performed as described in the manufacturer's instructions. Extracted DNA was used as the source for detection of meningococcal DNA.

Bacterial isolates were inoculated onto horse blood agar (Oxoid, Basingstoke, UK) and incubated overnight in 5% CO_2 at 37°C. Several single fresh colonies were inoculated into 0.5 mL sterile distilled water, boiled for 1 min, centrifuged at 15 000 x*g* for 2 min and the supernatant used for the detection of meningococcal DNA.

Automated PCR utilised the Roboamp-4200 robotic liquid-handling system, which permitted automation of the procedures required for the amplification of meningococcal DNA (Figure 1). Roboamp-4200 programming was performed according to the manufacturer's instructions.

All PCR reagents were maintained at 4°C on the refrigerated platform. Each reaction was performed in a final volume of 50 µL, using 1.1x Reddymix PCR master mix (ABgene, Surrey, UK). For a 50 µL reaction, 45 µL PCR master mix and 1 µL each of the *IS1106*[11] or *ctrA*[12] primer pairs (Table 1) were added together, to produce a 47 µL volume. These pre-prepared mixes were placed on the liquid-handling robot refrigerated reagent rack and the DNA-extracted samples were placed on the sample area of the liquid-handling robot. Into a refrigerated NCC 96-well plate, 47 µL of each master mix was added automatically, using a washable tip, together with 3 µL DNA preparation –

Table 1. PCR amplification primers used for amplification of *IS1106* and *ctrA* genes

Target gene	Forward primer (5' - 3')	Reverse primer (5' - 3')
IS1106	ATT ATT CAG ACC GCC GGC AG (850-869)	TGC CGT CCT GCA ACT GAT GT (1180-1161)
ctrA	ATG CGG TGG CTG CGG TAG GT (744-763)	CCG GCG AGA ACA CAA ACG ACA AG (1277-1255)

Table 2. Manual and automated non-culture confirmation of meningococcal disease by PCR

No. of samples	Target gene			
	IS1106		*ctrA*	
	Manual	Automated	Manual	Automated
Positive controls (n=20)	20/20	20/20	20/20	20/20
Negative controls (n=20)	0/20	0/20	0/20	0/20
Clinical samples (n=100)	100/100	100/100	97/100	97/100

resulting in a final volume of 50 µL. After each stage, the tip was washed automatically with 2 mL sterile distilled water, within an integrated tip-washing station.

The NCC 96-well plate was placed automatically into an integrated MWG-Biotech Primus 96 thermocycler (MWG Biotech), using PCR conditions described previously for either *IS1106*[13] or *ctrA*.[19] After thermocycling, the NCC plate was transferred automatically from the thermocycler to a refrigerated block, on which the samples were maintained at 4°C.

Agarose gel was prepared in a special tray to fit onto the robot platform for *IS1106* (1.5% agarose) and *ctrA* (3% agarose) detection. Both contained ethidium bromide. 10 µL of each sample was transferred automatically and loaded into the appropriate well of the gel block. After loading each sample, the tip was washed automatically with 2 mL sterile distilled water.

A power source was attached to the gel unit manually to continue the gel electrophoresis procedure, which was performed at 100 V for approximately 30 min. Finally, the gel unit was placed on an ultraviolet transilluminator to visualise the amplified meningococcal DNA.

Results

Semi-automated PCR was evaluated using 140 specimens, comprising 100 clinically confirmed samples, 20 bacterial isolates (positive controls) and 20 samples of sterile distilled water (negative controls) (Table 2).

Using the Roboamp-4200 with a washable tip, the entire process took approximately 2½ hours, from chemical preparation to gel visualisation. Initial results obtained with distilled water spiked with concentrations of a known suspension of *N. meningitidis* demonstrated that the automated PCR method, followed by automated agarose gel electrophoresis loading, could detect known positives at a sensitivity comparable with conventional manual techniques.

When used to examine the different clinical samples taken from patients with confirmed MD, all 100 previously confirmed as positive by the conventional manual *IS1106* insertion element method were also positive by the automated system. Of the 100 samples, 97 were *ctrA*-positive by both automated and manual methods.

All 20 negative controls placed randomly among the clinical samples and bacterial isolates proved negative by agarose gel electrophoresis, using both the automated and conventional manual methods (Table 2).

Automated PCR was found to be efficient and reproducible, and removed the laborious and occasionally inaccurate nature of this technique.

Discussion

Development of automated methods, such as the one described here, demonstrates that they are comparable with manual alternatives, and highlights several significant points. Firstly, automation is as accurate as manual methodology and, importantly, is constant in terms of quality and reproducibility – consistent high-quality manual results cannot be guaranteed.

Secondly, with increasing demand for PCR testing, automation can provide a rapid system and virtual 24-hour service for MD confirmation by PCR, which is beneficial in most circumstances. Owing to the minimisation of repeats, automation has proved to be cost-effective and maximises efficiency and accuracy.

Both short- and long-term disease surveillance studies depend on reliable molecular methods, which can be used to determine bacterial DNA in patient samples.[6,14] SMPRL has taken this a step further and introduced a rigid, reliable and reproducible automated PCR service for the detection of meningococcal DNA in all routine samples referred for analysis.

Many advantages are apparent when using an automated system for DNA detection in organisms such as *N. meningitidis*, and the integration of PCR within such a system provides greater flexibility than is available with time-consuming and laborious manual alternatives. □

Funding for the liquid-handling robot was generously provided by the Meningitis Association (Scotland).

References

1 Stefanelli P, Fazio C, La Rosa G, Marianelli C, Muscillo M, Mastrantonio P. Rifampicin-resistant meningococci causing invasive disease: detection of point mutations in the *rpoB* gene and molecular characterization of the strains. *J Antimicrob Chemother* 2001; **47**: 219-22.

2 Shi PY, Kauffman EB, Ren P *et al*. High-throughput detection of West Nile virus RNA. *J Clin Microbiol* 2001; **39**: 1264-71.

3 Fortin NY, Mulchandani A, Chen W. Use of real-time polymerase chain reaction and molecular beacons for the detection of *Escherichia coli* O157:H7. *Anal Biochem* 2001; **289**: 281-8.

4 Van Der Pol B, Quinn TC, Gaydos CA *et al*. Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG tests for detection of *Chlamydia trachomatis*. *J Clin Microbiol* 2000; **38**: 1105-12.

5 Sadler F, Borrow R, Dawson MM, Kaczmarski EB, Cartwright K, Fox AJ. Improved methods of detection of meningococcal DNA from oropharyngeal swabs from cases and contacts of meningococcal disease. *Epidemiol Infect* 2000; **125**: 277-83.
6 Clarke SC, Diggle MA, Reid JA, Thom L, Edwards GF. Introduction of an automated service for the laboratory confirmation of meningococcal disease in Scotland. *J Clin Pathol* 2001; **54**: 556-7.
7 Clewley JP. Automation of the polymerase chain reaction. Part 4. Detecting and characterising the product. *Commun Dis Public Health* 1999; **2**: 299-300.
8 Check WA. What automation offers microbiology. *CAP Today* 1997; **11**: 1, 20, 24-6.
9 Dimech W. Automation of an absorbed enzyme immunoassay for the detection of *Mycobacterium paratuberculosis* antibodies for an eradication program. *Vet Microbiol* 2000; **77**: 351-5.
10 Vasavada PC. Rapid methods and automation in dairy microbiology. *J Dairy Sci* 1993; **76**: 3101-13.
11 Adams V, Moll C, Schmid M, Rodrigues C, Moos R, Briner J. Detection and typing of human papillomavirus in biopsy and cytological specimens by polymerase chain reaction and restriction enzyme analysis: a method suitable for semi-automation. *J Med Virol* 1996; **48**: 161-70.
12 Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. *Cold Spring Harb Symp Quant Biol* 1986; **51** (Part 1): 263-73.
13 Ni H, Knight AI, Cartwright K, Palmer WH, McFadden J. Polymerase chain reaction for diagnosis of meningococcal meningitis. *Lancet* 1992; **340**: 1432-4.
14 Yakubu DE, Abadi FJ, Pennington TH. Molecular typing methods for *Neisseria meningitidis*. *J Med Microbiol* 1999; **48**: 1055-64.
15 Prats G, Margall N, Majo M. Diagnosis of meningococcal disease using PCR. *Rev Esp Salud Publica* 1998; **72**: 435-6.
16 Kotilainen P, Jalava J, Meurman O *et al*. Diagnosis of meningococcal meningitis by broad-range bacterial PCR with cerebrospinal fluid. *J Clin Microbiol* 1998; **36**: 2205-9.
17 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*: 111-7.
18 Borrow R, Claus H, Chaudhry U *et al*. siaD PCR ELISA for confirmation and identification of serogroup Y and W135 meningococcal infections. *FEMS Microbiol Lett* 1998; **159**: 209-14.
19 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.