JOURNAL OF CLINICAL MICROBIOLOGY, June 2003, p. 2440–2443 Vol. 41, No. 6
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.6.2440–2443.2003
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Comparison of Commercial DNA Extraction Kits for Extraction of Bacterial Genomic DNA from Whole-Blood Samples

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Received 26 September 2002/Returned for modification 25 November 2002/Accepted 27 February 2003

The demand for molecular diagnostic tests in medical microbiology has highlighted the need for efficient methods of DNA extraction. In addition, it is preferable for these methods to be automated. An example of such a requirement is for the confirmation of meningococcal disease where rapid, sensitive, and specific procedures are required for public health management purposes. Previous studies have shown that whole blood is the preferred method for the isolation of bacterial DNA in meningococcal disease, and in this study, we compare five commercially available kits for the extraction of bacterial genomic DNA from whole-blood samples. These include kits in a 96-well binding plate, 96-well filter plate, and metallic bead formats. The method for all five kits is described, and the sensitivity, specificity, ease of automation, and overall efficiency are determined.

In recent years, advances in molecular biology have promoted the routine use of techniques such as PCR and DNA sequencing for the improved diagnosis and surveillance of microbial disease (4, 12). Bacterial DNA can be extracted from the body fluids of patients with suspected bacterial disease and then be amplified by PCR and sequenced to enable detection and bacterial identification (4, 5). An efficient method of DNA extraction that produces pure, high-quality DNA is crucial to the success of PCR and sequencing reactions and the subsequent treatment of disease (11, 13). Current manual methods of DNA extraction are simple and reliable and are suitable for extraction of low numbers of samples (15). As the demand for molecular tests increases, new automated methods of DNA extraction will have to be developed to handle larger numbers of clinical samples (10). There are a number of DNA extraction kits available, based on a 96-well plate format, which allow integration into the workstation of a robotic liquid handler. This allows a much higher throughput of samples, is less work intensive, and produces PCR-ready bacterial DNA (10, 13). DNA extraction methods based on the absorption of DNA to metallic beads are also available and, again, can also provide high-throughput DNA extraction.

Purified bacterial DNA is required for many procedures in modern molecular biology, but one example is for the confirmation of meningococcal disease where rapid, sensitive and specific procedures are required for public health management purposes. Bacterial DNA can be amplified from clinical samples by using the PCR and information gained based on the serogroup, type, and subtype of the infecting organism as well as sequence type data (2, 3, 5, 6, 16). It has previously been shown that whole blood is the preferred method for the isolation of bacterial DNA in meningococcal disease (9), and in this study, a comparison of five DNA extraction kits was performed. Methods were developed for the kits where they were not initially designed for the extraction of bacterial DNA from whole blood. In addition, methods were developed so that all kits were fully automated and integrated into a previously described fluorescence PCR method (7, 8). These kits were compared for their relative effectiveness in purifying and recovering bacterial DNA from whole-blood samples. The kits operate by three basic principles. Two of the kits use 96-well binding plates for DNA binding and elution from blood samples. Two kits use metallic beads with a polymeric surface to bind DNA and a magnetic separator to extract these beads from solution. The fifth kit utilizes 96-well filter plates with progressively smaller pores to purify DNA from blood samples by size exclusion (10). The sensitivity, specificity, and ease of automation for each kit were compared.

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MATERIALS AND METHODS

Bacterial strains. Fully characterized reference strains of *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*, held at the Scottish Meningococcus and Pneumococcus Reference Laboratory, were used to inoculate whole-blood samples (Table 1). Two bacterial colonies were mixed in 1 ml of whole blood and incubated for 2 h at 37°C in 5% CO_2. The automated DNA extraction methods were performed on a Roboseq 4200 PE liquid-handling robot (MWG Biotech [United Kingdom] Ltd., Milton Keynes, United Kingdom). This robot possesses an integrated 96-well thermocycler, vacuum manifold, and Biotek FL600 fluorescence reader (MWG Biotech [United Kingdom] Ltd.). Programming of the robot was performed according to the manufacturer's instructions.

Promega Wizard SV96 System. One hundred microliters of whole blood was added to 200 μl of lysis buffer (SV RNA lysis buffer–1% Triton X-100) and vortexed to mix. The SV96 DNA binding plate was automatically placed on top of the vacuum manifold. One hundred fifty microliters of blood lysate was added to the wells of the binding plate. A vacuum of 600 mb was applied for approximately 2 min or until the solution had passed through the membrane. One milliliter of SV96 wash solution was added to each well, and a vacuum was applied as before for approximately 2 min. This washing step was repeated. The vacuum was again applied at 600 mb for 5 min to remove residual ethanol. A collection plate was then placed inside the vacuum manifold, and 75 microliters of nuclease-free water was added to each well on the binding plate. A vacuum of 600 mb was applied for 1 min to elute the DNA.

Macherey-Nagel Nucleospin robot 96 (plasmid) kit. The Macherey-Nagel Nucleospin robot 96 (plasmid) kit was used per the manufacturer's instructions, except that reagents from the Nucleospin blood kit were used in place of the

TABLE 1. Bacterial strains used in this study

Bacterial species	Strain no.	PCR result
Haemophilus influenzae		
Type a	NCTC 8465	Negative
Type b	NCTC 7279	Positive
Type c	NCTC 8469	Positive
Type d	NCTC 8470	Negative
Type e	NCTC 8455	Negative
Type f	NCTC 8473	Negative
Neisseria meningitidis		
Serogroup A	79-03901	Positive
	79-09267	Positive
Serogroup B	W60532	Positive
	W60430	Positive
Serogroup C	W60981	Positive
	W60987	Positive
Serogroup W135	W60411	Positive
	W60129	Positive
Serogroup Y	W64602	Positive
	W64542	Positive
Neisseria lactamica	W60010	Negative
	W2441	Negative
Neisseria mucosa	W64523	Negative
	W64556	Negative
Streptococcus pneumoniae	00-3674	Positive
	00-3175	Positive
	01-1282	Positive
	01-1241	Positive
	01-1231	Positive
	01-4176	Positive
Streptococcus oralis	93692A	Negative
	85737M	Negative
Streptococcus mitis	33861W	Negative
	99778V	Negative
Streptococcus parasanguis	460501P	Negative
	99226M	Negative
Staphylococcus aureus	W68639	Negative
	W68460	Negative
Escherichia coli	W68578	Negative
	W68392	Negative

plasmid kit reagents. Two hundred microliters of whole blood, 25 μl of proteinase K, and 200 μl of Nucleospin lysis buffer B3 were added to each other and vortexed for 20 s. The sample was then incubated for 15 min at 70°C. After incubation, 210 μl of 96 to 100% ethanol was added to each sample and the mixtures were vortexed again. The Nucleospin plasmid plate was placed on top of the vacuum manifold, and the samples were distributed to the appropriate wells. A vacuum of 400 mb was applied for 2 min or until the samples had been completely drawn through the filter. Each well was washed by adding 500 μl of Nucleospin BW washing buffer. A vacuum of 400 mb was then applied for 1 min, and the through-flow was discarded. Then 600 μl of Nucleospin buffer B5 was added to each well, and the vacuum was applied at 400 mb for 1 min. The vacuum was applied for a further 1 min to remove any residual ethanol. The DNA was eluted by adding 100 μl of Nucleospin elution buffer BE directly to the silica membrane in each well. The plate was incubated with this solution at room temperature for 1 min, and then a vacuum of 400 mb was applied for 1 min to elute the DNA.

Millipore Montage plasmid Miniprep$_{96}$ kit. The Millipore Montage plasmid Miniprep$_{96}$ kit is designed for the extraction of plasmids from bacteria, but information from the company suggested that the kit would work for blood extraction. Therefore, 150 μl of lysis solution was added to 100 μl of whole blood in a microcentrifuge tube and vortexed vigorously. The lysis mixture was incubated for 2 min at room temperature. After incubation, 150 μl of neutralization solution was added to the tube and mixed again. The Multiscreen plasmid plate was placed inside the vacuum manifold, and the Multiscreen clearing plate was placed on top of the manifold. Two hundred microliters of lysate was transferred from the sample tube and dispensed into the corresponding well of the clearing plate. The samples were extracted by applying a vacuum of 600 mb for 5 min or until the lysate had been drawn through the filter. The clearing plate was discarded. A collection plate was placed inside the vacuum manifold, and the Multiscreen plasmid plate was placed on top of the vacuum manifold. A vacuum of 800 mb was applied for 5 to 7 min or until the wells were empty. The filtrate was discarded. The plasmid plate was washed by adding 200 μl of wash solution 4 to each well and applying a vacuum of 800 mb for 5 min. The filtrate was discarded. A new collection plate was placed inside the vacuum manifold, and the DNA was recovered by adding 50 μl of solution 5 to each well and allowing the plate to sit for 30 min until the liquid ran through.

Bilatest bead DNA 2 extraction kit. A lysis solution was prepared by mixing 5 μl of magnetic particle suspension with 5 μl of lysis buffer B (containing 99 to 100% ethanol and guanidine thiocyanate [Sigma-Aldrich, Poole, England]). Fifty microliters of whole-blood sample was added to the wells of a Thermosprint 96-well plate (Bilatec AG, Mannheim, Germany). One hundred forty microliters of premixed lysis solution was added in a single rapid pipetting step to the blood sample in each well. The plate was then incubated at 55°C for 15 min to achieve lysis of the white blood cells in the sample. The plate was then placed on the magnetic separator for 2 min, and the metallic bead-DNA complex was allowed to sediment to the side of the well. The supernatant was discarded by gently pipetting from the bottom of the tubes. The tubes were removed from the magnetic separator, and 160 μl of washing solution 5 was added. The plate was placed on the magnetic separator, the particle-DNA complex was allowed to sediment, and the supernatant was discarded without disturbing the pellets. This process was repeated with washing solution 6. The particle-DNA complex was allowed to air dry by leaving the plate on the magnetic separator for 5 min. The plate was then removed from the magnetic separator, and 100 μl of sterile distilled water was added to the appropriate wells. The magnetic particles were resuspended by repeat pipetting until the suspension appeared homogenous. The DNA was eluted by incubating the bead suspension for 10 min at 65°C. The plate was placed on the magnetic separator, and the magnetic particles were allowed to sediment. The supernatant was recovered, containing the eluted DNA.

Promega Wizard Magnesil bead kit. The Promega (Southampton, England) bead kit is designed for the extraction of plasmids from bacteria, but information from the company suggested that the kit would work for blood extraction. Therefore, 120 μl of cell lysis solution was added to 100 μl of whole blood in a 1.5-ml microcentrifuge tube. The solution was mixed vigorously for 3 min, and then 120 μl of neutralization solution was mixed into the tube. Twenty-five microliters of Magnesil Blue magnetic beads were added to the tube and mixed again for 1 min. Two hundred microliters of neutralized lysate containing Magnesil Blue was transferred to the wells of a 96-well Greiner collection plate. The plate was placed on top of the magnetic separator, and the magnetized pellets were allowed to form for 90 s. One hundred microliters of the cleared lysate was transferred from each well of the 96-well plate to a new binding plate. The clearing plate was set aside with the remaining cleared lysate. Twenty-five microliters of Magnesil Red metallic beads were added to each sample on the new plate and mixed for 2 min. The binding plate was transferred to the bead separator, and pellets were allowed to form. The supernatants were discarded from each well. The plate was removed from the separator, and an additional 25 μl of Magnesil Red was added to each well on the plate. The remaining 100 μl of cleared lysate was transferred from the clearing plate to the corresponding wells on the binding plate. The binding plate was then placed on the magnetic separator again, and the pellets were allowed to form. The spent liquid from each well was removed and discarded. The plate was removed from the separator, 100 μl of 90 to 100% ethanol was added to each well, and the liquid was mixed by pipetting. The collection plate was transferred to the magnetic separator, pellets were allowed to form, and the spent liquid was discarded. This process was repeated for a total of three washes. The magnetized particles were allowed to air dry for 10 min at room temperature. One hundred microliters of elution buffer was added to each well and mixed. The plate was placed on the magnetic separator and allowed to stand for 5 min. The supernatant, containing the bacterial DNA, was transferred to collection tubes.

Fluorescence-based PCR. The presence of DNA in extracted samples was determined by fluorescence-based PCR. The level of fluorescence emitted in the reaction is directly proportional to the quantity of PCR product. This assay is highly sensitive and eliminates the need for gel electrophoresis. Each reaction was performed in a final volume of 25 μl as previously described (8). The fluorescence PCR method used in this study was that which detects meningococcal, pneumococcal, and *H. influenzae* DNA (7).

Sensitivity and specificity of DNA extraction kits. Blood samples were spiked with bacteria, and a series of 10-fold dilutions was prepared. Each dilution series was performed in triplicate. The sensitivity of each kit was determined by extracting bacterial DNA from every sample in the dilution series. One hundred microliters of each dilution was plated on blood agar (Oxoid, Basingstoke, United Kingdom) at 37°C in 5% CO_2 overnight. Following incubation, colony

counts were performed. Fluorescence-based PCR was also performed with 2 μl of each DNA extract from the dilution series. The sensitivity of each kit was then calculated from the minimum number of colonies necessary to provide a positive result through fluorescence-based PCR.

To test the specificity of each kit, whole-blood samples were spiked with *N. meningitidis*, *S. pneumoniae*, and *H. influenzae*. To ensure that the three primer sets used in the fluorescence-based PCR are highly specific to these three bacteria only, blood samples were also inoculated with other types of bacteria. These included oral streptococci, other *Neisseria* species, and several types of bacteria that are capable of causing septicemia (Table 1). The DNA from each spiked whole-blood sample was extracted with each kit. The extracts were then tested by fluorescence-based PCR, with 2 μl of DNA in each reaction mixture, for the presence of each of the bacterial species.

Quantitation of bacterial DNA with the PicoGreen assay. The PicoGreen assay (Molecular Probes, Poortgebouw, Netherlands) uses an ultra-sensitive fluorescent nucleic acid stain for quantitating double-stranded DNA in the DNA extract solutions (1, 14). First, a standard curve was constructed by serially diluting the lambda DNA standard (Molecular Probes). The final volume of all samples was 100 μl. A working solution of the PicoGreen reagent was prepared by diluting the concentrated stock by 200-fold in 1× Tris-EDTA buffer. One hundred microliters of this reagent was added to each sample and incubated for 5 min at room temperature. The samples were protected from light to avoid photobleaching. After incubation, the fluorescence level of the samples was measured with a fluorescence microplate reader at wavelengths of ~480 and ~520 nm. A reagent blank consisting of sterile distilled water was used, and the value of this blank was subtracted from each of the test values to create the standard curve. Whole-blood DNA extracts were diluted in 1× Tris-EDTA buffer to give a final volume of 100 μl. One hundred microliters of PicoGreen reagent was added to each sample and incubated for 5 min as before. Fluorescence was measured at ~480 and ~520 nm, and again, the reagent blank value was subtracted from the values produced. The DNA concentration of the sample was determined by plotting each value on the standard curve.

RESULTS

Ease of use and automation. All commercially available DNA extraction kits were used successfully and were fully automated. Where kits were not specifically designed for the extraction of bacterial DNA from whole blood, the kits were successfully modified to enable this by using the appropriate reagents. Overall efficiency was determined by throughput time, DNA yield, and labor intensity. Although all kits successfully extracted bacterial DNA from whole blood and were automated, there were differences in their ease of use and automation. The 96-well plate format of the Promega Wizard SV96 kit allowed it to be easily adapted for use on a liquid-handling robot. A large number of samples could be processed with little or no manual intervention. The kit was extremely simple to use and could extract up to 96 samples in under 20 min. The kits based on the use of metallic beads were also very simple to automate. They worked well on a 96-well plate format that could be easily controlled by a liquid-handling robot. The most efficient of these kits was the Bilatest DNA 2 system which could rapidly extract high-quality DNA from a large number of samples automatically.

Sensitivity and specificity. The sensitivity of each kit was calculated as the minimum number of CFU required to produce a positive result through fluorescence-based PCR. The results for the approximate sensitivity of each kit are as follows. The Bilatest bead method was the most sensitive and could detect down to 1 genome copy per 100 μl of sample. The least sensitive method was the Millipore Montage kit, which could only detect down to 6 genome copies per 100 μl of sample. The Promega Wizard SV96 system and Promega Magnesil Wizard bead kit could detect 2 and 3 genome copies, respectively, per 100 μl of sample. The Macherey-Nagel system could detect 5 genome copies per 100 μl of sample. Gel electrophoresis was also used to display the amplified PCR product. The specificity of each kit was also tested by using samples inoculated with several different species of bacteria. The results of the specificity tests are shown in Table 1. Fluorescence-based PCR with all DNA extraction kits was shown to be highly specific for strains of *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* types b and c (the multiplex assay only detects these types of *H. influenzae*) and did not give false-positive results for the other bacterial strains used.

DNA quantitation by PicoGreen assay. The concentration of DNA extracted by each kit was determined by the PicoGreen assay. The average amounts of DNA detected by each kit per 100 μl of sample (± standard deviations) are as follows: Bilatest system, 9.06 ± 0.922 μg; Promega SV96 system, 7.4 ± 1.7523 μg; Millipore Montage kit, 4.9 ± 1.323 μg; Macherey-Nagel system, 3.9 ± 2.012 μg; and Promega Magnesil bead kit, 2.45 ± 0.1874 μg. Upon comparison of the results, it is clear that the Bilatest DNA 2 kit extracted the highest concentration of DNA per 100 μl of sample. The concentration of DNA extracted by the Promega bead kit was lower than desired, although this kit is not specifically designed for the extraction of bacterial DNA from whole blood.

DISCUSSION

A rapid diagnosis is crucial to the treatment and recovery of individuals suffering from bacterial septicemia or meningitis. The development of new methods is important for the improvement of the sensitivity and specificity of such diagnosis. Although rapid and sensitive PCR methods have been developed in recent years, there is a lack of fully automated methods for the extraction of bacterial DNA from body fluids, particularly whole blood. In this study, a comparison of five DNA extraction kits was performed and methods were developed for the kits which were not initially designed for the extraction of bacterial DNA from whole blood. The sensitivity, specificity, and efficiency of the kits were compared. The two most successful kits in all areas were the Bilatest bead DNA 2 kit and the Promega Wizard SV96 kit.

Particular problems encountered with the kits were related to the Millipore Montage and Promega bead kits. With the former, the plate bent in the middle when the required vacuum pressure was applied, leading to cross-contamination of samples. This is undesirable in a situation where clinical samples have to be extracted for diagnostic testing. With the latter kit, the DNA concentration was lower than desired, although this kit is not specifically designed for the extraction of bacterial DNA from whole blood. It is more suitable for the extraction of plasmid, so this may have been the cause of the poor yield.

The choice of kit depends on the needs of the user, whether he prefers a 96-well filter plate format or a metallic bead-based system. Both kits can successfully produce good quality bacterial DNA. These kits are both highly sensitive and produce DNA at a desirable concentration for use with PCR and DNA sequencing techniques. Furthermore, both kits are easily automated and very simple to use. They are capable of processing 96 samples in a short time period, without the need for manual intervention. This allows labor to be concentrated in other areas and leads to a more rapid diagnosis of disease. When

used for the detection of meningococcal, pneumococcal, and *H. influenzae* DNA from whole blood by fluorescence PCR, the method remained specific and sensitive.

ACKNOWLEDGMENTS

We thank Steve Hughes at MWG Biotech (United Kingdom) Ltd. and Steve Keeney at Manchester Royal Infirmary, Manchester, United Kingdom, for help and advice.

This project was supported by MWG Biotech AG, Ebersberg, Germany, and Wyeth, Maidenhead, United Kingdom. Funding for the liquid-handling robot was generously provided by the Meningitis Association (Scotland) and National Services Division of the Scottish Executive.

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