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A Magnetic Nanoparticle Based Nucleic Acid Isolation and Purification Instrument for DNA Extraction of *Escherichia Coli* O157: H7

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The objective of this study was to evaluate the performance of a nucleic acid isolation and purification instrument using *Escherichia coli* O157:H7 as the model. The instrument was developed with magnetic nanoparticles for efficiently capturing nucleic acids and an intelligent mechanical unit for automatically performing the whole nucleic acid extraction process. A commercial DNA extraction kit from Huier Nano Company was used as reference. Nucleic acids in 1 ml of *E. coli* O157: H7 at a concentration of 5×10^8 CFU/mL were extracted by using this instrument and the kit in parallel and then detected by an ultraviolet spectrophotometer to obtain *A260* values and *A260/A280* values for the determination of the extracted DNA's quantity and purity, respectively. The *A260* values for the instrument and the kit were 0.78 and 0.61, respectively, and the *A260/A280* values were 1.98 and 1.93. The coefficient of variations of these parallel tests ranged from 10.5% to 16.7%. The results indicated that this nucleic acid isolation and purification instrument could extract a comparable level of nucleic acid within 50 min compared to the commercial DNA extraction kit.

Keywords: DNA Isolation and Purification, Magnetic Nanoparticle, *Escherichia Coli* O157:H7, Ultraviolet Spectrophotometer.

1. INTRODUCTION

First identified in 1982 as a human pathogen, *Escherichia coli* O157: H7 is one of the most important foodborne pathogens nowadays, since it has been responsible for severe outbreaks worldwide.[1] Therefore, rapid and sensitive methods are needed by regulators and the food industry to detect very low numbers of pathogens in foods in the effort to protect consumers from foodborne illness.[2] Polymerase Chain Reaction (PCR) is a technique in molecular biology, developed by Kary Mullis in 1983,[3] and has been widely used in the detection of *E. coli* O157:H7 due to its rapidness, high specificity and sensitivity.[4–8]

In our previous research, a microfluidic real-time PCR system research prototype for rapid detection of pathogens was developed. The prototype was composed of a thermal cycler for precise and fast control of the PCR temperatures, a microfluidic chip for loading PCR reagents and a fluorescence detector for real-time monitoring of the fluorescent signal. It had been evaluated by using avian influenza virus as a model and consistent results were obtained compared to standard viral isolation method. The evaluation study on foodborne pathogens is ongoing.[9]

Obtaining sufficient amount of genomic DNA that is suitable for PCR is a critical factor.[10] Conventional methods for nucleic acid extraction include phenol-chloroform method, alkaline extraction method, and CTAB extraction method.[11] These methods have limitations, such as time-consuming, labor-intensive, high requirements on facilities and technicians, or variability caused by manual operation,[12] and these limited PCR technology are to be extended in the basic food safety public service agencies in China. With the development of micro-electro-mechanical systems (MEMS) and microfluidic chip or Lab-on-a-chip, studies on microfluidic chip for DNA extraction have been reported recently and shown great potentialities for the practical applications.[13–16] Microfluidic chip based DNA

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extraction is a new approach to fully automate the DNA extraction procedures on a centimeter or smaller platform. The benefits of these sample-in–answer-out devices include shorter analysis time, reduced reagent use and reduced sample consumption. However, they have limitations as well, such as low sensitivity, high fabrication cost, and complex deep reactive ion etching protocol.

With the development of nanotechnology for synthesis of SiO_2 coated magnetic nanoparticles,[17, 18] magnetic nanoparticle based nucleic acid extraction method has been widely used in purification of nucleic acid nowadays.[19] Compared with those conventional methods, this method has many advantages, such as time-saving, cost effective without using toxic compounds, e.g., phenol or chloroform,[20] and automatic process.[21] In this study, we intended to develop a nucleic acid isolation and purification instrument with magnetic nanoparticles and evaluate it with *E. coli* O157: H7. This instrument will be integrated with the microfluidic PCR system through a micropump to provide a systematic solution for a rapid screening of foodborne pathogens.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Phosphate-buffered saline (PBS, 0.01 M, pH 7.4) was obtained from Sigma-Aldrich (St. Louis, MI, USA). Isopropyl alcohol was supplied by Beijing Chemical Works (Beijing, China). Deionized water (18.2 MΩ · cm) produced by a Millipore Mill-Q system (A10, Bedford, MA, USA) was used throughout. HRXJ-0030 DNA extraction kit was purchased from Henan Huier Nano Technology Company (Henan, China).

2.2. Culture and Plating of Bacteria

Forzen stock of *E.coli* O157: H7 ZDBE 2001 (Nanchang University, Nanchang, China) was maintained in brain heart infusion broth (BHI, Beijing Land Bridge Technology Co., Ltd., Beijing, China) at −70 °C. The culture was harvested in BHI maintained at 37 °C for 18–22 h. For enumeration, pure cultures were serially diluted in PBS and surface plated on sorbitol MacConkey (SMAC) agar (Beijing Land Bridge Technology Co., Ltd., Beijing, China), which was incubate at 37 °C for 20–22 h.[22]

2.3. Magnetic Nanoparticle

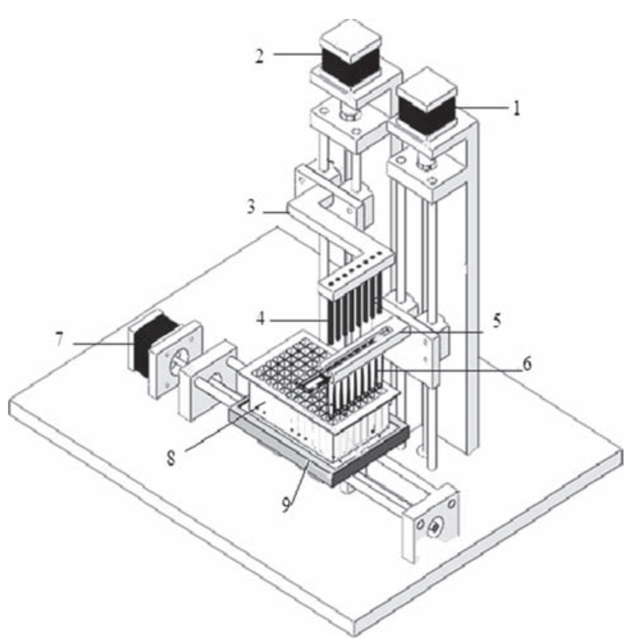
The magnetic nanoparticles with an average diameter of 200 nm were obtained from Henan Huier Nano Technology Company (50 mg/mL, Henan, China) and stored at 4 °C. They are made of Fe_3O_4, and coated with a matrix of silica with terminal functionalized groups. The procedure for magnetic nanoparticle to isolate nucleic acid included four steps:

(1) Lysis of the bacterial cells to release nucleic acids;
(2) Binding of the nucleic acids with the magnetic nanoparticles;
(3) Washing of the magnetic nanoparticles and the bound nucleic acids;
(4) Elution of the nucleic acid and removal of the magnetic nanoparticles.

2.4. Nucleic Acid Extraction Instrument

The magnetic nanoparticle based nucleic acid isolation and purification instrument included:

(1) three step motors (42BYGH, Wuxi Huisitong Company, Jiangsu, China) using an STM32F103VET6 microprocessor to precisely control the up-and-down motion (120 mm) of magnetic bars ($D = 3$ mm, $h = 60$ mm, Grade: N38 NdFeB), mixing bars ($D = 5$ mm, $d = 4$ mm, $h = 45$ mm), and the side-to-side motion (125 mm) of reagent cartridge respectively, and the control strategy was investigated by following magnetic particle based nucleic acid extraction method;
(2) a temperature controller using a Pt100 thermal sensor (±0.15 °C, 2.3 × 2.0 × 0.9 mm, Shen Zhen RTD Sensors Technology Co. Ltd., Shenzhen, China) to monitoring the temperature of the lysis and elution columns in the cartridge (96-well plate, Xi'an Tianlong Company, Shaanxi, China) and a high temperature ceramic heater (70 × 7 × 1.3 mm, Beijing Youpusi Company, Beijing, China) to heat the columns;
(3) a computer software developed in Visual Studio 2008 with C# language for parameter setting, running status monitoring, and data analysis.



1. Motor 1 **2.** Motor 2 **3.** Mechanical arm 1
4. Magnetic bars **5.** Mechanical arm 2 **6.** Mixing bars
7. Motor 3 **8.** Reagent cartridge **9.** Reagent cartridge base

Figure 1. The schematic drawing of the magnetic nanoparticle based nucleic acid isolation and purification instrument.

As shown in Figure 1, the first column of the 96-well plate on the left was used as the lysis column, followed by a binding column, three wash columns and an elution column, respectively. The instrument was capable of extracting DNA from eight samples per run within 50 min.

2.5. Nucleic Acid Extraction

As the recommended method to extract nucleic acid in the manual of the extraction kit, ten-fold dilution of pure *E. coli* O157: H7 culture at a starting concentration of 5×10^9 CFU/mL was prepared in PBS. Then, 1 mL of *E. coli* O157: H7 suspension at a concentration of 5×10^8 CFU/mL was transferred to a 1.5 mL centrifuge tube. Quadruplicate nucleic acid extractions were performed according to the recommended method. The input volume was 1 mL, and elution volume was 200 μL.

For nucleic acid extraction by this developed nucleic acid isolation and purification instrument, triplicate nucleic acid extractions were performed using HRXJ-0030 DNA extraction kit. Briefly, 1 mL of *E. coli* O157: H7 suspension was added to a 1.5 mL centrifuge tube and centrifuged at 12,000 g for 1 min. After the supernatant was aspirated, 315 μL of lysis buffer was transferred to the tube to resuspend the bacteria and then transferred to the lysis column in the cartridge and incubated for 20 min at 70 °C. The instrument accelerated the lysis of bacteria cells by moving a mixing bar on the mechanical arm up-and-down. Then, the lysis solution was transferred to the binding column containing 350 μL of isopropyl alcohol and 10 μL of 200 nm silica-modified magnetic nanoparticles (50 mg/mL). After mixing for 2 min and magnetic separation for 2 min, the magnetic nanoparticles were transferred to the wash columns with 500 μL of wash buffer using a magnetic bar on the mechanical arm to capture the nanoparticles with nucleic acids. The nanoparticles were released and recaptured after suspending in wash solution for 3 min. The wash step was repeated three times. Dry the magnetic nanoparticles in the air for 2 min, then transfer the magnetic nanoparticles to the elution column containing 200 μL of elution buffer and incubate for 10 min at 65 °C. In the end, remove the magnetic nanoparticles from elution column and collect the eluate into the tubes.

2.6. Nucleic Acid Yield and Purity Measurement

The yield and purity of the isolated and purified nucleic acids were determined using NanoDrop 1000 spectrophotometer (ND Technologies, Wilmington, DE, USA) to measure the absorbance of the nucleic acids in the elution buffer. The elution buffer in the extraction kit was used as blank. The absorbance at 260 nm (*A260*) was measured for each sample and used to assess the yield of nucleic acid. The ratio of A260 to A280 (*A260/A280*) was measured for each sample and used to estimate the purity. *A260/A280* values of 1.8–2.0 are generally considered relatively free of protein contamination.[23]

2.7. Magnetic Separation

The magnetic nanoparticles at different concentrations were prepared by separating the magnetic nanoparticles and resuspended by certain volume of PBS. The nanoparticles were pipetted into the cartridge, captured by the magnetic bar for certain time and transferred to a centrifuge tube containing the same volume of PBS. Prior to optical measurement, the nanoparticles were vortexed for 30 s and the absorbance at the wavelength of 400 nm was measured using a USB4000 spectrometer from Ocean Optics. Deionized water was used as blank. The tests were repeated twice for each measurement.

The separation efficiency was calculated by the following equation:

$$SE(\%) = \frac{C_a}{C_b} \times 100 \tag{1}$$

where, SE is the separation efficiency; C_b is the concentration of magnetic nanoparticles before separation, μg/mL; C_a is the concentration of magnetic nanoparticles after separation, μg/mL.[24]

3. RESULTS AND DISCUSSION

3.1. Magnetic Separation

3.1.1. Calibration

The magnetic nanoparticles at the concentrations of 25, 50, 75, 100 and 125 μg/mL were used for calibration curve. As shown in Figure 2, the calibration curve showed a linear relationship between the absorbance (A) at 400 nm and the magnetic nanoparticles concentration (C) and was described as $A = 0.0132\text{C}–0.1145$ ($R^2 = 0.99$).

3.1.2. Magnetic Separation Efficiency

The separation efficiency of the nanoparticles is vital for the nucleic acid extraction. Magnetic nanoparticles at different concentrations of 1, 1.5 and 2 mg/mL and different separation time from 0.5 min to 2.5 min were used for measuring the magnetic separation efficiency. As shown in

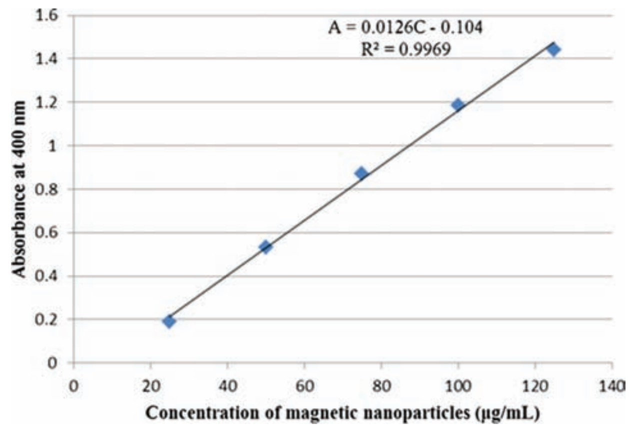


Figure 2. The linear relationship between the absorbance at 400 nm and the magnetic nanoparticles concentration.

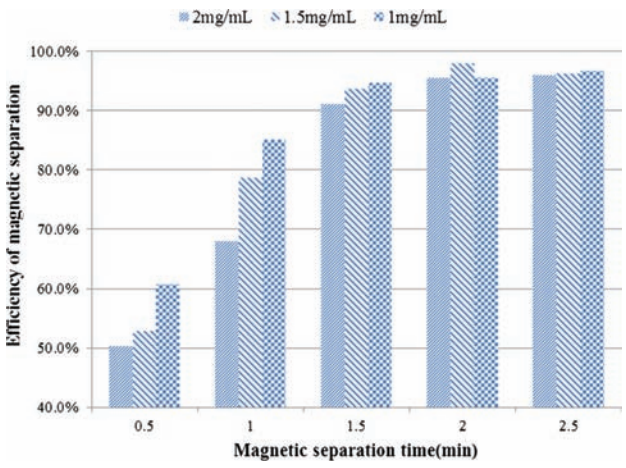


Figure 3. Magnetic separation efficiency at different separation time.

Figure 3, the turning point of separation time was between 1.5 min and 2 min, and the magnetic separation efficiency was greater than 90%. Thus, the separation time of 2 min was applied for nucleic acid extraction tests.

3.2. Nucleic Acid Extraction

Comparisons were made between the yield and purity of nucleic acid extracted by the recommended method and this developed instrument from *E. coli* O157: H7 suspension at a concentration of 5×10^8 CFU/mL. As shown in Figure 4, the average *A260* for the recommended method and this developed instrument were 0.61 and 0.78, respectively, indicating that the amount of the nucleic acids extracted by this developed instrument was more than that by the recommended method. Both *A260/A280* values were between 1.8 and 2, indicating that the extracted nucleic acids did not suffer contaminations.

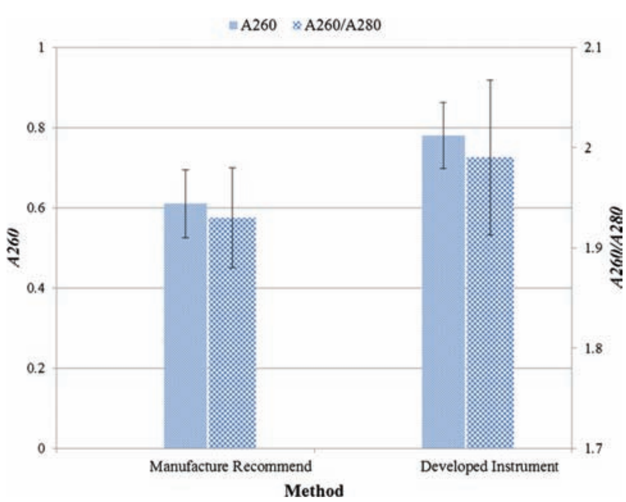


Figure 4. Yield and purity of nucleic acid extracted from *E. coli* O157: H7 by recommended method and the developed instrument.

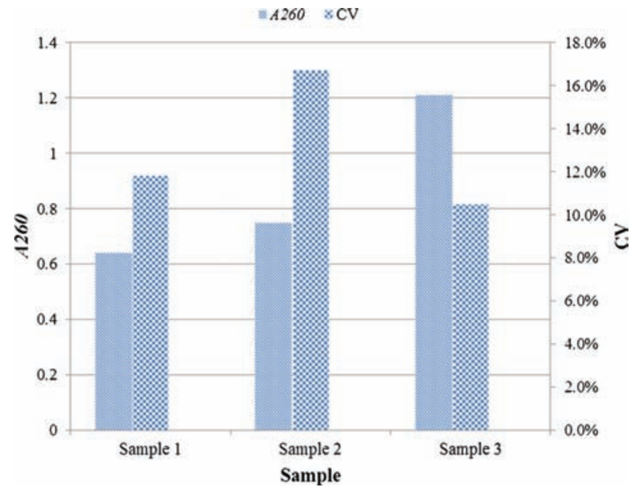


Figure 5. Repeatability of the developed instrument.

3.3. Repeatability

Triplicate tests of different samples were executed in parallel to evaluate the repeatability of the developed instrument. As shown in Figure 5, the coefficient of variation (CV) of the *A260* value for sample 1, sample 2 and sample 3 were 11.8%, 16.7% and 10.5%, respectively, indicating that the repeatability of the developed instrument was fair.

4. CONCLUSIONS

In this study, magnetic nanoparticles based nucleic acid isolation and purification instrument was developed for efficient and automated extraction of nucleic acids from the bacterial specimen. Compared to the recommended method by the manufacture of the commercial DNA extraction kit, the developed instrument showed a better extracting efficiency in the extraction of *E. coli* O157:H7, but the repeatability of this developed instrument were not very ideal probably due to the diversity of the parallel samples. This developed instrument will be improved and integrated with the microfluidic PCR instrument to provide a simple, low-cost and fast screening of foodborne pathogens in the near future.

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