Analytica Chimica Acta 906 (2016) 1–6

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

A novel automated device for rapid nucleic acid extraction utilizing a zigzag motion of magnetic silica beads

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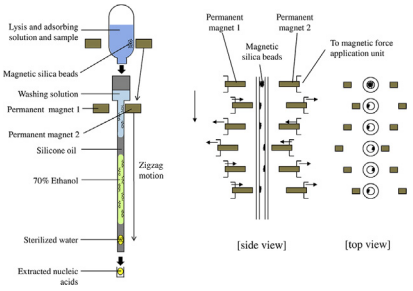
HIGHLIGHTS

- Automatic nucleic acid extraction is performed in 3 min.
- Zigzag motion of magnetic silica beads yields rapid and efficient extraction.
- The present our device provides better performance than the conventional procedure.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 August 2015
Received in revised form
8 October 2015
Accepted 17 October 2015
Available online 24 October 2015

Keywords:
Nucleic acid extraction
Magnetic silica beads
Zigzag motion
Automated device

ABSTRACT

We report a novel automated device for nucleic acid extraction, which consists of a mechanical control system and a disposable cassette. The cassette is composed of a bottle, a capillary tube, and a chamber. After sample injection in the bottle, the sample is lysed, and nucleic acids are adsorbed on the surface of magnetic silica beads. These magnetic beads are transported and are vibrated through the washing reagents in the capillary tube under the control of the mechanical control system, and thus, the nucleic acid is purified without centrifugation. The purified nucleic acid is automatically extracted in 3 min for the polymerase chain reaction (PCR). The nucleic acid extraction is dependent on the transport speed and the vibration frequency of the magnetic beads, and optimizing these two parameters provided better PCR efficiency than the conventional manual procedure. There was no difference between the detection limits of our novel device and that of the conventional manual procedure.

We have already developed the droplet-PCR machine, which can amplify and detect specific nucleic acids rapidly and automatically. Connecting the droplet-PCR machine to our novel automated extraction device enables PCR analysis within 15 min, and this system can be made available as a point-of-care testing in clinics as well as general hospitals.

1. Introduction

Genetic tests based on polymerase chain reaction (PCR) are widely used for the diagnosis of viral and bacterial infections. For

Abbreviation: PCR, polymerase chain reaction.

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http://dx.doi.org/10.1016/j.aca.2015.10.011
0003-2670/

early and accurate identification of the causative pathogen, these tests need to be rapid and sensitive. However, the conventional PCR procedures require several steps, namely, extraction, amplification, and identification of specific nucleic acids, and therefore take a long time to obtain the results. Moreover, skilled laboratory technicians are required, and the research analysis facilities are available only in relatively large hospitals. If rapid and automated PCR tests can be made available, they can be used as point-of-care testing for the diagnosis of infectious diseases even in outpatient clinics.

Nucleic acid extraction is one of the essential steps in genetic analyses. Various methods of nucleic acid extraction have been reported so far [1–4]. One of the commonest methods uses a mixed bed solid-phase nucleic acid extraction method [2–4], which consists of the following 3 steps: (1) adsorbing nucleic acids to silica beads in the presence of a chaotropic salt; (2) washing to remove inhibitors and chaotropic salts; and (3) eluting nucleic acids from silica beads. In this method, the silica membrane or magnetic silica beads was used as a solid-phase carrier. Centrifugation or pipetting is necessary in each step to wash, remove, and elute, which impedes the miniaturization of the extraction device and automation of the processes. Many studies have been performed with magnetic silica beads that are actuated by permanent magnets; in such cases, the devices for rapid extraction are miniaturized systems [5–13]. Although the methods using the beads do not require centrifugation, the reagents around the beads cannot be removed, thereby potentially inhibiting PCR efficiency.

Clinical samples contain many inhibitors such as viscous substances (mucin and serum), hemoglobin, lysozyme, and lactoferrin, which interfere with PCR analysis [14,15]. Additive reagents and chaotropic salts also inhibit PCR. Therefore, the washing step is another very important process in nucleic acid extraction. Unnecessary proteins and salts are efficiently eliminated by using solid-phased silica.

In this paper, we describe a novel automated device that consists of a mechanical control system and a disposable cassette, which enables nucleic acid extraction within 3 min. We compared the extraction efficiency and the detection limit of our device with that of the conventional manual procedure.

2. Materials and methods

Our novel automated device for nucleic acid extraction is composed of two parts, which are a mechanical control system and a disposable cassette (Fig. 1A).

2.1. Mechanical control system

The mechanical control system has a motor, a magnetic force application unit, and a pair of permanent magnets (permanent magnet 1 and permanent magnet 2). The motor transports the magnetic silica beads in the longitudinal direction while the magnetic force application unit vibrates the beads in the transverse direction, thus influencing the transport speed and the vibration frequency of the magnetic beads. The transport speed of the magnetic beads through the washing reagents depends on the longitudinal movement of the permanent magnets (Fig. 1B). The permanent magnets outside the capillary tube also vibrate the magnetic beads, which are gathered and dispersed within the capillary tube by repeated shifts of the permanent magnets 1 and 2. This zigzag motion of the magnetic beads is a specific feature of our device (Fig. 2).

2.2. Disposable cassette

The disposable cassette is composed of a bottle, a capillary tube, and a chamber (Fig. 1B), all of which are made of polypropylene. The inner diameter of the capillary tube is 1 mm. Nucleic Acid Extraction Kit MagExtractor™ – Genome – (Toyobo Co., Ltd., Osaka, Japan) was used as the reagent for nucleic acid extraction. The maximum concentration of the magnetic silica beads is 50% in 5 M of lithium chloride solution. The bottle holds 700 μL of the solution for lysis and adsorbing, 4 μL of magnetic silica beads, and 100 μL of the sample. The capillary tube holds 280 μL of the washing solution, 22 μL of 70% ethanol, and 4 μL of sterilized water. Each of these reagents in the capillary tube is separated by silicone oil as plugs. The sample (100 μL) is put into the bottle and mixed by shaking for 30 s by hands until the nucleic acids are adsorbed on the surface of the magnetic

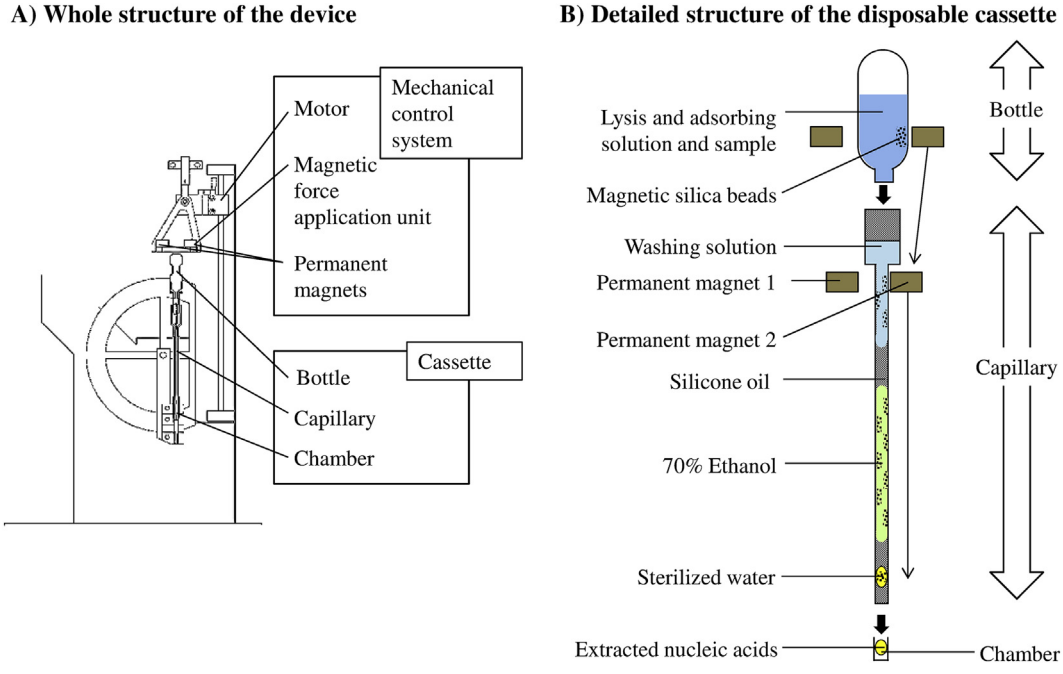

Fig. 1. Diagram of the automated nucleic acid extraction device.