Flow-through cell for DNA extraction

© D.G. Petrov, I.E. Antifeev, M.V. Zaitseva, N.N. Germash, K.A. Simonova

Institute of Analytical Instrument Making, Russian Academy of Sciences, St. Petersburg, Russia
E-mail: dimoon88@mail.ru

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DNA sample preparation before testing is a routine process. However, significant human-related risks and errors arise when one works with samples containing a small amount of the target product or with large-volume (more than 5 ml) samples. The only way to eliminate the human factor is to design a fully automatic device. A flow-through chamber or cell, which offer a number of advantages over closed-volume systems, may form the basis of such a device. The results of experiments with a cell modification providing a nucleic acid extraction efficiency above 80 %, which is sufficient for a reliable result of the real-time polymerase chain reaction, are presented.

Keywords: DNA, flow-through systems, sample preparation, extraction, purification, concentration.

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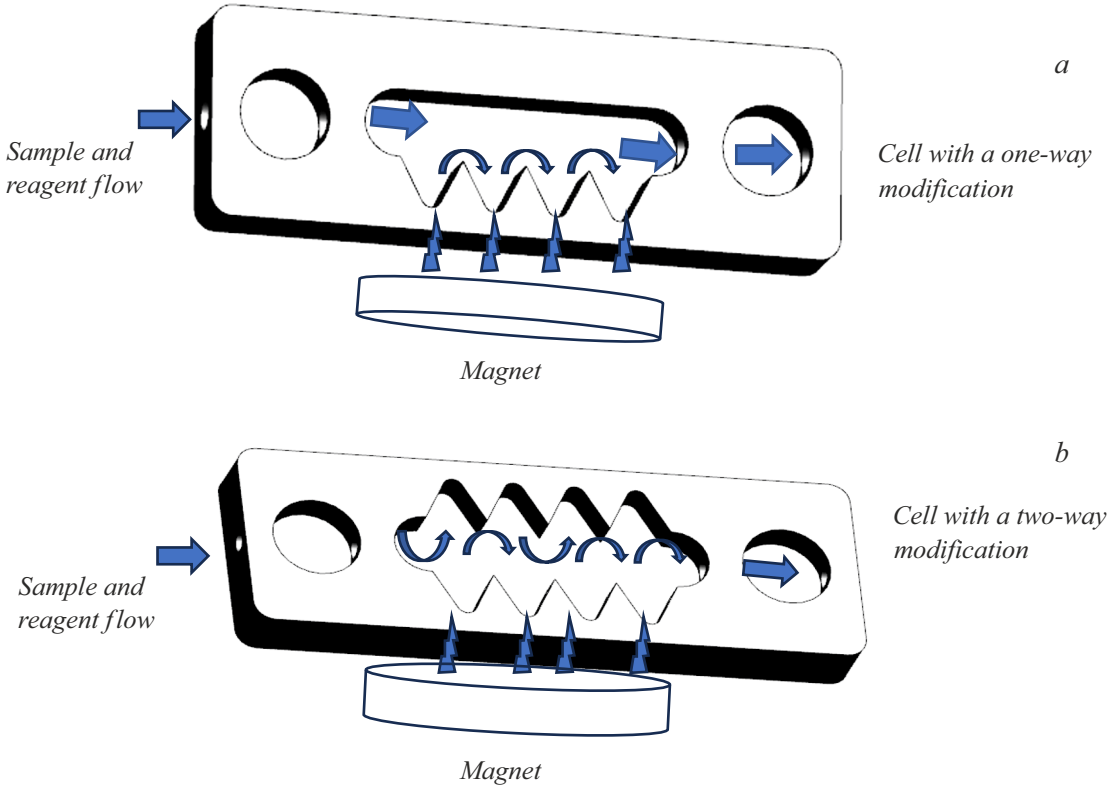
Sample volumes greater than 1−2 ml are typically used in the analysis of environmental samples and in such cases where the target product amount is close to or outside the detection limit of methods of subsequent analysis. The maximum sensitivity of the real-time polymerase chain reaction (RT-PCR) method is 1−10 molecules per 1 μl. If a laboratory technician needs to analyze the entire volume of the received sample, duplicate sample preparation is inevitable, which has a significant effect on the reliability of further analysis, since the human factor is crucial in flow studies of samples [1]. This leads to loss of the target product; in the case of nucleic acid (DNA/RNA) analysis, a common corollary is a false negative result of the entire analysis, since such methods as RT-PCR are highly sensitive and react to changes in the amount of analyzed DNA/RNA [2].

One way to solve this problem is to develop a flow-through method for extraction, purification, and concentration of nucleic acids that would allow for efficient (more than 80 % of the initial amount) extraction of DNA from samples larger than 1 ml and ensure a degree of concentration sufficient for reliable analysis by RT-PCR (i. e., more than one molecule of the target product per 1 μl [3]), which is one of the most sensitive current techniques.

The key element of such a flow-through system is a modified cell with a sorbent or filter section where DNA/RNA molecules from the sample are retained. Extraction of the target product is achieved this way; it is also necessary to choose the proper conditions for efficient extraction process with flow-through concentration. To design such a flow-through cell, we analyzed several types of geometry of the channel through which the sample is passed. Diagrams of flow-through cells are shown in the figure. In literature, flow-through cells for concentrating the target product are found mostly in analytical chemistry problems [4]. Various disposable cartridges, where the input sample volume is also limited, are normally used for DNA concentration [5].

The diagrams present cell designs for high DNA/RNA concentration. The cell is made of polydimethylsiloxane (PDMS). It may also be fabricated from such materials as polypropylene, polycarbonate, etc., that are resistant to reagents for the extraction of nucleic acids (isopropyl alcohol, guanidine thiocyanate). PDMS is convenient for rapid prototyping and replication of products by thermoforming [6,7].

Prior to performing experiments for evaluation of the efficiency of DNA extraction in the flow-through cell, we needed to choose the shape of the flow-through channel that retains the magnetic sorbent at a sample flow rate of 1 ml/min. The way to do this is to determine (select) the geometric size and shape of irregularities of one or two walls of the cell to which the magnet, which restrains the magnetic sorbent, is adjacent. When the sample and subsequent reagents are introduced, modification of the wall geometry provides a degree of mixing that directly affects the efficiency of extraction. The option with a change in the direction of motion of liquid passing through the cell was analyzed. Experimental data revealed no increase in the efficiency of extraction with intensification of mass exchange processes, which seems obvious. The results of measurements of the efficiency of nucleic acid extraction at a flow rate through the cell of 1 ml/min are listed in the table. It also presents the results of measurements where the geometric dimensions of the modified wall of the flow-through cell were maintained and the distance between the walls was changed (i. e., the size of the main free channel of the cell was increased). Triangular irregularities with a height of 2 and 3 mm (see the figure) were formed in the walls of the flow-through channel of the cell. The linear size of the cell was constant (length, 15 mm; width, 8 mm). The distance between the walls (channel width) was 2 mm in the unmodified part and 5 or 7 mm in the modified part. The channel height was 2, 3, and 5 mm. The main objective of the experimental part of the study was to determine the difference in the efficiency of DNA extraction in cells with



Flow-through cell diagrams. *a* — Cell with a one-way modification; *b* — cell with a two-way modification.

Efficiency of nucleic acid extraction (in %) at a liquid flow rate through the cell of 1 ml/min

Modification of cell	Channel height					
	2 mm		3 mm		5 mm	
	Modification height					
	3 mm	2 mm	3 mm	2 mm	3 mm	2 mm
One-way	53	57	78	75	56	48
Two-way	64	69	81	79	67	62

different wall modifications at the same sample flow rate and thus identify the most efficient cell design.

The presented data make it clear that the greatest efficiency is achieved in a cell with a two-way modification and a modification height of 3 mm.

This result illustrates the feasibility of application of such a flow-through cell in the production of instruments for DNA sample preparation in the flow mode.

During operation, the cell is positioned horizontally with the magnet adjacent to the modified wall. Each value presented in the table is the average result of three repetitions. The error of each extraction did not exceed 5%, which is comparable to the error provided by a laboratory dispenser. Comparing the cells with one-way and two-way channel modifications, one sees that the cell with a two-way modification is preferable, since it provides a higher efficiency. The presented schematic diagrams of liquid flow illustrate a certain ideal model; since solutions with different properties are used in extraction, the distribution of flows within the cell will change, but the key features will be preserved with variation of the channel height. The data were obtained by modeling in COMSOL Multiphysics.

E. coli cells grown in a nutrient medium (i. e., a sample of laboratory origin) were used in the present study.

Conflict of interest

The authors declare that they have no conflict of interest.

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