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A Membrane-based Sample Preparation Kit for Waterborne Pathogens: Design and Manufacture

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Abstract. Membrane-based microfluidic devices have been demonstrated in recent literature to show a significant potential in developing low-cost but high-efficient analytical devices. Usually, the step of sampling and sample preparation is the most importance processes in the whole analytical experiments. This study designed and manufactured a low-cost kit for water sampling and sample preparation of waterborne pathogens, especially protozoan parasites. Subsequently, *Saccharomyces cerevisiae* was employed as the model microbe to verify the function of kit. The concepts of green design and agile manufacturing were reflected throughout this work. In the devices, membrane filters were fixed and locked in a pair of disposable filter holders, and then the filter set would be assembled with a volumetric sample container to filter the microorganism in water samples. After the sampling process, the used filter holder with microbes on the membrane would be taken out and conserved in a preservation buffer, which could protect the DNA/RNA molecules inside the cells. When these filter holders were transported to a remote laboratory, the sample preparation cassette will be used in the on-site extraction of the DNA/RNA from the cells on the membrane. At last, the eluate was made for further identification, i.e. NASBA tests. Eight kinds of candidate membrane filters were evaluated in the kit, and the function of the kit was verified.

Introduction

One of the most importance natural resources in the world is water, especially fresh water. Great efforts and funding have been invested in keeping the water clean and safe. However, in spite of the advanced achievement in water treatment technologies, the outbreak of waterborne diseases still threatens the water safety in both developed and developing countries. A main threat comes from waterborne pathogens in the drinking water, which relates to "waterborne disease". Within waterborne pathogens, there are many parasites that have been documented to cause a lot of the waterborne disease cases, i.e. cryptosporidia, giardia etc. [1] It's necessary to carry on the routine examinations on parasites in the water source and drinking water system, which will be very helpful to the control of diseases. Generally, the current method of sampling water is very primitive. The water sample usually is measured with a container and sent to a laboratory. When an out-of-door investigator for water environment has to carry on dozens of sampling processes within one day, the big volume of samples will be an important factor to the operator.

To solve this operational problem, a set of sampling devices based on membrane filters and its sample preparation kit were designed. These devices aims at various microorganisms, only by the replacement of the membrane filter with a different pole-size. To study the protozoan parasites which diameters are around 10μm, cultured yeast cells (Saccharomyces cerevisiae) with similar sizes were selected here as a model to demonstrate the function of the devices, because S. cerevisiae is a safe and well-studied microorganism and its properties have been made clear. Water samples

can be easily collected and filtered by the device on the spot, and then the microorganisms in 1 or 2 liters water have been condensed onto the membrane filter, so that the investigator no longer need to carry a large volume of liquid samples. The sampling and transport process could be facilitated greatly; then the membrane filter will be soaked in about 10ml transport medium buffer after the filtration immediately to preserve the DNA/RNA. After replacement of the filter set, the sampling device can be used again. In order to facilitate the operators and avoid sample loss caused by disassembly of filter holder, a sample preparation cassette that can directly accommodate the filter holder was also designed, which can operate the DNA/RNA extraction processes on the membrane. The device can avoid sample loss as much as possible in the sample handling. Furthermore, the entire operation is performed in a closed, standardized, automated device, which could also reduce contaminations.

Materials and methods

Green design of the sampling device and sample preparation cassette. This study aimed to develop a low-cost sample preparation kit for out-door environmental investigators. One basic design strategy is to employ as many commercialized products as possible in the kit, so as to reduce the R&D expenses and the manufacture cost of single product in the future. Therefore, the standard membrane filters with 25mm diameter were selected for this kit. The design of other components was based on this precondition.

The sampling device is composed of two components, the filter set and volumetric container, shown in Fig. 1. The volumetric container is originally modeled on a 2L container for edible oil. Thereupon, the container from the commercial package of edible oil could be used with a little modification in the testing of prototypes. The cost of blow molding could be saved at the R&D stage. The filter set is the smallest component. It consists of two pieces filter holder with a central symmetry and self-locked structure. Since two pieces filter holder is totally the same, this design could save 50% of the cost on the mold for filter holders. A pair of filter holders can accommodate one or two pieces of membrane filters (Ø=25mm, thickness≤0.7mm), which usually include a piece of pre-filter and a piece of working filter with a definitive pore size. The filter holders and membrane filters are disposable, while the other parts of the sampling device are designed to be reusable, so the consumption of each sampling process is rather low. The filter set could be fixed on the bottle of a volumetric container by two waterproof nuts, see Fig 1. There is a plug cock at the bottom of the container, which could turn open during the filtration. The filter set can be disassembled from the container after the filtration, see Fig 2.

The sample preparation cassette has a compact multi-layered structure built upon four thin layers of Polydimethylsiloxane (PDMS) and two stainless steel layers (top and bottom). The total thickness of the cassette is about 10mm. The self-locked filter holders will be inserted precisely in the slot on the middle PDMS layer. The other 3 PDMS layers are to form two flow channels on the both sides of the filter holder, and each channel lead to the inlet or outlet port in the top layer. In the central of top layer, there is an additional port for a plastic syringe, which is the port to load samples, see Fig 3. The top 3 layers adhered to each other to form a whole top part. So did the bottom 3 layers.

Fabrication of devices. The devices were designed using Solidworks® software and then performed fluid field analysis by finite element simulation as the previous work [2-4]. The prototypes of the sampling kit were fabricated by SLS rapid prototyping using polyamide as the primary material, which is able to approximate common engineering plastic materials. The prototype of volumetric container was rebuilt from a commercial container for edible oil. But blow molding technology would be employed in the production of enormous quantities.

As to the sample preparation cassette, the stainless steel components were processed by numerically-controlled machine tools. PDMS wafers were prepared on a silicon wafer by spinning the mixture of polydimethyl-siloxane(DMS-V22, Gelest© Inc., USA), methylhydrosiloxane-

dimethylsiloxane copolymer(HMS-151, Gelest© Inc.,USA) and the catalyst (Platinum/Vinylsiloxane catalytic complex, Abcr© Inc., Germany)[5]. After that, the thin films of PDMS were cut by a laser cutting machine (JK320, Jingke© Laser equipment Co. Ltd, Liaocheng, China) and bonded together by oxygen plasma as described before.[6] There are holes for screws at four corners.

Operation of the sampling device and sample preparation cassette. A proper membrane filter was loaded into two filter holders, and then they were aligned and rotated to be locked; water sample was filled in the volumetric container as much as possible, and then the volume of water samples was read at the first time, recorded as V_1; after the filter set was assembled to the bottle of the container, the entire container would be placed upside down in a bracket vertically; open the plug cock of the container at the bottom, so that the liquid could go through the filter set under its own gravity; Wait for approximately 10 minutes, the plug cock could be closed to terminated the filtration. Read the volume of water again, recorded as V_2, then the difference of V_1 and V_2 was the volume of water that is filtered. After that, the filter set can be disassembled from the container, and be soaked in a small container with 10ml transport medium buffer immediately. And then a new sampling process can restart.

Once the filter set was inserted into the bottom slot, the top part and bottom one will be coordinated and fixed by screws at four corners. Then the inlet and outlet ports were connected to OMNIFIT© adapters and then a valve and pipe system. NucliSens Magnetic Extraction Reagents (BioMerieux®, Franch) was used in this device. The procedures of extraction DNA/RNA in the cassette are as followed, (all the following reagents were added from the inlet port and discards from the outlet port, except transport medium buffer)

- The transport medium buffer that was used to conserve the sample was injected by a 10ml plastic syringe (BD®, USA) through the pore on the top of the cassette. Some pathogens may fall down from the membrane due to a long time soaking; Fix the piston using a clamp;
- Sterile air is pumped into the cassette to drive the liquid out;
- Close the outlet, but take away the clamp on the piston, then add 1ml lysis buffer to cassette; excess air will go into the syringe, but the membrane filter was immersed in the lysis buffer; Incubate at room temperature for 10min.Vortex the silica suspension (NucliSens Magnetic Extraction Reagents), Add 50μl silica to the cassette, and Incubate for 10 minutes at room temperature;
- Open the outlet again, press down the syringe and fix the piston using a clamp again; sterile air is pumped into the cassette again to drive the liquid out;

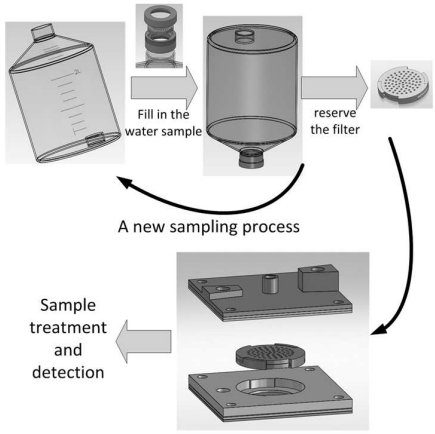

Figure 2 The diagrammatic sketch of sampling process

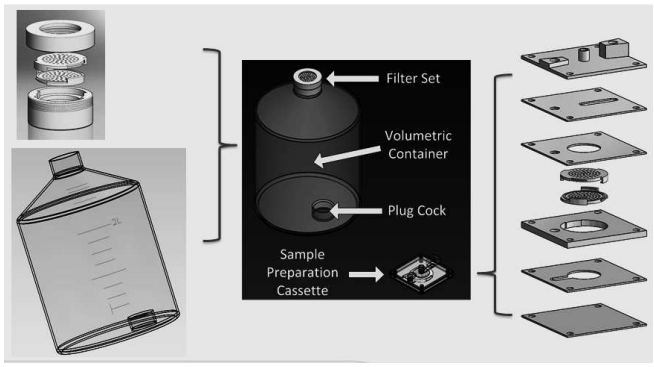

Figure 1 The components of the sampling device and sample preparation cassette

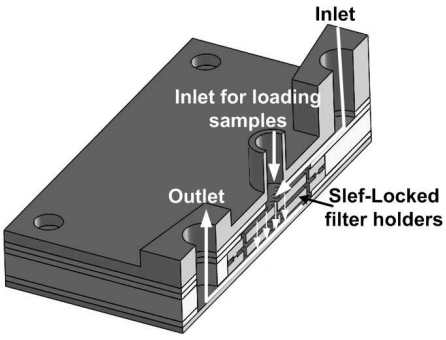

Figure 3 The structure of sample preparation cassette

- Add wash buffer 1(NucliSens Magnetic Extraction Reagents) 0.5ml and wash for 30s, and then dry the channel by air; and then repeat this step again;
- Add 500 µl wash buffer 2 to repeat the above steps;
- Wash once with 500µl wash buffer 3 for 15s and dry the channel again;
- Add 500µl Elution buffer and put the cassette on a Thermoshaker (Eppendorf®, Germany) for 5 minutes at 60°C;
- Using sterile air to drive the eluate (containing isolated nucleic acids) out of the cassette. Collect it in a RNase/DNase free tube, and use in an amplification procedure.

Testing of different membrane filters in the device. For the initial testing, a model system was set up to demonstrate the function of these sampling devices. In this model, *S. cerevisiae* cells were cultured in YPD mediums by ordinary methods. After the harvest, the cells were diluted and quantified using a haemacytometer under a microscope. The *S. cerevisiae* cells were used as the analog of dangerous protozoan parasite. Besides, eight kinds of membrane filters were tested in the cassette to deal with the artificial samples, including Nylon filter (10µm pore-size, from Millipore®), Cellulose filter (depth filter, from Millipore®),. Glass fiber filter (depth filter,~2µm pore-size, from Millipore®), Cellulose acetate(CA) filter(0.8µm pore-size,, from Whatman®), Polypropylene (PP) filter (2µm pore-size, from Tisch®), PP filter (0.45µm pore-size, from Tisch®), PP filter (0.45µm pore-size, from Whatman®) and PP filter (25µm pore-size, from Millipore®). In each testing, the concentrations of *S. cerevisiae* cells in the artificial sample were set at 1×10^6 cells·L^{-1}. The phenomenon during the filtration process would be observed carefully and the DNA/RNA extraction of each test was analyzed by NASBA assays using pan-fungal probe and primers according to previous studies.[7] Other reagents in NASBA assays were obtained from the PreTect™ HPV Proofer kit (Norchip©, Norway).

Results and discussion

Different membrane filters showed different performance in the filtration processes (Table 1). The samples prepared by this device can also be detected by NASBA tests perfectly, see Fig 4. The testing suggested that 3# glass fiber filter has an outstanding capability to capture almost all the targets in the sample, which proves that this filter is sufficient to meet the requirements of filtration physically. However, the glass fiber filter showed a weak mechanical strength in the testing, and it seems that DNA/RNA in the lysate can easily bind with the glass fibres. 6# PP filter (0.45µm, Tisch®) showed very low fluid resistance, good chemical inertness, strong mechanical strength and low absorption to DNA/RNA. After testing the combinations of different filters, we found the best combination for this device is 3# Glass fiber filter (Millipore®) as the pre-filter and 6# PP filter (0.45µm, Tisch®) as the working filter. Furthermore, it was found that the glass fiber is able to enhance the efficiency of sample extraction process. The operation time of sampling is usually less than 10 minutes. And the operation time of sample preparation is also half of the similar manual processing.

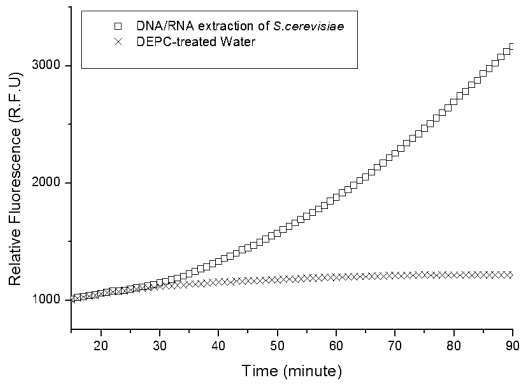

Figure 4 The typical result of NASBA test.

TABLE 1 SUMMARIES OF TESTING RESULTS ON FILTERS

Property	Type of Membrane filters							
	1#	*2#*	*3#*	*4#*	*5#*	*6#*	*7#*	*8#*
Fluid Resistance	L	L	L	H	L	L	H	L
Leakage of cells	L	H	L	L	L	L	L	H
Mechanical strength	H	L	L	L	M	M	M	H
Chemical Inertness	H	M	M	N	H	H	H	H
Absorption to DNA/RNA	L	L	H	N	L	L	L	L

a. 1# Nylon filter (10µm, Millipore®); 2# Cellulose filter (Millipore®);.3# Glass fiber filter (2µm Millipore®); 4# CA filter(0.8µm, Whatman®); 5# PP filter (2µm, Tisch®); 6# PP filter (0.45µm, Tisch®); 7#PP filter (0.45µm, Whatman®); 8# PP filter (25µm, Millipore®)

b. L: low; H: high; M:middle; N:uncertain.

Summary

The sampling and sample preparation kit was design and agilely manufactured. The testing results indicated that the sampling device is suitable for the investigation of pathogens in water environment. It is a convenient sampling tool with a wide range of applications. This paper not only reported a robust sample preparation kit for waterborne pathogens, but also demonstrated the concept of green design and fast prototyping, which is the key to such an object-oriented R&D program. The preliminary results indicated that the function of these devices has reached the primary objective of the study. More in-depth research is required for the optimization of the sample preparation kit.

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