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Advanced Application of Molecular Biology

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Abstract

Microbial contamination can affect any kind of water in any way: water from the surface, ground, sea, and even ice. Numerous sources of contamination exist, the majority of which are connected to human activities: reuse of effluents that have not been sufficiently treated, the use of animal waste as manure, and the disposal of wastewater that has not been treated for all living things to survive, water is essential. However, various types of water pollution, including chemical, microbiological, and other types can harm human and creature wellbeing and disturbs the trustworthiness of the climate many infectious pathogens that are excreted by infected hosts (humans or animals) can be transmitted through water to new hosts. Water-borne diseases, such as gastroenteritis, cholera, typhoid, and amebiasis, can be caused by these pathogens. These infectious diseases are typically spread through direct or indirect contact. These diseases are thought to be the primary cause of human morbidity and mortality worldwide and they may occasionally cause epidemics.

Keywords: Water • Concentration • Microorganisms • Filtration • Centrifugation • PCR

Introduction

The existence of microorganisms, such as bacteria, protozoa, and viruses in water is an important determinant of its safety and quality. In laboratories for monitoring water quality, conventional and classical methods are frequently used to identify these microorganisms; Nonetheless, the speed and precision of these methods' results are limited. The methods for analyzing water have greatly advanced as a result of the application of molecular biology. Nonetheless, the decision of the focus convention considering the best pace of microorganism recuperation in a suspension stays a genuine test. The goal of this exploratory review is to look at the recuperation pace of three unique conventions of water fixation (film filtration, filtration on cloth cushion and centrifugation) for tests planned for investigation by polymerase chain response PCR. Which can then act as a source of perspective convention for water quality control labs. The exploratory outcomes have shown that the film filtration convention yields the best recuperation rate and convergence of microorganisms followed by filtration on dressing cushion, while the centrifugation convention gives the most reduced pace of recuperation out of the three conventions. We are able to make a contribution to the standardization and optimization of concentration techniques for water samples intended for Molecular Biology analysis thanks to the experimental results obtained in this study [1].

Literature Review

The presence of microorganisms in the water stays a significant mark of the wellbeing of the populaces and climate. In laboratories that are responsible for controlling and monitoring the quality of the water, classical and conventional methods of analysis are frequently utilized. Be that as it may, these techniques are unreasonably tedious (possible, corroborative test) and at times a few microorganisms, for example, infections might be challenging to distinguish or they are not in an adequate amount in the water tests to have the option to be recognized. An alternative to culture-based microbiological methods for the detection and quantification of microorganisms is the use of the quantitative polymerase chain reaction and provides an efficient tool for quickly identifying and quantifying microorganisms in water.

Water samples absolutely need an initial phase of sample concentration, in contrast to the majority of biological samples for which PCR analysis involves extraction followed by amplification and detection. Microorganisms, such as bacteria, protozoa, and viruses are found dispersed in water matrices. The presence of suspended matter and other elements makes it difficult to analyze them, so they must be separated and concentrated first before being studied and found [2].

Discussion

Concentration protocols include membrane filtration, filtration on a gauze pad, centrifugation, and others. Analysts face a real challenge in selecting a method that will result in satisfactory recovery rates. Particle separation techniques used in a variety of microbiology fields include centrifugation and filtration with various supports.

The use of centrifugal force is necessary for centrifugation. Using this method, particles in a solution are separated according to their size, shape, density, medium viscosity, and rotor speed. The straightforward protocol that makes it possible to isolate more than two distinct types of cells is this method's primary benefit; anyway centrifugation is restricted to little volumes of water. Furthermore, centrifuged microorganism cells may be harmed by this method's low purity.

Numerous advantages of membrane filtration include: It is a quick and easy method that can be used with any volume of underbid water. The selection of the microorganisms to be tested may be influenced by the filtration medium's size and structure. According to the microorganisms tested, it can be utilized with a variety of membranes of varying composition and porosity and is inexpensive. However, the most significant drawback of this method is the possibility of membrane clogging. As a result, turbid water cannot be filtered. Additionally, in order for this protocol to function, a high differential pressure is required. Filtration on a gauze pad has the same advantages as membrane filtration, but it can also be used as a measure for turbid water (sewage and wastewaters) for which it was originally developed. Additionally, the performance of filtration on a gauze pad can be enhanced by adding an adjuvant

An experimental study is presented in this research paper to assist water analysis lab analysts in selecting the most effective concentration protocol for better microorganism recovery and subsequent use as a reference protocol [3].

Materials and procedures the molecular biology-based methods for analyzing water samples necessitate an initial concentration phase. It is crucial to select a method that provides a higher microorganism recovery rate. Three concentration

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protocols for water samples intended for Molecular Biology analysis are compared in this experimental study. The principal approach is called layer filtration utilizing a film with 0.45 µm pore size and 47 mm of breadth. The second method is gauze pad filtration and the final one is the centrifugation protocol employing.

This experimental work will be carried out on two different matrices: 1) a wastewater matrix characterized by a high concentration of microorganisms and suspended matter; 2) a natural water matrix (surface water) with a low concentration of microorganisms. The goal is to guarantee that the outcomes got are free from the sort of network and test the viability of the fixation conventions in the instances of tests rich or poor in suspended matter.

The two water samples (natural and wastewater) used in this study were doped with a specified concentration of an E. coli strain before being divided into three parts and subjected to the three distinct concentration protocols outlined above, followed by a common extraction phase, in order to compare the recovery rates of various concentration protocols and ensure positive results that can be compared later [4].

Chemical lysis enzymatic lysis (proteinase K), and other protocols using temperature (boiling) can all be used to extract nucleic. The E. coli DNA was extracted using magnetic beads technology in this study. Finally, real-time PCR was used to do the amplification and detection, and the results were compared to find the concentration protocol with the highest recovery rate. A comprehensive explanation of the experimental protocol used in this study is provided in the sections that follow.

Exposure of the experiment's concentration protocols. The process of filtration is a type of separation that enables the concentration of suspended species on a support, typically a sterile filter membrane depending on the size of the microorganism being examined or utilizing a sterile bandage pad(Manor et al., A sterile adjuvant of filtration can also be added.

Sonication is the process of agitating a solution containing particles with sound waves. Typically, an ultrasonic bath or probe is used to apply it. Solicitors can be probes that are directly attached to the sample to be solicited or they can produce sound waves in a water bath where samples are placed [5].

One of the most common methods utilized in the laboratory of molecular biology is centrifugation. Its foundation is the separation of particles based on their size and density. Rotors determine the variety of centrifuges available. Rotors come in a variety of shapes and sizes, and their applications vary. For instance, due to their ability to pellet, the fixed-angle rotor is the one that is utilized the most frequently in laboratories that collect environmental water samples.

The PCR cycle threshold (Ct) values and amplification curves were used to make a comparison between the recovery rates of these three concentration protocols, which were used to concentrate water samples for PCR analysis.

The basis for an accurate and repeatable quantification of fluorescent PCR techniques is the concept of the "threshold cycle." The thermal cycle number at which the fluorescent signal surpasses the background signal and thus reaches the positivity threshold is known as the cycle threshold (Ct). Ct worth can be straightforwardly associated to the beginning objective fixation for the example.

In a continuous PCR measure, a positive response is identified by the gathering of a fluorescent sign. The number of cycles required for the fluorescent signal to cross the threshold is referred to as the Ct. Ct levels are conversely corresponding to how much objective nucleic corrosive in the example. The amount of genetic material in the sample is proportional to the Ct value. Ct values got in this manner are semi-quantitative and can recognize high and low bacterial heaps of *E. coli*.

The centrifugation method produced the highest Ct values; then the gauze filtration, where the membrane filtration's ct values are lowest. Both the waste water and the natural water matrix reached the same conclusion. The distinction in the Ct values gives direct data on the distinction in the underlying heap of E. coli strain and thusly on the recuperation of every fixation convention. Both the waste water and the natural water matrix reached the same conclusion. The recovery of each concentration protocol is directly affected by the variation in the Ct values, which reveal the variation in the initial load of the E. coli strain.

The detection kit used also includes an internal positive control (IPC), which consists of reference genes that will be amplified with the target sequence during the same PCR reaction run to guarantee the quality of the results.

The goal is to make sure that the right amount of DNA was taken out of the sample, as well as to look for extremely negative results (when the pathogen being tested shows amplification but there is no internal control DNA amplification) or the presence of substances that make the test less effective.

All of the internal positive controls had a positive amplification, indicating that there were no inhibitory substances and that the extraction and amplification processes were successful. Additionally, the cycle's positive and negative controls produced conforming outcomes. Which makes it possible to verify the obtained results.

The limitations of traditional water sample microorganism detection methods include low specificity and accuracy, lengthy incubation times, and so on. Also, can't cover all boundaries. The use of molecular methods to identify these microorganisms is highly recommended as a novel strategy that will enable very precise and speedy detection. A crucial step is the samples' concentration phase. In this experimental study, the recovery rates of three concentration protocols were compared. The membrane filtration concentration protocol had the highest recovery rate for microorganisms, followed by the gauze filtration and centrifugation protocols.

Although centrifugation is regarded as a robust method, when compared to filtration methods, the concentration protocol for water using centrifugation results in a lower loss of bacterial biomass. These factors include: the selection of centrifugation speed and duration, the method of discarding the supernatant and the centrifugal compaction-induced alteration of bacterial cell surface properties and internal structures, including DNA [6].

Conclusion

To unite the outcomes acquired, extra tests involving different kinds of microbes for doping and utilizing different grids (treated water, ocean side water) are all around prescribed and to assess the misfortunes connected to the exhibition of the centrifugation convention, other trial review including different states of centrifugation can be dealt with. For the same samples (natural water or waste water) doped with the same concentration of E. coli strain and subjected to three distinct concentration protocols membrane filtration, filtration on gauze pad, and centrifugation the Ct values were different, despite the similar extraction and PCR detection procedures. In addition, for all of the tested matrices (natural water, waste water), these values were always higher for the centrifugation procedure, which was followed by filtration on gauze and membrane filtration. In order to compare various water concentration protocols, additional experiments were carried out. Compared two concentration protocols for 40 ml of Seeded water samples under various conditions: Centrifugation. Their research demonstrated that filtration recovery is generally superior to centrifugation. Additionally, the results affirm that filtration strategy for the disengagement of mycobacteria from water tests is a more delicate technique for focus than centrifugation.

Acknowledgement

None.

Conflict of Interest

None.

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