Journal of Research in Medical and Dental Science
2019, Volume 7, Issue 2, Page No: 210-213
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Available Online at: www.jrmds.in
eISSN No.2347-2367: pISSN No.2347-2545

How to Reuse Pipette Tips and Tubes in PCR and Electrophoresis Procedures? A New In-house Method Development

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ABSTRACT

This survey aimed to assess the roles of pipette tip's contamination and usage of one second-hand tip in the loading of all samples in the electrophoresis procedure, and use of second-hand tubes in PCR procedure. Three genes with different band sizes were amplified with the PCR method. Three dilutions of 1x, 0.5x and 0.1x were made for all three PCR products. After loading of each PCR products in its first well, pipette tip has not replaced and washed in 5 µl distilled water. Then, 5 µl of the washed water was loaded in the second well. Two kinds of staining methods were done, including safe stains and Ethidium-Bromide. At the same time, Non-sterile and second-hand vials were washed with 6% NaOCl and used for PCR method, again. Results showed that among all three dilutions (1x, 0.5x and 0.1x), only 1x dilutions had bands in first wells for each gene. In addition, none of them has presented bands in the second wells (which was done without replacing the pipette tips). In addition, 6% NaOCl had the ability to eliminate contamination from second-hand vials among PCR and vials could be re-used for second times. We conclude that it is not necessary to replace the pipette tips among the loading of products in electrophoresis and second-hand tubes are usable after washing with NaOCl (6%-10%) for PCR.

Key words: Second-hand tube, Replacing of pipette tip, Electrophoresis, PCR

HOW TO CITE THIS ARTICLE: Elghar Soltani, Mohammad Ahangarzadeh Rezaee, Pourya Gholizadeh*, How to reuse pipette tips and tubes in PCR and electrophoresis procedures? A new in-house method development, J Res Med Dent Sci, 2019, 7(2): 210-213

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Received: 22/02/2019
Accepted: 20/04/2019

INTRODUCTION

During the past three decades, polymerase chain reaction (PCR) and several other DNA/RNA amplification techniques were recently developed for *in vitro* amplification of genomic elements [1-4]. PCR has advantages in both basic and diagnostic aspects of molecular biology. Since it has the ability to produce large amounts of a specific DNA fragment from small amounts of a template DNA [5-7]. PCR, amplification-based techniques had some applications to detect organisms that cannot grow in conventional culture media. These techniques facilitate the assessing of the epidemics and detection of genes associated with drug resistance in bacteria, viral and fungal [8,9]. Electrophoresis is a technique to use for separating of macromolecules such as proteins, DNA and RNAs. These macromolecules have different migration rates through agarose gel depending on their total charges, sizes, and shapes [10,11]. Nucleic acid molecules are moved by applying an electric field to separate the negatively charged molecules through the agarose. Shorter molecules move faster than longer ones since shorter molecules move more easily through the pores of the agarose gel [12,13]. Amplification products contamination and visualization of multiple bands in electrophoresis gels have been the main impediment to using these techniques routinely in diagnostic laboratories [14]. Amplification sterilization techniques have been needed in order to prevent contamination from PCR. Useful techniques were used to eliminate the carryover of amplified materials from well to well in electrophoresis [15-17]. Most researchers from different fields have the idea that sterile disposables should use in both PCR and electrophoresis steps [18]. Almost most of the laboratory researchers believe that if pipette tips would not replace among the loading of PCR products in the electrophoresis gel, the results will be undesirable with extra bands. In addition, from their point of view, second-hand washed tubes could not be used for the second time in PCR steps. The aims of this study have assessed the roles of pipette tip's contamination in the electrophoresis procedure and advantages usage of one second-hand tip in the loading of

all PCR products, as well as, assessing how the use of second-hand tubes in PCR procedure.

MATERIALS AND METHODS

Bacterial isolates

The present study is qualitative observational study. In this work, three isolates of *Klebsiella pneumoniae* were used for the identification of three genes, which were considered three bands in different sizes in the electrophoresis gel. Mentioned isolates were PCR positive for the three genes including k54 (capsular serotype) with the size of 881 bp (as a heavy band), [19] rmpA (regulator of mucoid phenotype A) with 516 bp (as a medium band) [20], and *K. pneumoniae* 16S/23S ITSD (the internal transcribed spacer region) with 130 bp (as a light band) [19].

PCR condition

All genes had the same PCR condition, as previously described [19]. After the preparation of reaction mixtures and performing PCR, sterile vials were prepared for making different dilutions. In post PCR steps, three dilutions of 1x, 0.5x and 0.1x were made with distilled water for all three PCR products.

Electrophoresis

To compare the sensitivity of bands visualization in electrophoresis, two kinds of staining methods were done, including safe-stains (Thermo-Fisher Scientific, USA) and Ethidium-Bromide (Merck, Germany). After preparation of agarose gel, two wells were considered for each concentration. After loading of 1x concentration in the first well, pipette tip (Progen Scientific Ltd., UK) has not replaced and washed by pipetting up and down for three times in 5 µl distilled water. Then, 5 µl of the washed water was loaded in the second well. This process was repeated for 10 times. Finally, agarose gels were visualized by UV transillumination (UVP, USA) to see the band's presence [21].

Second-hand vials checking with washed distilled water and NaOCl

To investigate the transmission of DNA contamination in the vials, two series of PCR vials were kept from the previous stage. One series was washed with 300 µl of distilled water and other series with 300 µl of 6% NaOCl (Sigma, UK). Again, the same PCR mix was prepared in non-sterile vials and DNA was not added at the end. These vials were put in the thermocycler with the same condition for PCR (Bio-Rad. Singapore). The contamination was checked by electrophoresis, which was stained with separated safe-stain and Ethidium-Bromide.

RESULTS

Two types of electrophoresis for evaluating of transmission of contamination among gel wells were done. During these processes, when PCR products were loaded into the wells, the pipette tips were not replaced for each gene in the second well. The results of electrophoresis with safe-stain staining were showed that among all three dilutions (1x, 0.5x and 0.1x) only 1x dilutions had bands in the first wells for each gene. In addition, none of them has presented bands in the second wells (the wells loaded by the washed tip, which was done without replacing the pipette tip) (Table 1).

Table 1: Accuracy of pipette tips replacing for the detection of bands in the electrophoresis.

Bands	After replacing of P.T.[1] (N)[2]	Not-replacing of P.T. (N)	False-positive bands (not replacing of P.T.)	Smeared bands (not replacing of P.T.)
Heavy bands	100% (10) detection	100% (10) detection	None	None
Medium bands	100% (10) detection	100% (10) detection	None	None
Light bands	100% (10) detection	100% (10) detection	None	None

[1]P.T.: Pipette tips; [2]Repeat of each PCR products loading

Our findings of electrophoresis with Ethidium Bromide staining were provided among all three dilutions (1x, 0.2x and 0.1x). The 1x and 0.5x dilutions showed bands in their first wells for each gene. Again, none of them had the band in second wells, which were loaded by the washed tip. The results showed that the Ethidium bromide staining has high sensitivity for stained small amounts of PCR products.

In addition, the results of vials washed by distilled water and 6% NaOCl showed that in both types of electrophoresis's staining, 6% NaOCl had the ability to eliminate contamination among PCR products. In contrary, distilled water had not this ability and transmitted the contamination.

DISCUSSION

PCR is one of the most common technique that has many applications including detection and diagnosis of infectious and genetic diseases, genetic fingerprints, detection of different mutation and oncogenes, personalized medicine, and genetics researches [22-24]. Molecular works have a high ability for detection of low amounts of targeted DNA. However, the high sensitivity of the molecular tests, prone them to false positive results and makes it difficult to interpret these experiments [25,26]. In addition, much unexpected sources of contaminants are varied including reagents, water, disposables, amplicon, and sample carryover, which can cause problems in the identification of targeted DNA [15,27]. Hence, the prevention of contamination in these tests had a great importance, which can be controllable. The contamination can be reduced by observing some items such as careful handling of waste disposable and using filter disposable to prevent aerosol formation [28,29]. Heat sterilizes disposables such as pipette tips and reaction tubes can carry RNA/DNA in their contents.

In addition, one of the important sources of contamination in electrophoresis procedure is Tris-Borate-EDTA, Tris-Acetic acid-EDTA, Tris-EDTA and Sodium-Borate buffers [15]. Methods that provide elimination contaminating DNA are enzymatic treatment such as using of DNase I, exonuclease III, and restriction enzymes [30-32], irradiation [3,33], HCl or hydroxylamine hydrochloride [27,34,35] and the use of sodium hypochlorite [28,36]. Other procedures which could destroy amplification products by agents like modified primers [1], irradiation with the addition of (iso) psoralen and the uracil-DNA-glycosylase/dUTP approach [37,38]. Researchers believe that disposable items like pipette tips and tubes are essential with the high amount in molecular tests. For example, pipette's tip swapping is time-consuming when loading PCR products and a high number of consumable materials require high costs. Molecular techniques are so common in developed and wealthy countries. In contrary, the conditions are completely different in developing countries with low income. The low budget is dedicated to research activities, especially molecular experiments. The low budget is one of the preventing factors in the development of molecular tests in developing countries.

Loading of all PCR products only with one pipette tip could speed up the work and it makes the process of work so easy for researchers. The only thing to keep in mind is that to consider the product inside the pipette tip is completely drained. In another condition, in contrast to the researcher's concepts, second-hand tubes could be washed with NaOCl 2% to 10% and re-used in PCR, again. According to another study which has suggested that cleaning agents such as ethanol, and commercial cleaners like Extran® (cleaner for laboratory use) were not effective to eliminate the contaminating DNA [26,39,40]. However, our findings confirmed that washed second-hand tubes with NaOCl can be useful in PCR process and never made contamination.

CONCLUSION

This is the first study, which directly proves that replacing of pipette tips among the loading of PCR products in electrophoresis had any effect on creating of multiple bands. In addition, this finding could be a great way for researchers at third-world countries to solve a dilemma between replacing and not replacing the pipette tips in electrophoresis. As well as, not replacing the pipette tips when loading the PCR products, which leads to a high consumption of the pipette tips, can be cost effective in conducting the researches. In this investigation, we conclude that all nucleic acid amplification assays are functional both in research as well as in the clinical diagnosis. Therefore, reducing the cost of consumables is important for promoting molecular techniques in developing countries. Hence, according to our results, it is not necessary to replace the pipette tips among the electrophoresis procedure and second-hand tubes are usable after washing with NaOCl (6% to 10%). On the other hand, the speed of the work rises and the work gets easier by loading only one pipette tip.

ACKNOWLEDGMENT

This work was supported by Department of Medical Microbiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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