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Original Article

A simple long term storage solution for human tissues as continuous source of DNA/RNA for PCR analysis

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Abstract

Aim: In molecular biology, there is a need for tissue storage solutions for long term analysis in order to have a suitable source of nucleic acids (RNA and DNA). There are many reports to store the tissue samples in different solutions like ethanol, DMSO as well as with storage in liquid nitrogen, but a suitable storage solution for tissue at room temperature is still missing. Therefore, a solution called Genekam Tissue Storage Solution (GTSS) is developed, which can be used to store the tissues at room temperature for a long time.
Materials and Methods: Human tissues were stored in this solution for 4 years. as these are tested with conventional and real time PCR for the presence of internal controls after the isolation of RNA and DNA over the period of 4 years, along with spectrometric testing.
Results: Results of internal controls for human housekeeping genes like beta-globin and beta-actin were successful in nucleic acid isolations. Spectrophotometric values of all nucleic acid isolation range from 1.03 to 33.8 ng/µl (1.03 to 33.8 µg/ml).
Conclusion: In this work, it may be the first time about the successful development of a simple long-term noncryogenic storage solution for human tissues at room temperature as a continuous source of DNA and RNA for molecular biological analysis.

Keywords: Tissue storage solution, polymerase chain reactiontissue, nucleic acid isolation, pathology

INTRODUCTION

Molecular biological methods like polymerase chain reaction (PCR) for the detection and expression of different gene targets are part and parcel of modern medicine. Nucleic acid isolation is an essential step to conduct these studies (1).

During the PCR analysis, one uses different carriers, like buccal swabs to isolate the DNA and RNA from the nasal as well as mouth cavity for conducting various molecular biological testing e.g., detecting human RNA viruses (2-4). These buccal swabs can be stored for many days at room temperature and in cooling conditions. Moreover, these can be sent to other laboratories at other destinations with the postal services. The problem arises, when it comes to store the tissue for long-term use, as there is no standardized method available and the degradation of nucleic acid in the tissue puts a lot of pressure on the laboratories to conduct the studies as early as possible. Therefore, scientific community is searching for a suitable solution to store the tissue for long-term applications. There are reports that one can store the tissues at -20°C also, which is not feasible under field conditions, and there are costs of power supply (5). There are many reports that one can store the tissues in DMSO (dimethyl sulfoxide) solutions as well as ethanol (6-8). The biobanks need a solution to store the tissues, and they used many different commercial solutions to solve the storage issue, but found that storage at room temperature is possible for a few hours during the quality assessment studies (9,10). Freezing temperatures are being used to store the tissues to maintain the integrity of nucleic acid, as well as a report about comparing of storage of tumor tissues at -80°C or in liquid nitrogen in order to have a constant supply of DNA and RNA for studying gene expression as well as gene sequencing in cancer tissue biobank (11,12). In reality, the liquid nitrogen cannot be used for the transportation of tissues everywhere in the world.

Therefore, our laboratory decided to develop inexpensive noncryogenic solutions for the long-term storage of human tissues for different molecular biological applications.

MATERIALS AND METHODS

The ethical committee of the institute Genekam Biotechnology AG gave permission (GEN01-23) for conducting the studies. A solution containing different chemicals is created with a particular pH. The composition of this solution can not be disclosed as patent rights are being assessed. This is available under the brand name Genekam Tissue Storage Solution (GTSS).

The placenta and umbilical cord were donated, and donor gave consent to use these for scientific purposes. Both were stored at 4°C for 2 weeks. After that, a part of these samples was cut in small pieces of 3 mm each and put in 20 ml of GTSS into two 50 ml plastic tubes. They were stored for 4 years at room temperature.

During the storage period, the RNA and DNA were isolated with mini column nucleic acid isolation kits (Genekam, Germany). If GTSS in tissue was reduced, an extra 5 ml of GTSS was added. 100 µl of stored tissue solution was used as input for isolation, and the protocol was followed as per the manufacturer and discussed in previous research work. The eluted volume of 50 to 100 µl per isolation was achieved (1).

The presence of human housekeeping genes like beta-actin and beta-globin in isolated RNA and DNA samples was determined with real time PCR tests (Genekam Kit number FR799 and FR118) in ABI 7500 and Quantstudio 5 (Thermofischer, USA) (1). 2 µl of isolated nucleic acid was used as input for conducting the PCR testing, and total volume was 20 µl. The assay was conducted in 96 wells plates. Negative and positive controls were used. They were also conducted according to instructions of ready to use PCR kits from Genekam, Germany. The procedures are available as manuals from Genekam.

The conventional PCR tests were performed with internal control PCR kits (Genekam, Germany) for housekeeping genes like beta-actin and beta-globin genes. The input DNA or RNA per PCR reaction was 2 µl. The total volume of reaction was 20 µl. The results were read on 2% gel agarose (Roth, Germany) after 30 minutes of ethidium bromide staining.

Negative and positive controls were included. These bands were at 268 bp for DNA and 154 bp for RNA internal controls. The instructions are available as manuals from the manufacturer.

Spectrometric analysis of isolated RNA and DNA was done on Nanodrop (Thermofischer, USA). Calibration was done with 2 µl of elution buffer from Genekam mini column nucleic acid isolation kit.

After that, 2 µl of isolated nucleic acid RNA or DNA were used to measure the amount twice per sample, and average amount per sample as well as ml was calculated.

RESULTS

Tissues stored in GTSS are continuous sources of RNA and DNA for different analyses done over a period of 4 years. They are stored at room temperature (21°C) in a room without any direct exposure to sunlight. Once the solution is reduced, 5 to 10 ml GTSS to rest of stored tissue was added so that there is a continuous supply. Pieces of 5 to 20 grams per tissue in 10 to 20 ml GTSS at the beginning were used.

Housekeeping genes for beta-actin and beta-globin in real time PCR in 14 samples show that there is hardly any difference between the isolations done over a period of 4 years in a real time setting (Table 1 and 2). The ten-fold dilutions during PCR analysis indicate that isolated nucleic acid can be diluted up to fivefold or even more. There was no signal in the negative controls.

Table 1. The presence of RNA and DNA with real time PCR internal controls in different nucleic acid isolations

Sample number	Tissue	RNA internal control	DNA internal control
1	Umbilical cord	++	++
2	Umbilical cord	++	++
3	Umbilical cord	++	++
4	Umbilical cord	++	++
5	Umbilical cord	++	++
6	Umbilical cord	++	++
7	Umbilical cord	++	++
8	Umbilical cord	++	++
9	Umbilical cord	++	++
10	Umbilical cord	++	++
11	Placenta	+	+
12	Placenta	+	+
13	Placenta	+	+
14	Placenta	+	+
15	Negative control	-	-

Table 2. Spectrometric values of isolated RNA and DNA from different isolations after 4 years of storage time

Sample number	Tissue	Total RNA ng/µl	Total DNA ng/µl
1	Umbilical cord	26.23	33.8
2	Umbilical cord	19.76	26.6
3	Umbilical cord	24.45	32.25
4	Umbilical cord	26.7	37.15
5	Umbilical cord	19.8	25.3
6	Umbilical cord	23.08	29.6
7	Umbilical cord	27.75	34.45
8	Umbilical cord	26.15	33.65
9	Umbilical cord	21.85	28.5
10	Umbilical cord	28.6	36
11	Placenta	1.03	1.15
12	Placenta	1.16	2.1
13	Placenta	1.43	2.2
14	Placenta	1.45	2.65

Conventional PCR for presence of housekeeping genes in 14 samples showed also successful bands in gel agarose and indicated the presence of RNA and DNA i.e., there were successful isolation of nucleic acids. The intensities of these bands indicate that there should be a high amount of the nucleic acid in the samples, but there was no band in the negative controls.

Spectrometric studies indicate that there is variation ranging from 2 to 32 µg/ml in 14 isolated samples from human placenta and umbilical cord with mini column isolation kits (Table 2). The values vary between 19.8 to 28.6 µg/ml for isolated RNA and 25.3 to 38 µg/ml of isolated DNA for umbilical cord tissue, but the values for the placenta were in single digit range. Umbilical cord tissue provided more nucleic acid than the placenta, as it was almost 5 times more (Table 2).

100 µl solution from the stored issue was used to isolate the nucleic acid with mini column kit. 5 to 10 ml GTSS was added to tissues, once solution was used, which provided a continuous source for analysis. The addition of extra GTSS seems not to affect the concentration of isolated DNA and RNA. It means that more than 100 isolations for RNA and DNA were done till today from these samples. Moreover, the supernatant was only used for isolation, not the tissue pieces.

DISCUSSION

We are presenting a long-term storage solution for the human placenta and umbilical cord as examples. These stored tissues act as a long-term source for RNA and DNA. 100 µl supernatant of the stored tissue as input for mini column isolation is sufficient to provide 50 to 100 µl eluted nucleic acid for conducting various real time and conventional PCR studies. This solution does not need any freezing during storage as this is the case in other studies in literature. The cost of this solution is Euro 30 for 100 ml. This solution can be stored at room temperature. Moreover, GTSS is used to store the tissues for 4 years, and still it is acting as source of nucleic acid in our laboratory against the solutions mentioned in the literature, which provides only for very short period ranging from a few days to a few months (7,9). except for liquid nitrogen for long term storage, which is not feasible in most of laboratories around the world (10). The results of this research work indicate that this storage solution may be a suitable solution for many institutes, biobanks, and other laboratories looking to store the tissue over years to conduct different analysis around the world. To our surprise, umbilical cord provides higher output of nucleic acid than that of placenta, this variation of nucleic acid needs to be studied further. We isolated till today more than hundred times the nucleic acid to be used in our laboratory research work, but in this work we are showing the results of a part of our experiments as an example. Our lab is working to use this method for buccal swabs storage and other tissues of human and other species to be stored for longterm.

CONCLUSION

To our knowledge, it may be a first noncryogenic long-term storage solution for human tissues, which are to be continued source of RNA and DNA for real time and conventional PCR analysis. The storage period is more than 4 years at room temperature.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
This permission has been provided under the number (GEN01.23) from the ethical committee of institute (Genekam Biotechnology AG, Duisburg, Germany).

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