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Development of conservation techniques for liver samples from rodents of the genus *Rattus* in Abidjan

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Abstract

Tissue samples should be stored in liquid nitrogen immediately after excision and/or kept at -80°C until analysis. In relation to this requirement, researchers in Côte d'Ivoire have difficulty collecting and transporting tissue samples to areas far away from testing laboratories due to the unavailability, use and cost of preservation equipment. In this study, we prepared two preservation solutions and evaluated their ability to protect liver tissue RNAs for two months for collection and transport to a testing laboratory. Two preservative solutions named solution S_1 and S_2 were prepared to perform this experimental study. As an animal model for the experiment, we used 15 rats of the genus *Rattus*. These rats were sacrificed and liver tissue samples were collected and aliquoted according to the solutions, temperatures and storage times. A total of 1500 samples were analysed. Liver RNA can be stored without solution at +18°C for 6 hours, at +4°C for 3 days and at -20°C for 5 days. Solutions S_1 and S_2 have been shown to preserve liver RNA for 12 hours at +18°C, for 3 days at +4°C and for 30 days at -20°C. Therefore, solution S_2 can preserve liver RNA for up to 60 days at -20°C in contrast to solution S1 which does so for 30 days at the same temperature. Solution S2 could be indicated for the preservation of liver RNA for 2 months.

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Introduction

Formalin-fixed paraffin-embedded tissues (FFPE) and ultra-low temperature frozen tissues (-80°C and -190°C) are the most commonly used preservation sources for diagnostic and research purposes in histology and molecular biology (Naber, 1996 ; Naber, 1992 ; Hood, 2006). In Côte d'Ivoire, research on zoonotic diseases requires researchers to capture and collect animal organs in areas far away from the screening laboratories. The collection and transport of tissue samples in these locations is usually done with liquid nitrogen (-196°C) to avoid degradation of nucleic acids and proteins. However, liquid nitrogen is dangerous to handle and failure to maintain storage containers in good conditions can lead to leakage and loss of molecular characteristics. In addition, liquid nitrogen also represents a significant investment (Jerry *et al,* 2014).

Formalin-fixed paraffin-embedded tissues (FFPE) are the commonly used storage techniques in the clinic at room temperature. However, these samples have degraded and denatured nucleic acids and proteins (Srinivasan *et al,* 2002). The Ribonucleic Acid (RNA) is also fragmented and the RNA yield is low, although the samples can be analysed by RT-PCR (Srinivasan *et al,* 2002). As a result, researchers face difficulties in preserving tissue samples under good conditions when collecting and transporting samples from areas far away from testing laboratories.

In recent years, new tissue preservation solutions to avoid tissue damage have become available. They allow the preservation of both tissue morphology for accurate diagnosis and nucleic acids, proteins and cells for research. Recently, some solutions with such properties have been described. These include transplantation solutions such as the Stanford solution (Drinkwater *et al,* 1995), the histidine-tryptophan-ketoglutarate (HTK) solution (von Heesen *et al,* 2012), the St. Thomas solution (Rosenfeldt *et al,* 1996), the University of Wisconsin (UW) solution (Jiang *et al,* 2012), and the Euro-Collins solution (Den Toom *et al,* 1991). The UW solution is the most widely used of these solutions. However, as these solutions are specifically used in organ transplantation, there is a lack of data describing their potential nucleic acid protection capacity in the literature. The UW solution is easy to prepare with an available chemical composition (Compagnon, 2011). Therefore, we used the chemical composition of the UW solution as a basis for making two UW solutions. These preservative solutions were used as experimental solutions to protect the RNA of the tissue samples for 2 months.

In this study, we prepared two preservative solutions (S_1 and S_2) and determined whether these solutions protect the RNA in Rattus rodent liver tissue samples for a sufficient period of time for the samples to be collected and transported to a laboratory for possible biological testing. Then, a validation of the preservation technique according to the ISO 15189 standard and the SH GTA 04 will be carried out with the use of the best performing solution in terms of preservation. This study is part of the research program on COVID-19 surveillance in animals in Côte d'Ivoire.

Materials and methods

Experimental animals

Animals captured

Five *Rattus rattus* and five *Rattus norvegicus* adults and juveniles of both sexes were captured at the Pasteur Institute of Côte d'Ivoire (PICI) site in Adiopodoume from December 2019 to September 2020. The capture equipment consisted of Sherman trap and wire trap (Figs. 1 and 2), protective goggles, fluorescent waistcoats, single-use gowns, red and white signaling tape, thin sterile gloves, personal protective equipment (PPE), and protective masks with filters and Waste from Healthcare Activities with Infectious Risks waste bags.

Breeding animal

Five 16-week-old *Rattus norvegicus* of the Wistar strain (laboratory rats) of both sexes were used in our study. These rats were reared at the Animal Resource Management Unit of PICI from August to December 2020.

Materials and reagents for euthanasia, necropsy and organ harvesting

Euthanasia, necropsy and organ removal of rats were carried out with the following equipment: necropsy table, Class II Microbiological Safety Cabinet (Baker company), trap washing station, pit for incineration of infectious materials, protective glasses sterile dissecting scissors, sterile forceps, sterile dissecting instruments, viscera helmet, single-use gown, PPE, thin sterile gloves, protective mask with N95 filter, waste bags, cotton wool, 55 x 14.2 mm Petri dishes and absorbent paper. The reagents used for euthanasia were forene (1-chloro-2,2,2-trifluoroethyl-difluoro methyl ether). Alcohol 70° (antiseptic) and incidin 1% (detergent) were used to decontaminate the dissecting instruments.

Material for aliquoting

The equipment used for aliquoting consisted of Class II Microbiological Safety Cabinet (Baker company), single-use gown, sterile forceps, sterile dissection scissors, single-use sterile scalpel blade, sterile gloves (Delta Plus Group, France), waste bag, PPE, sterile 2ml cryotubes, sterile 2ml Eppendorf tubes (Eppendorf AG, 22331 Hamburg, Germany), absorbent paper, 55 x 14.2mm Petri dish and the prepared tissue sample preservation solutions.

Material of storage

The equipment used for the conservation of the liver tissue samples consisted of a -20°C freezer (Thermo Fischer Scientific, USA) and a +4°C refrigerator (Facis S.A., France), a 1100 litre RCB cryoconservator (CryopAL, France) for conservation in liquid nitrogen, racks, 81-well cyoboils (Nalgene®), liquid nitrogen supplied by Air Liquide Côte d'Ivoire, and PPE.

Material for the preparation of conservation solutions

The equipment used for the preparation of the preservative solutions consisted of a precision balance for weighing the chemical compounds, a Chemical Safety Cabinet, a sterile scalpel, a sterile flask, a graduated cylinder, sterile distilled water, a sterile magnet bar, a magnetic stirrer, aluminium foil, a pH meter, PPE, a 1L bottle, an autoclave and the chemicals and their quantities (Table 1). The chemicals used were Sigma-Aldrich products.

Laboratory materials

Materials and reagents for RNA extraction

The extraction of RNA from the liver tissue samples was performed using the Zymo Research Kit Direct-zolTM RNA MiniPrep. In addition to the kit materials and reagents, sterile 2 ml Eppendorf tubes (Eppendorf AG, 22331 Hamburg, Germany), TRI Reagent® (Molecular Research Center, Inc.) as lysis buffer, 95-100% ethanol (VWR International S.A. S, France), micropipettes (P1000, P200, P100, P20, P10), sterile filter cones (P1000, P200, P100, P20, P10, P5), a vortex mixer (Ohaus, Germany), Class II Microbiological Safety Cabinet (Baker company), sterile gloves (Delta Plus Group, France), absorbent paper, refrigerated centrifuge (Ohaus, Germany), waste bags and PPE.

Materials and reagents for RNA quantification

RNA quantification was performed using the spectrophotometer (Nanodrop One C), a 2 µl micropipette, RNAase free solution and new laboratory wipes for cleaning the sample deposit station, 1 µl sterile filter cones, sterile gloves (Delta Plus Group, France) and PPE.

Materials and reagents for RNA agarose gel electrophoresis

The preparation of the 1.5% migration gel was carried out using agarose, a precision balance, sterile distilled water, measuring cylinder, combs, Tris Acetate EDTA (TAE) buffer, Syber Safe, PPE and a microwave. Subsequently, deposition of samples into wells and agarose gel electrophoresis of RNA extracts was performed using a migration tank (Cleaver scientific Ltd, England), aluminium foil, Tris Acetate EDTA (TAE) buffer, Syber Green, sterile gloves (Delta Plus Group, France), micropipettes (P20, P10), sterile filter cones (P20, P10). Visualisation of the RNA bands was obtained using the band visualisation device (Gel DocTM EZ Imager, USA).

Methods

Type of study

This is an experimental study on the application and validation of tissue preservation techniques and the ability of solutions to preserve nucleic acids from tissue samples for a period of 2 months. We used Rattus liver tissue as experimental sample.

Capture of R. rattus and R. norvegicus

Before setting the traps, a field inspection was carried out in the morning to locate favourable landscape matrices for setting the baited traps. After inspection, the Sherman and wire traps were set for five consecutive nights in different selected sites. The day after trapping, the presence of rodents in each trap was checked.

The traps containing rodents were collected and transported to the necropsy site (Fig. 3). Macroscopic identification by the Chapellier method was then carried out after euthanasia. Rodents other than R. rattus and R. norvegicus were released.

Autopsy and organ removal

The autopsy and organ harvesting were carried out in compliance with biosafety rules with the use of PPE and Class II Microbiological Safety Cabinet. All measures were taken to ensure that the study was conducted under ethical conditions.

The rats were sacrificed by inhalation with isoflurane in accordance with the guidelines of the Canadian Council on Animal Care. In particular, this study received the approval of the National Health and Life Sciences Ethics Committee on the number: 043-21/MSHP/CNESVS-km.

Before dissection, cotton soaked in 70° alcohol is used to disinfect the rodent's abdomen. Then, dissection began by opening the animal's rib cage (Fig. 4) followed by organ harvesting.

The liver of each rat was used in our study, while the other organs were placed in physiological water and then stored at -80°C and in liquid nitrogen for later studies. Dissecting instruments were disinfected with 70° alcohol at each organ removal from the same animal and soaked in a tray containing a detergent (1% incidin) at the end of the dissection of each animal. Between each autopsy, the work surface was disinfected with incidin and 70° alcohol and the contaminated material was transferred into a waste bag for later incineration.

Aliquoting and preservation of liver tissue

Each harvested liver organ was placed in a sterile Petri dish and cut into small pieces using sterile forceps and a sterile scalpel blade (Fig. 5). Each piece of liver corresponded to 10 mg and the time between death of the animal and preservation of the tissue was on average 20 minutes. Liver pieces were stored with or without solution at +18°C, +4°C, -20°C and -196°C. The liver tissue samples stored at -196°C were used as a control in our study.

The volume of the preservative solutions was 1 ml in each tube. This meets the requirement that the volume of a preservation solution should be at least 5 times the volume of the tissue to be preserved.

Preparation of the preservative solutions

We prepared two preservative solutions (S_1 and S_2) in our study. These solutions were prepared based on the chemical composition of UW solution (Compagnon, 2011).

This is a most commonly used preservation solution for organ transplantation. It is easy to prepare with less expensive chemical compounds. Solutions S_1, S_2 and UW are similar, but have some differences in their compositions (Table 1). The modified UW solution aims to improve the preservation quality of the tissue by adding chemical compounds, including glycerol, polyethylene glycol and histidine (Table 1).

Preservative solutions S_1 and S_2 were prepared by mixing the chemical compounds with their corresponding amounts (Table 1) in one litre of distilled water. The resulting mixture was sterilised, cooled and stored at +4°C for later use.

Validation of a preservation method on tissues according to ISO 15189 and HS GTA 04.

This is a study on the validation of preservation techniques for tissue samples with solution S_2. For this study, we followed the methodology described by Cofrac in SH GTA 04 (Revision 0 - April 2011) for the experimental design necessary for method validation. We have adapted this methodology to molecular biology. For this method, we evaluated the repeatability, intermediate fidelity and contamination of liver tissue samples of the genus *Rattus* preserved in solution S_2. To perform this method validation, we needed 3 laboratory people (1 technician, 1 PhD student and 1 researcher).

Checking the sample for contamination

We assessed the absence of contamination during the manipulation by using the classical method. That is, performing an extraction without using a tissue sample. After extraction of this blank sample, it appears that only the extraction solvent is recovered. We then carried out a spectrophotometric measurement, followed by agarose gel electrophoresis.

Evaluation of repeatability

RNA extraction, quantification and quality were performed on liver tissue samples from Rattus rats. We used 8 pieces of 10 mg liver from the same animal for the repeatability and intermediate fidelity study. For the repeatability study, five pieces of liver were randomly selected and numbered 1, 3, 5, 6 and 8. The selected samples were analysed on the same day by the same operator using the same protocol.

Assessment of intermediate precision

For the intermediate precision evaluation, three pieces of liver were randomly selected and numbered 2, 4 and 7.

The extraction, quantification and RNA quality of these selected samples were performed according to the same protocol but by 3 different operators (1 technician, 1 PhD student and 1 researcher).

Biology tests

RNA extraction from preserved liver tissue samples with or without preservative solution

RNA extraction from liver tissue samples was performed using the Zymo Research kit (Direct-zolTM RNA Miniprep). Each piece of liver tissue was placed in 600 µL of lysis buffer (Trizol®Reagent, Invitogen), and then total RNA isolation was performed according to the manufacturer's instructions provided in the Zymo Research (Direct-zolTM RNA Miniprep) kit.

RNA quantification

The RNA extract obtained was quantified using a Spectrophotometer (Nanodrop One C). 1 µl of the RNA extract was deposited on the lower base, then the nanodrop arm was lowered to measure the concentration.

Checking the quality of the RNA

After quantification, RNA quality was checked by 1.5% agarose gel electrophoresis and visualisation of ribosomal RNA bands (28S and 18S) using a compact, automated gel imaging system (Gel DocTM EZ Imager).

Statistical analysis

To conduct statistical analyses, samples of analysed liver tissue were divided into ten groups (including a control group, group T). Statistical tests were then performed to compare the variability of the mean concentrations of liver RNA extracts between these different groups.

The information on its groups is set out in the table below: (Table 3):

Results

A total of 100 pieces of liver from each rat (i.e. 1500 pieces of liver) were used in this study.

The concentrations of RNA extracts were measured for each of the groups 1 to 9 as well as for the control group. The RNA concentrations of groups 1-9 were compared to control group.

Table 1. Preservation solution for tissue samples.

	Preservation solution		
	UW	S_1	S_2
Powder (g/L)			
Lactobionic acid	35.83	-	-
Raffinose pentahydrate	17.83	17.83	17.83
Adenosine	1.34	1.34	1.34
Allopurinol	0.136	0.136	0.136
Glutathione	0.922	-	-
Glutamate	-	1.47	0.922
Hydroxyethylamidon	50	-	-
Potassium hydroxyde	120	-	-
Sodium chloride	30	1.2	4.8
Potassium chloride	-	8.95	1.86
Magnesium sulfate heptahydrate	1.23	1.23	1,23
Histidine	-	-	1.73
Monophasic potassium phosphate	3.4	-	-
Liquid (mL/L)			
Monophasic potassium phosphate	-	1	3.4
Glycerol	-	35.83	35.83
Polyethylene glycol (PEG)	-	0.5	1
pH	7.4	7	7.2
Price	223,014 FCFA (409,2 $)	103,910 FCFA (190,66 $)	105,610 FCFA (193,78 $)

Table 2. Times and temperatures of liver tissue samples stored with or without solution.

		Duration									
		6 hours	12 hours	24 hours	3 days	4 days	5 days	7 days	14 days	30 days	60 days
Temperatures	+18°C	1	1	1	1	1	1	1	1	1	1
	+4°C	1	1	1	1	1	1	1	1	1	1
	-20°C	1	1	1	1	1	1	1	1	1	1
	-196°C	1	1	1	1	1	1	1	1	1	1

In our study the concentration of the extracts corresponded to the RNA quality. Groups with higher or lower concentrations compared to control group had degraded RNA after agarose gel electrophoresis. Fig 6 showed that the RNA concentrations of groups 1-9 compared to control group had no significant difference at 6 hours of storage with $p>0.05$.

This explains that RNA from liver tissue can be stored at +18°C, +4°C and -20°C with or without solutions S_1 and S_2. At 12 hours of storage, only the RNA concentrations of group 1 tissue samples were significantly higher than those of control group with $p<0.05$ (Fig 7). Figs 8 and 9 showed that groups 1, 2 and 3 showed a significant difference in concentrations compared to control group at 24 hours and 3 days of storage. At 4 days of storage, groups 1, 2, 3, 4, 5 and 6 showed a significant difference from control group (Figs 10 and 11). Similarly, at 5 days of storage Fig 12 showed that there was a significant difference between group 7 and control group; unlike groups 8 and 9 which showed no significant difference after 30 days of storage (Figs 13 and 14). In our study, only group 9 showed no significant difference from control group up to 60 days (2 months) of storage (Fig 15).

Table 3. Division of the analysed tissues into sample groups for statistical testing.

Group 1: tissues stored at +18°C for 2 months without solution	Group 2: tissues stored at +18°C for 2 months in solution S1	Group 3: tissues stored at +18°C for 2 months in solution S2	Group 4: tissues stored at +4°C for 2 months without solution	Group 5: tissues stored at +4°C for 2 months in solution S1
Group 6: tissues stored at +4°C for 2 months in solution S2	Group 7: tissues stored at -20°C for 2 months without solution	Group 8: Tissues stored at -20°C for 2 months in solution S1	Group 9: Tissues stored at -20°C for 2 months in solution S2	Group T: tissue stored at -196°C for 2 months without solution (control group)

Meaning of the groups

G1: Group 1; G2: Group 2; G3: Group 3; G4: Group 4; G5: Group 5; G6: Group 6; G7: Group 7; G8: Group 8; G9: Group 9; GT: Control Group.

After quantification, RNA quality assessment was performed by 1.5% agarose gel electrophoresis. Ultraviolet revelation allowed the observation of total RNA bands. For the intact tracks, the 2 bands of 28S and 18S rRNA can be seen above with an intensity ratio of 2:1. For degraded tracks, the 28S and 18S bands are no longer visible and streaks are observed towards the very low sizes (Fig 16).

The results obtained during the quantification and the quality of the RNA allowed us to observe that the intact samples had concentrations close to those of the control.

Table 4. Integrity of RNA as a function of storage time and temperature with and without solution.

Parameters	Tissues stored without solution at +18°C (Group 1)	Tissues stored in solution S_1 at +18°C (Group 2)	Tissues stored in solution S_2 at +18°C (Group 3)	Tissues stored without solution at +4°C (Group 4)	Tissues stored in solution S_1 at +4°C (Group 5)	Tissues stored in solution S_2 at +4°C (Group 6)	Tissues stored without solution at -20°C (Group 7)	Tissues stored in solution S_1 at -20°C (Group 8)	Tissues stored in solution S_2 at -20°C (Group 6)	Tissues stored without solution at -196°C (Control Group)
ARN integrity	6 hours	12 hours	12 hours	3 days	3 days	3 days	5 days	1 month	2 months	2 months and more

Table 5. Repeatability assessment: quantification of extracted RNA assessed by spectrophotometry.

	Concentration C_1 (µg/µl)	Concentration C_2 (µg/µl)
Sample 1	300.6	299,4
Sample 3	298.7	305.1
Sample 5	301.1	303.9
Sample 6	302.7	299.3
Sample 8	298.2	300.9
Mean	300.26	301.72

In contrast to the degraded samples, which have higher or lower concentrations than the control, their P-value is less than 0.05 ($p<0.05$).

In short, liver RNA can be stored without solution at +18°C for 6 hours, at +4°C for 3 days and at -20°C for 5 days. Solutions S_1 and S_2 have been shown to preserve liver RNA for 12 hours at +18°C, for 3 days at +4°C and for 30 days at -20°C. Therefore, solution S_2 can preserve liver RNA for up to 60 days at -20°C in contrast to solution S_1 which does so for 30 days at the same temperature (Table 4).

Table 6. Wilcoxon and Mann-Whitney tests for repeatability.

K (Observed value)	0.535
K (Critical value)	3.841
DF	1
p-value (bilateral)	0.99
alpha	0.05

Table 7. Repeatability assessment: quantification of extracted RNA assessed by spectrophotometry.

	Operator 1 Concentration (µg/µl)	Operator 2 Concentration (µg/µl)	Operator 3 Concentration (µg/µl)
Sample 2	297.8	299.1	292.9
Sample 4	296.6	300	304.8
Sample 7	301.1	299.3	305.5
Minimum	296.600	299.100	292.900
Maximum	301.100	300.000	305.500
Mean	298.500	299.467	301.067
Ecart-type	2.330	0.473	7.081

Validation of a preservation method on tissues according to ISO 15189 and SH GTA 04

Evaluation of the source of contamination

After spectrophotometric measurement and agarose gel electrophoresis, no bands were found in the well-used for the evaluation of the source of contamination. The experimental conditions used are consistent with the absence of contamination in the material and the internal procedures used ensure the absence of contamination.

Table 8. Wilcoxon and Mann-Whitney tests for intermediate reliability.

K (Observed value)	0.800
K (Critical value)	5.991
DF	2
p-value (bilateral)	0.98
alpha	0.05

Evaluation of repeatability

The 5 assays show that the amount of RNA extracted is equivalent for each sample (Table 5).

Fig. 1. *Rattus norvegicus* caught in a wire trap (25 cm x 10 cm)

Similarly, agarose gel electrophoresis did not reveal any difference in RNA integrity. Thus, we consider that this extraction method is repeatable.

Interpretation of the test

H0: The average concentrations are identical

Ha: The average concentrations are identical.

The calculated p-value is above the significance level alpha=0.05, the hypothesis that the mean concentrations are identical cannot be rejected.

Intermediate Fidelity Assessment

The spectrophotometric assays did not show any significant difference (p>0.05) and the agarose gel electrophoresis did not show any degradation on the quality of the extracted RNA (Table 7 and Fig. 17). Thus, we can consider that our extraction method enjoys a satisfactory intermediate fidelity.

Fig. 2. Sherman trap (H. B. Sherman Inc., Tallahassee, and Florida. 9 cm x 7.5cm x 23 cm).

Interpretation of the test

H0: The variances are identical.

H0: The average concentrations are identical

Ha: The average concentrations are identical.

The calculated p-value is above the significance level alpha=0.05, the hypothesis that the mean

concentrations are identical cannot be rejected.

Fig. 3. *Rattus rattus* on the autopsy table in an MMP II.

Fig. 4. Dissection and removal of organs.

Discussion

Tissue samples are an important tool for the molecular, cellular and biochemical diagnosis of diseases. Research on zoonotic diseases requires researchers in Côte d'Ivoire to capture and collect animal organs in areas far away from testing laboratories. The collection of tissue samples in these areas is mostly done in liquid nitrogen (-196°C). However, the use of liquid nitrogen (LN2) represents a significant investment and also poses rare but serious risks, including burns, supply tank explosions, and LN2 leaks during sample transport (Jerry *et al,* 2014). Organ degradation begins after the death of the animal, therefore, inadequate storage may lead to unreliable results after performing the biological tests.

Fig. 5. Liver pieces in a sterile Petri dish.

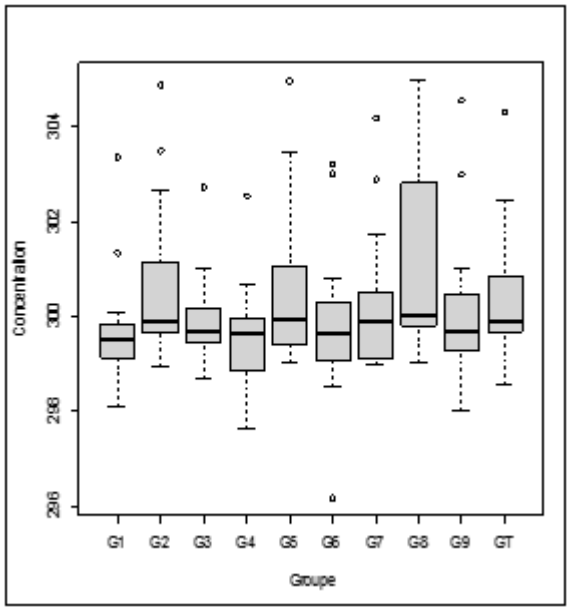

Fig. 6. Tissues stored for 6 hours.

In this study, we prepared two preservation solutions based on the chemical composition of the UW solution. We then investigated the ability of these solutions to eliminate the need for emergency

freezing of samples at very low temperatures (-196°C). A solution that protects the molecular properties of tissue until it reaches a diagnostic laboratory is important.

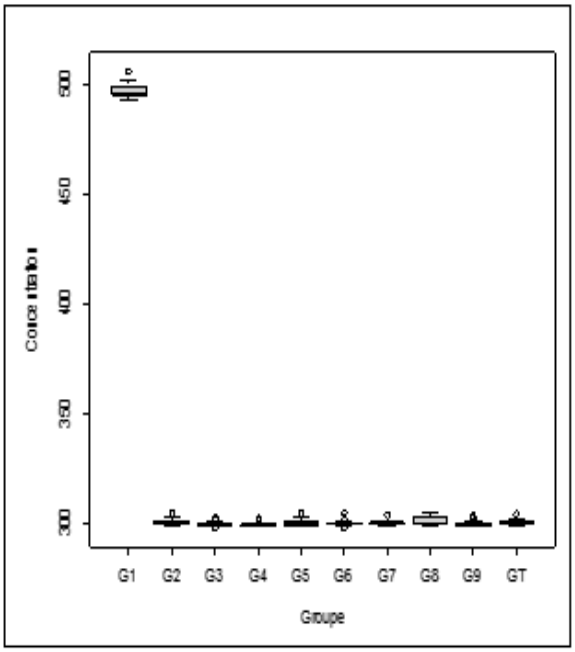

Fig. 7. Tissues stored for 12 hours.

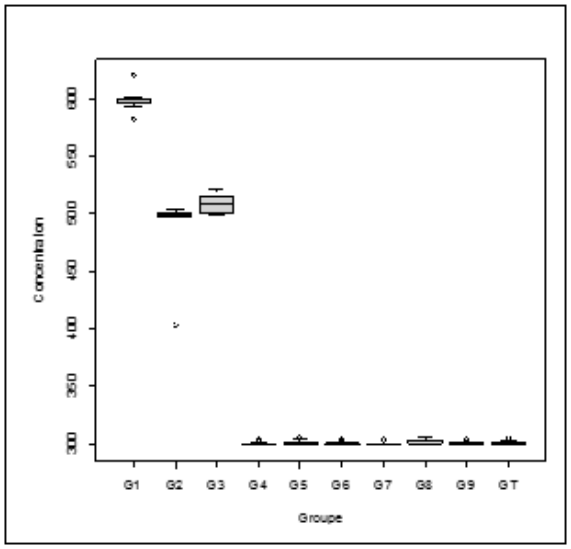

Fig. 8. Tissues stored for 24 hours.

In this study, we used the liver of specimens of the genus Rattus as an experimental sample. In current practice, collected tissue samples are immersed in liquid nitrogen for a few minutes and kept at -80°C for molecular examination (Swash and Schwartz, 1984). Formalin fixation and paraffin embedding (FFPE) of tissues is a very old practice and leads to degradation of nucleic acids (RNA, DNA).

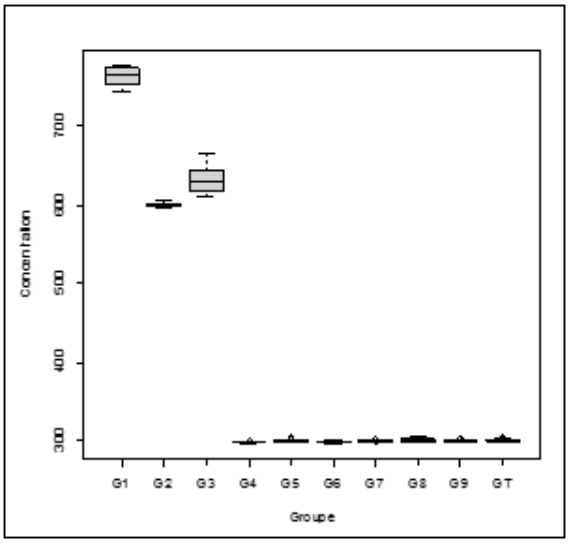

Fig. 9. Tissues stored for 3 days.

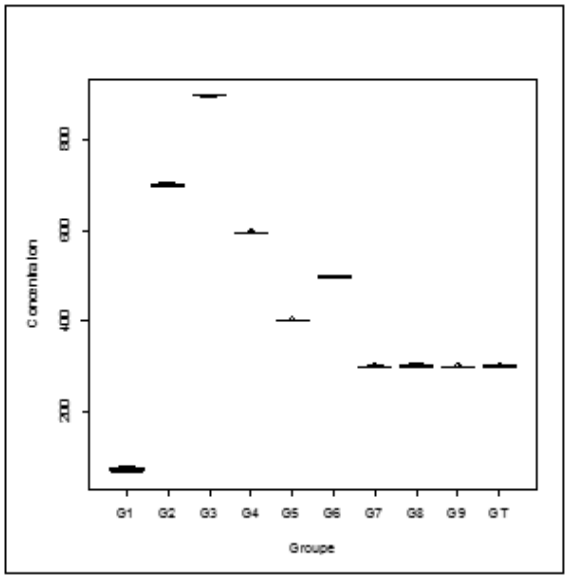

Fig. 10. Tissues stored for 4 days.

Therefore, FFPE samples are not ideal for molecular biology, cellular and biochemical analyses that require better preserved nucleic acids, proteins and cells. In addition, formalin is a known class I carcinogen (Lou *et al,* 2014).

In recent years, new tissue preservation procedures to avoid tissue damage are available. They allow the preservation of both tissue morphology for accurate diagnosis as well as nucleic acids, proteins and cells. Recently, some solutions with such properties have been described. These include organ transplantation solutions such as the University of Wisconsin (UW)

solution. And it is the most widely used of the organ transplant solutions. However, since these solutions are specifically used in organ transplantation, there is not enough data describing their potential ability to protect liver RNA in the literature.

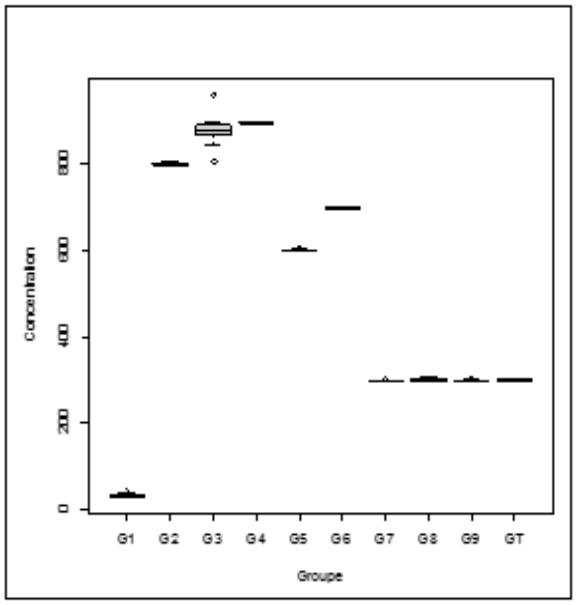

Fig. 11. Tissues stored for 5 days.

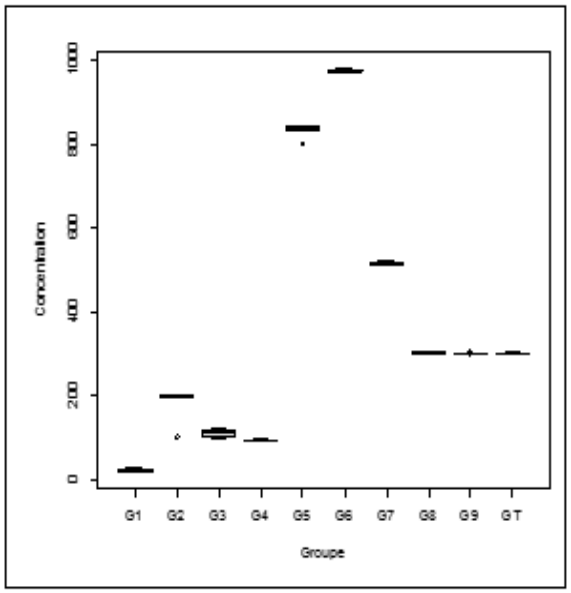

Fig. 12. Tissues stored for 7 days.

The results of our study show that RNA from rat liver tissue can be stored for 6 hours at +18°C without a preservation solution. In contrast, a study by Almeida *et al* in 2004 assessed the effect of factors on RNA integrity and mRNA expression levels by storing freshly obtained mouse liver tissue at room temperature for periods of 0 to 4 hours.

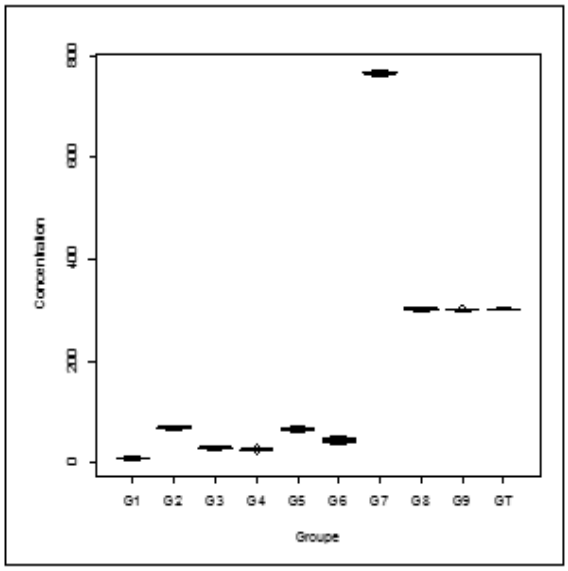

Fig. 13. Tissues stored for 14 days.

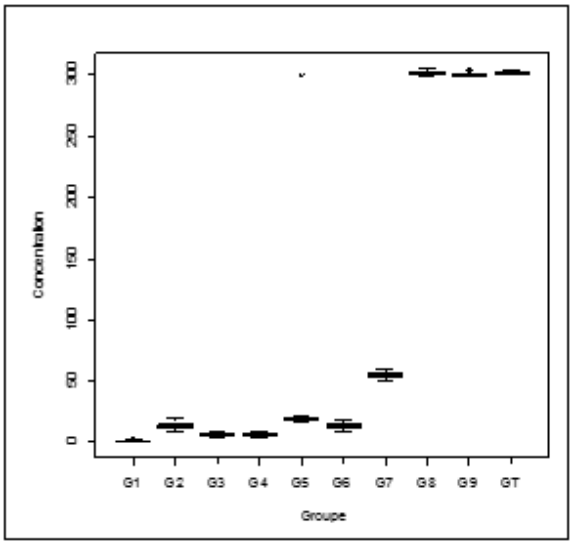

Fig. 14. Tissues stored for 30 days.

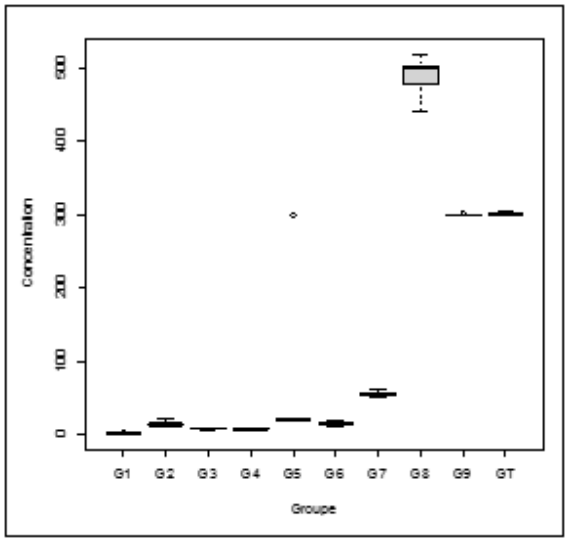

Fig. 15. Tissues stored for 60 days.

The results showed a slight degradation of RNA after 4 hours (Anna *et al,* 2004). A study on colon samples showed no decrease in RNA integrity with either warm or cold ischaemia for up to 4 hours (Bao *et al,* 2013). RNA was also stable in breast tumour samples stored at room temperature for up to 24 hours before freezing (De Cecco *et al,* 2009) and in tonsil tissue after overnight storage (16 hours) at room temperature (Micke *et al,* 2006).

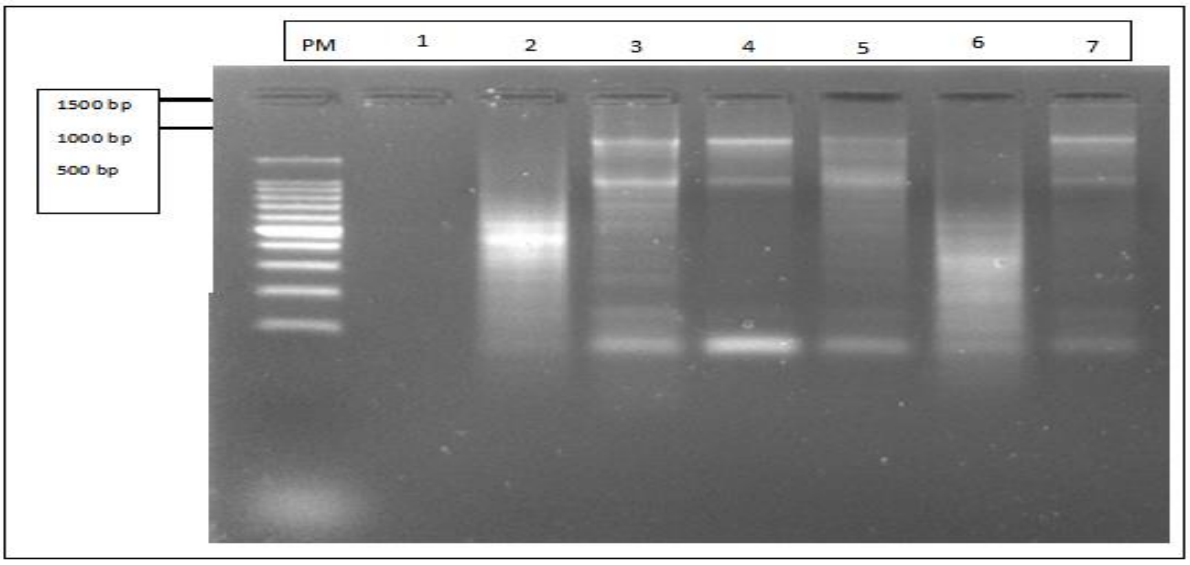

Fig. 16. rRNA band development on 1.5% agarose gel. (MW: molecular weight marker; well 1: negative control; wells 2 and 6: group 1 sample (samples stored at +18°C for 12 hours without solution (degraded RNA); wells 3 and 4: group 2 and 3 samples (samples stored at +18°C in S_1 and S_2 respectively for 12 hours) (intact RNA); well 7: control group sample (sample stored in liquid nitrogen).

In contrast, several other studies have shown a progressive deterioration of RNA integrity with increasing time between tissue excision and freezing (Van Maldegem *et al,* 2008; Barnes *et al,* 2008; Hong *et al,* 2010; Bray *et al,* 2010; Sampaio-Silva *et al,* 2013).

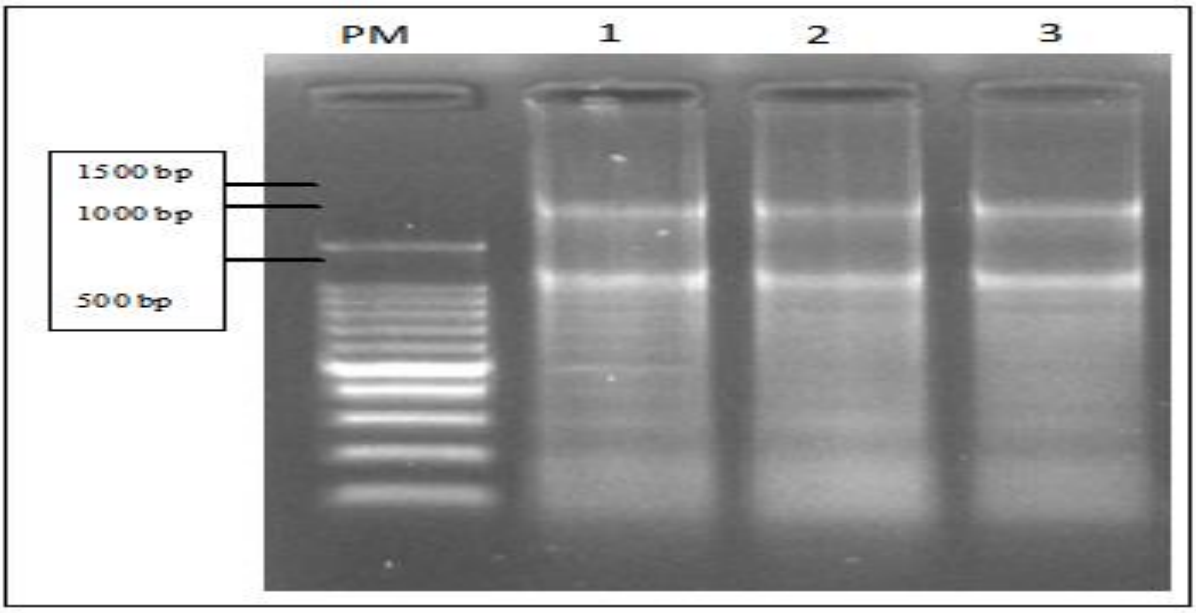

Fig. 17. Revealing rRNA bands on 1.5% agarose gel. (MW: molecular weight marker; well 1: sample 4 from operator 1; well 2: sample 4 from operator 2; well 3: sample 4 from operator 3.

In 2015, a study was conducted on the comparison of the effects of Kurt-Ozcan (KO) and University of Wisconsin (UW) preservation solutions on skeletal muscle biopsy samples. Rat muscle tissue samples were stored at room temperature and +4°C for 3, 6, 18 and 24 hours with (KO and UW) or without solution.

The integrity of the mRNA was measured to determine the tissue samples in which RNA degradation occurred as soon as possible. And they showed that mRNA was best preserved in UW and KO for 3 hours at room temperature and for 6 hours at +4°C (Yasemin *et al,* 2015). In contrast, our study showed that S_1 and S_2 solutions were able to preserve RNA in liver tissue at +18°C and +4°C for 12 hours and 3 days, respectively.

Based on the results obtained, solutions S_1 and S_2 may have the ability to protect RNA molecules from the liver of rats of the genus Rattus for 12 hours at +18°C, for 3 days at +4°C. However, storage at -20°C in solution S_2 protects the RNA for 60 days, in contrast to solution S_1 which preserves the RNA for 30 days. Nevertheless, liver tissue can be transferred within 6 hours at +18°C and within 3 days at +4°C without using a preservation solution.

Conclusion

In this study, we prepared two solutions (S_1 and S_2) at a lower cost than the UW solution. These solutions were used to preserve liver tissue samples for a period at least long enough to reach a screening laboratory. These solutions are the result of a modification of the UW solution based on their chemical compositions. Solutions S_1 and S_2 have been shown to have the same ability to preserve the integrity of liver tissue RNA at +18°C and +4°C for 12 hours and 3 days respectively. But solution S_2 preserves RNA better than solution S_1 at -20°C for 60 days. For most countries, these periods will be sufficient to transport a sample from the collection site to a diagnostic laboratory. Next, validation of a tissue preservation technique with solution S_2 was performed according to ISO 15189 and SH GTA 04. Contamination, repeatability and intermediate fidelity tests were satisfactory for this validation of the preservation technique. At the end of our study, the S_2 solution could be indicated for the conservation of liver RNA for 2 months. Thus, the evaluation of cell viability and enzymatic activity of liver tissues of the genus Rattus with these two solutions proved to be interesting.

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