Advancement in Preserving Human Tissue Morphology and Gene Expression Profiling

AQIX RS-I Technology

www.AQIX.com

Introduction

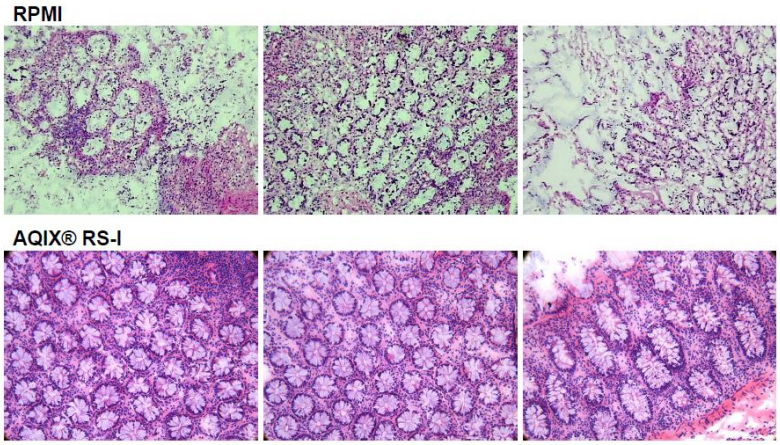
The quality of your results is directly impacted by the quality of the sample when working with human tissues. Current preservation techniques range from stopping all processes (formalin) to suspending all processes (feeezing) to targeting specific processes (RNAlater®).

Previous attempts by biobanks to preserve the integrity of RNA in human tissue biopsies have focused on using non-fixed, frozen tissues in order to overcome the fragmentation of RNA profiles. However, the logistics of preparing frozen tissue samples is compromised by having to process the *ex vivo* tissue samples almost immediately (1-3 hours) in adverse conditions (the operating theatre environment).

Micke et al (2006) produced high quality RNA profiles from fresh, frozen human tissue biopsies which allowed the application of molecular tools to enable tissue profiling of normal and deseased tissues, e.g., expression microarrays (miRNA's), real-time PCR and RNA integrity (RIN) in terms of gene expression. They showed that tissue samples stored, immersed in 'cold' 0.9% sodium chloride solution, in comparison to storage in RNA*later* solution (Ambion, USA), exhibited less tissue shrinkage and, more significantly, minimal changes in gene expression patterns following incubation periods of up to 16 hours, with the strongest results up to 6 hours.

Another implication of their work is to suggest that time is limited and RNAlater may "alter the outcome" by increasing variability and reducing tissue viability. The value of gene expression profiling relates to an understanding of pathogenetic mechanisms which govern diagnostic and prognostic outcomes (van't Veer et al., 2002). It is an essential requisite in the preservation of tissue viability that any aberrant changes in gene expression profiling or replication be negated and that RNA integrity and mRNA expression are at representative levels

H & E of Normal Human Colon Biopsies Transported and Stored in RPMI and AQIX® RS-I media



•30h post operation
•Transportation on ice

Source: Cureline Inc.

It was hypothesised that if fresh, frozen human tissue biopsies could be immediately immersed in a solution that closely resembles the milieu that lies juxtposed to every human cell membrane, namely, the interstitial fluid, then the cells comprising these biopsied tissues would maintain a homeostatic balance during transportation under hypothermic conditions.

The findings reported in this document indicate that AQIX® fluid technology can achieve such an outcome.

Trial A: AQIX® RS-I compared to RPMI

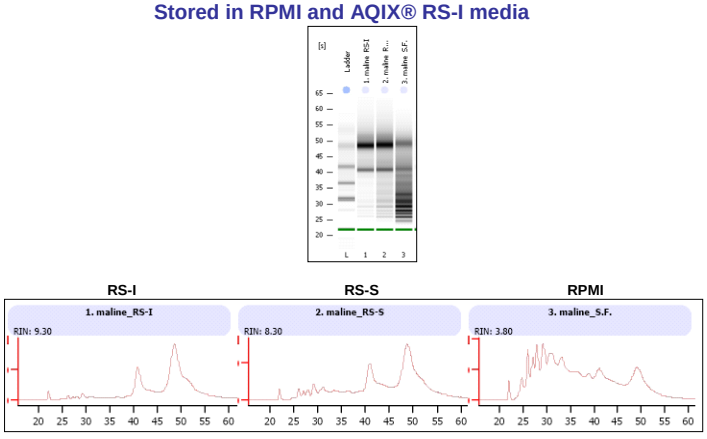
Aims. To compare the preservation of human colon tissue biopsies stored and transported over ice for a period of 30 hours using RPMI culture media verses those samples immersed in either AQIX® RS-I or RS-S solutions. All samples were transported utilising AQIX® Storage & Transportation Kits (Code: RSI/KIT).

Method. Human Colon Tissue Biopsies: Biopsied samples (1cm x 1cm) of normal human colon tissue were procured and placed directly into Nalgene® bottles containing either 125 ml of AQIX® RS-I, AQIX® RS-S or RPMI solutions (see SOP/2009, below) and transported over ice for a period of 30 hours.

Histological examination of tissue samples was carried out using conventional H & E techniques in association with an evaluation of RNA parameters (RNA concentration; rRNA ratio;RIN) using Agilent Technology Inc. analysis procedures.

Results. Human Colon Tissue Biopsies: Tissue samples stored and transported in AQIX® RS-I solution showed superior preservation of both morphology and RNA integrity in comparison to those tissue samples stored and transported in RPMI solution (Figs.1 &2).

Fig. 2 RNA QC of Normal Human Colon Biopsies Transported and Stored in RPMI and AQIX® RS-I media



•30h post operation
•Transportation on ice

Source: Cureline Inc.

Trial B: AQIX® RS-I compared over time

Aims. To evaluate the preservation of normal and cancerous human breast tissue biopsies stored and transported over ice for periods of 24, 36 and 48 hours using AQIX® RS-I solution and contained in AQIX® Storage & Transportation Kits (Code: RSI/KIT).

Method. Normal and Cancerous Human Breast Tissue Biopsies: Biopsied samples (1cm x 1cm) of normal and cancerous human breast tissue were procured and placed directly into Nalgene® bottles containing 125 ml of AQIX® RS-I solution (see SOP/2009, below) and transported over ice for periods of 24, 36 and 48 hours. Histological examination of tissue samples was carried out as for Trial A.

Results. Normal and Cancerous Human Breast Tissue Biopsies: Tissue samples stored and transported in AQIX® RS-I solution showed excellent preservation of both morphology and RNA integrity over 24, 36 and 48 hours. Noteably, the ratio of 28S:18S ribosomal subunits (rRNA) remained high in all three breast cancerous tissue samples at 24 hours and two out of three samples at 36 hours. Of particular note was the observation that during the periods of storage in AQIX® RS-I solution, the RNA concentration remained high in the majority of the breast cancerous tissue samples over the 24-48 hours in comparison to normal breast tissue samples Equally, RNA integrity (RIN) was better preserved in the majority of the breast cancerous tissue samples over the 24-48 hours in comparison to normal breast tissue samples.

[SOP/2009] Storage & Transport of Human Tissue Biopsy Samples

a) AQIX® RS-I solution is packaged in a 125 mL ml Nalgene® PETG Container Kit.

b) Store AQIX® RS-I solution Container Kits in a fridge @ 3 – 8 ºC for not longer than 6 weeks.

c) Dispatch AQIX® RS-I solution Container Kits to tissue retrieval site.

d) Maintain AQIX® RS-I solution Container Kits in a fridge @ 3 – 8 ºC at the tissue retrieval site.

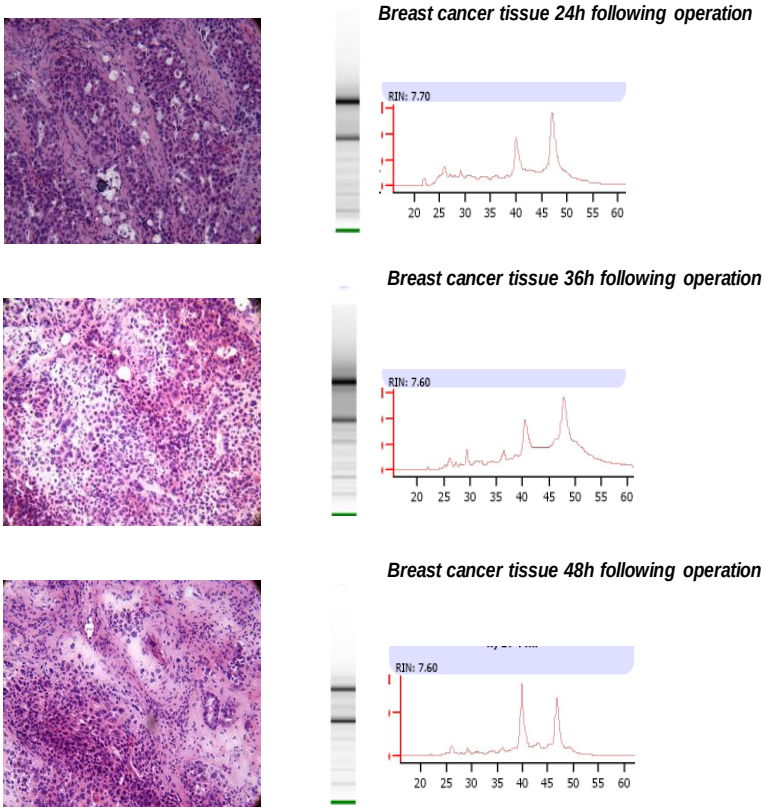
e) Open AQIX® RS-I solution Container Kit for the minimum time possible before inserting the human tissue biopsy sample (Sample size 1cm x 1cm).

f) Quickly attach the lid closure on the Container Kit and close tightly.

g) Transport the 125 mL Container Kit(s) back to laboratory @ 0-4 ºC (over 'wet' ice) in a polystyrene, outer box.

h) Conduct experimental procedures immediately upon arrival of the tissue biopsy samples in the laboratory.

Fig.3 Fresh breast cancer tissue morphology and RNA integrity following 24 - 48h of transportation in AQIX® RS-I solution



Source: Cureline Inc.

Discussion

Acknowledging that RNA is considered a most fragile molecule along with the instability of the cytoarchitecture of malignant or cancerous cells upon isolation (Ohashi et al., 2004), both the morphology and RNA composition of the human breast tissue samples in this study show a remarkable preservation over the 24 – 48 period of storage and transportation over ice in AQIX® RS-I solution. These findings are significant as they compare well with similar findings of biopsies from human colon and breast malignant tissues over a much lesser period of16 hours (Micke et al., 2006).

AQIX® RS-I solution has been designed to simulate the composition of human interstitial fluid and thereby afford isolated cells and the tissues thereof, to maintain homeostasis of biophysical and metabolic parameters during periods of both hypothermic and normothermic preservation which enables individual cells to retain a normal cytosolic balance and therein their cellular morphology. In this study, the maintenance of the cytoarchitecture and metabolism in the fragile, cancerous cells is indicated by their ability to maintain high levels of RNA content during the long periods of hypothermic storage, a comparable observation reported in human cancerous colon and breast tissue cells (Micke et al., 2006). Equally, the ratios of 28S:18S RNA subunits were greater (rRNA:3.0 – 4.5) in the malignant breast tissue samples stored in AQIX® RS-I solution than that found in normal breast tissue samples (rRNA:2.0 – 2.6), again indicating that AQIX® RS-I solution has an ability to enhance maintainance of RNA levels even during suppressed metabolism when stored under hypothermic conditions.

Conclusion

The results presented indicate that AQIX® RS-I solution technology significantly advances the ability to store both normal and human, malignant biopsied tissues and transport such tissue samples over periods of time that overcome geographic distances in a condition that will preserve their morphology, RNA integrity and thereby facility more accurate expression of gene profiles to advance diagnostic and prognostic outcomes.

References:

1. Patrick Micke, Mitsuhiro Ohshima, Simin Tahmasebpoor, Zhi-Ping Ren, Arne Östman, Fredrik Pontén and Johan Botling. Biobanking of fresh frozen tissue: RNA is stable in nonfixed surgical specimens. Laboratory Investigation (2006);86:202–211
2. van't Veer, L.J., Dai, H., van de Vijver, M.J., et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530-536.
3. Ohashi, Y., Creek, K.E., Pirisi, L., et al. RNA degradation in human breast tissue after surgical removal: a time-course study. Exp Mol Pathol 2004;77:98-103.

Authors:
Dr. Douglas Rees[1]
cso@aqix.com
1. Correspndence

Matthew Durdy

Aqix Limited
Imperial Incubator,
London, SW7 2AZ
United Kingdom

The images and data used in this note were kindly supplied by Cureline, Inc.

Human Tissue for Research

www.cureline.com