

## RNA Purity and Yield of Frozen Tumor Tissues from the Biorepository Unit, Chulabhorn Royal Academy

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**Background:** Tumor specimens are valuable and beneficial for medical research. Effective storage is critical for the quality of frozen tumor specimens for successful outcomes. Long-term preservation of tissues can impact RNA more than DNA or protein. Thus, the quality of RNA needs to be maintained from the beginning of tissue collection until its utilization.

**Objective:** The present study evaluated the purity and yield of tumor specimens stored at -80°C in the Biorepository Unit from 2010 to 2019.

**Materials and Methods:** Fresh frozen tumor tissues of colon, liver, cervix, and lung from several cases were annually qualified using a Nanodrop spectrophotometer.

**Results:** We found that the optical density (OD) 260/280 ratio of RNA from all specimens remained more than 1.8. Preservative periods of 1 to 9 years did not affect purity of RNA from tissues. Additionally, RNA yields of tumor tissues were in the range of 0.89 to 15.10 µg/mg depending on the tissue type.

**Conclusion:** The results of the present study suggest that the frozen specimens from Biorepository Unit were properly preserved for the long-term and yielded quality RNA.

**Keywords:** RNA purity, RNA yield, Tumor tissue, Biorepository

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The Biorepository Unit at HRH Princess Chulabhorn College of Medical Science, Chulabhorn Royal Academy has been constituted since 2010 for the preservation and management of human biological specimens. The specimens were obtained with consent from participants and cancer patients of Chulabhorn Hospital and stored at -80°C. Almost 600,000 frozen samples have been stored, including serum, plasma, buffy coat and urine samples, sputum cell pellets and fresh tissues. These samples, in particular the frozen tissues, could be used for tissue profiling for genomics, epigenomics, transcriptomics, proteomics or metabolomics<sup>(1-3)</sup>.

To prevent the degradation of tissues, tissue processing should be carefully performed. Fresh tissues should

be processed after resection until freezing within 30 to 120 min<sup>(3,4)</sup>. The tissues were cut at 0.5×0.5×0.5 cm per piece to reduce the freeze-thaw cycles<sup>(1,5,6)</sup>. The storage temperature was also recommended at -80°C to extend the conservation of molecular substances. However, some studies reported that RNA from tumor specimens may possibly degrade after 5 years of preservation<sup>(1,7,8)</sup>. Thus, many biorepository units are recommended to assure the quality of stored specimens<sup>(1-4)</sup>.

The present study aimed to evaluate the quality of frozen tumor tissues in The Biorepository Unit. Tumor RNA was extracted from fresh frozen tissues and evaluated using optical density (OD), which is a basic approach used to quantify and qualify RNA of tissues<sup>(1)</sup>.

### Materials and Methods

#### Tumor tissue specimens

Before the preserving processes, all cancer patients provided consent to donate their specimens to the Biorepository Unit, HRH Princess Chulabhorn College of Medical Science, Chulabhorn Royal Academy. The cancer care programs have deposited tumor specimens for research since 2010. Patients were under treatment by standard procedures; resected tissues were obtained by a surgeon and a pathologist transported the tissues in a sterile container as rapidly as possible. Fresh tumor tissues were collected and cut into 0.5×0.5×0.5 cm per piece and immediately frozen at -80°C.

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The authors selected liver, colon, cervix and lung tumor specimens from patients who did not receive chemotherapy or radiotherapy before operation. The authors performed both prospective and retrospective evaluations. For the prospective evaluations, the samples were randomized two percentages of new specimens in 2014 from colon cancer (35 cases) and liver cancer (15 cases), so we used one case of colon cancer (FT570084) and one case of liver cancer (FT570042). One piece of frozen tissue from each individual patient was evaluated each year from 2015 to 2018. For the retrospective study, the authors used the result of RNA quality test from our laboratory. The data covered 9 years of tissue preservation that were reinterpreted RNA purity and concentration, including three cases of colon cancer (FT530005, FT530054, and FT550036), two cases of lung cancer (FT550039 and FT570031), one case of liver cancer (FT530012) and one case of cervix cancer (FT570017). However, some samples cannot examine throughout freezing period because of inadequate frozen tissue for test. The protocol of this research was reviewed and approved by the Human research ethics committee, Chulabhorn Research Institute No. 012/2558.

#### **RNA extraction and quality analysis**

Parts of the frozen tissues were weighed and homogenized by pestle (Axygen, CA, USA) and RNA was extracted using 1 ml TRIzol reagent (Thermo Fisher Scientific, CA, USA) according to the manufacturer's instructions. Next, chloroform (VWR International S.A.S, Fontenay-sous-Bois, France) was added and the sample was centrifuged for 15 min at 12,000 rpm at 4°C. The aqueous phase containing RNA was precipitated by 2-propanol (Merck, Darmstadt, Germany) with centrifugation for 10 min at 12,000 rpm at 4°C. RNA pellets were washed with 75% ethanol and diluted in diethyl pyrocarbonate water. The concentration and purity of total RNA was evaluated by a Nanodrop spectrophotometer (Thermo Fisher Scientific). An OD 260/280 ratio more than 1.8 was considered to indicate good quality of RNA. The RNA yields (µg/mg) were calculated using the formula (volume of RNA (µl) × RNA concentration (ng/µl) × 10<sup>-3</sup>) / tissue weight (mg).

#### **Results**

The RNA purity from frozen tissues of colon cancer was evaluated. The OD 260/280 ratios of RNA covering the sixth month to the ninth year of preservation from the first patient (FT530005) were slightly decreased from 2.05 to 1.97. The second case (FT530054) was tested starting in the third year and yielded readings of 2.01 to 1.98 at the end. The third (FT550036) case was examined after 1 year of preservation and showed a OD 260/280 ratio of 2.05. The last prospective sample (FT570084) yielded OD 260/280 ratios nearly constant at 1.97. These results were all higher than 1.8 (range of 1.95 to 2.06) and considered as good quality of RNA, as shown in Figure 1A.

The RNA yields of colorectal tissues were calculated. The results of the first patient increased from

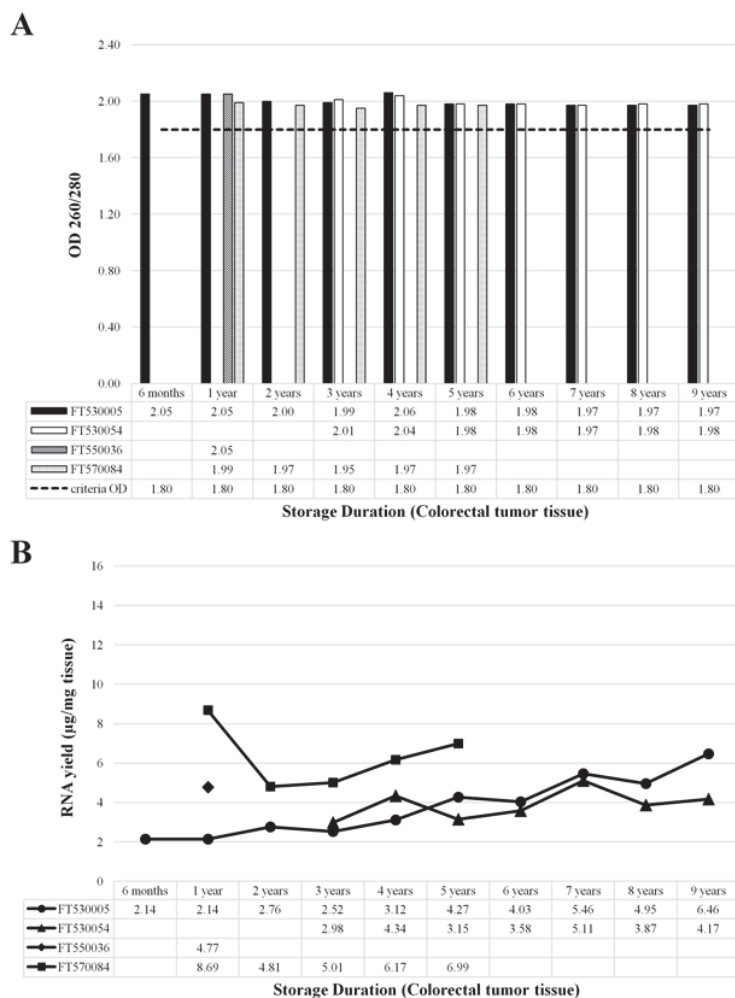
2.14 to 6.46 µg/mg. Tumor tissues of the second patient had RNA yields slightly fluctuate from 2.98 to 4.17 µg/mg. RNA yields of the third case showed 4.77 µg/mg in the year of preservation. The last tumor tissue had RNA yields decreased from 8.69 to 6.99 µg/mg within 5 years of examination (Figure 1B).

The liver tumor tissue of the first patient (FT530012) was collected from the year 2010. The OD 260/280 ratios had narrow changed between 1.89 and 2.05 from the second to eighth year. The prospective case (FT570042) was investigated over a period of 5 years. The purity values closed to 1.98. These results revealed no difference in RNA quality among the storage periods, as shown in Figure 2A. Furthermore, the fluctuated results of RNA yield were found both cases of liver cancer (Figure 2B).

The OD 260/280 ratios of the first year storage from the two lung cancer cases (FT550039 and FT570031) were 2.02 and 1.84 (Figure 3A). The RNA yield of the second cases was 0.89 µg/mg; we did not obtain sufficient RNA from the first case to determine the yield (Figure 3B). Cervical tumor tissues (FT570017) were qualified from the sixth month until the third year after collection. The purity outcomes dropped from 2.01 to 1.93 and RNA yields of these tissues changed between 2.95 and 6.07 µg/mg. These data are shown in Figure 3A and 3B, respectively.

#### **Discussion**

Biospecimens are potentially important materials in medical research. To ensure the quality of stored specimens, the entire processes of preservation must be carefully managed<sup>(5,6)</sup>. Quality assurance should be provided as much as possible as in each biobank<sup>(1-4)</sup>. After examining the quality of frozen tumor RNA, the authors found all samples of colorectal, liver, cervical, and lung cancer yielded RNA with an OD 260/280 ratio above 1.8. Additionally, RNA yields of colorectal tumor tissues ranged from 2.14 µg/mg to 8.69 µg/mg over the period of 9 years. RNA from liver tumor tissues ranged from 1.34 µg/mg to 15.10 µg/mg from the first to the eighth year of preservation. While yields of cervical tumor RNA ranged from 2.95 µg/mg to 6.07 µg/mg within 3 years. The authors only examined one sample (0.89 µg/mg) of lung tumor tissues with a year of storage time. The variation of RNA yield may depend on many factors such as tissue types and components of tissue<sup>(9)</sup> or processes in the laboratory. Although the maximum period of preservation to ensure RNA quality is not known, our results may partly support the stability of RNA under -80°C storage for 9 years in liver and 8 years in colon tumor tissues. Moreover, the findings reveal RNA purity and yield of lung and cervical tumor tissues. Cervical tissues can be preserved at least 3 years without RNA purity change. However, some studies showed that the tissues of normal and cancerous colon along with pancreas cancer show stability under 3 years of preservation<sup>(5,10)</sup>. In contrast, gynecologic tumor tissues and brain tissues showed a decrease in RNA quality after 5 years of storage<sup>(8)</sup>. The longest tissue conservation that still maintains RNA quality is 27 years of



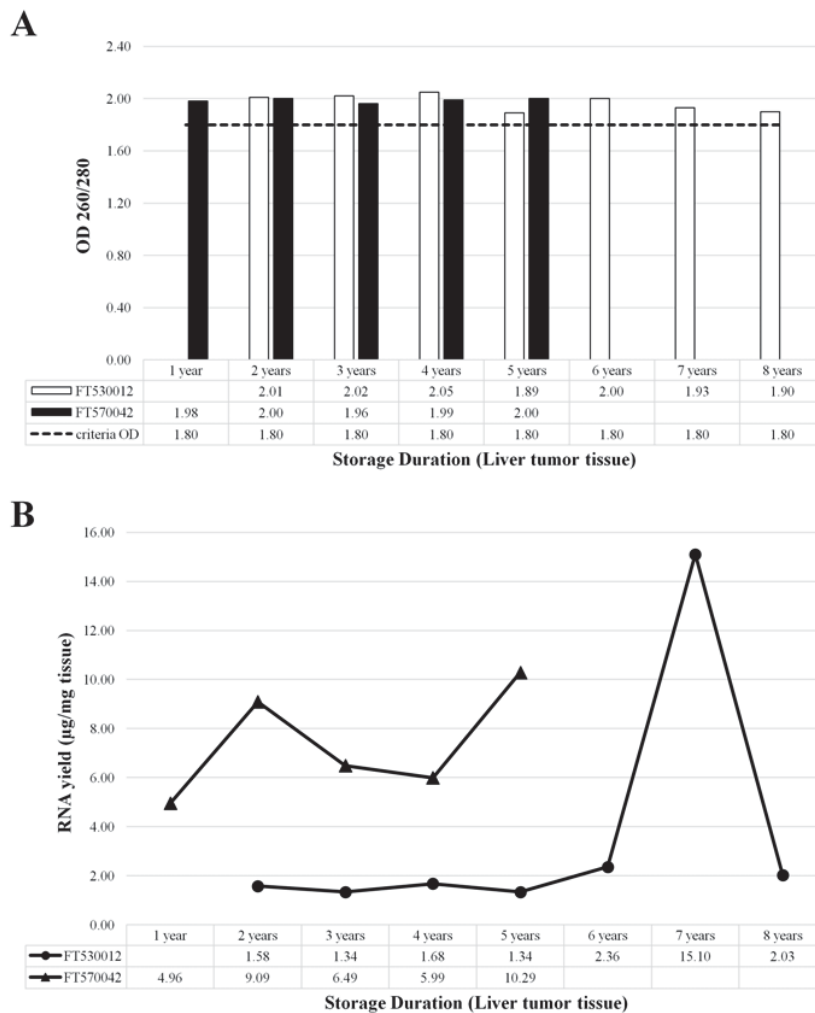
**Figure 1.** Effect of long-term storage on RNA purity and yield of colorectal tumor tissues. The fresh tissues were collected and stored at  $-80^{\circ}\text{C}$  until analysis using Nanodrop spectrophotometer from the sixth month to the ninth year after collection. A) The OD 260/280 ratio of RNA from four colorectal cancer samples. B) The RNA yield from four colorectal cancer samples.

storage for endocrine tissues<sup>(6)</sup>.

A major limitation of current study is the few samples of each tissue type. Because of the number of annual cases and remaining specimens in Biobank, the randomization followed the standard guidance using 2% of newly collected samples. Moreover, the number of aliquot tissues from cancer patients was unequal for each patient. For instance, the authors collected a majority of frozen tissues from 35 colorectal cancer patients but not all cases had more than two preserved pieces. Consequently, the insufficient outcome could not apply to statistic analysis.

The present study had provided the approximate

quality and quantity of frozen tissues that stored at  $-80^{\circ}\text{C}$ . Some types of cancer tissue have been collected for 9 years. The data of these specimens will be of some benefit in further research. Notably, RNA degradation is not directly assessed by Nanodrop spectrophotometers. Hence, techniques with better specificity such as bioanalyzers are commonly used to assess the RNA integrity of samples<sup>(1)</sup>. This approach will be used in future studies. However, not only were the stored samples examined, but the sample records were also counterchecked<sup>(3,4)</sup>. Our biorepository has established facilities for the long-term stability of temperature with an emergency electric supply,  $\text{CO}_2$  back up, and temperature



**Figure 2.** Effect of long-term storage on RNA purity and yield of liver tumor tissues. The fresh tissues were collected and stored at  $-80^{\circ}\text{C}$  until analysis using Nanodrop spectrophotometer from the first year to the eighth year after collection. A) The OD 260/280 ratio of RNA from two liver cancer samples. B) The RNA yield from two liver cancer samples.

detection probes with SMS alert. The standard laboratory procedure of each tissue type processing has also been defined, and time of tissue transportation and ischemic or freeze-thaw events are minimized<sup>(3)</sup>. Some studies suggested that the processing time before freezing can be extended to 16 h, with no degradation effect in tissues<sup>(11)</sup>.

### Conclusion

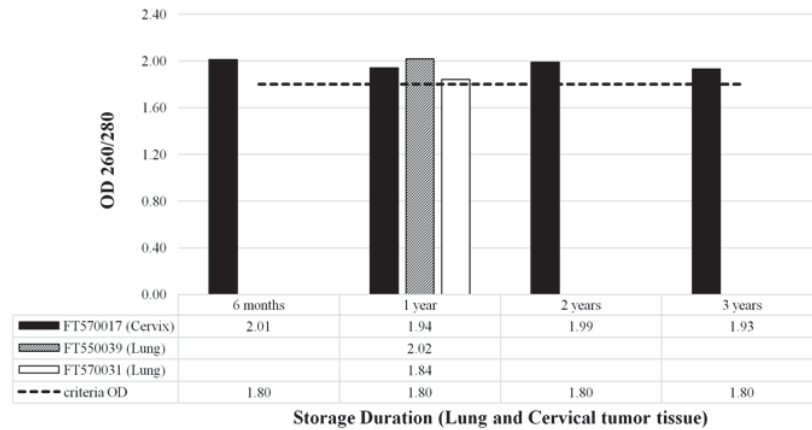
The present study suggested that the procedures of specimen processing are suitable for long-time preservation. The RNA purity of frozen tumor tissues was retained in specimens from lung, cervix, liver, and colon cancer

patients collected after 1 year, 3 years, 8 years and 9 years, respectively. We also showed a range of RNA yield from each cancer type. Biobanks should be providing consistent services for researchers in storing samples. Moreover, quality assurance will be regularly performed for the improvement of better storage protocols.

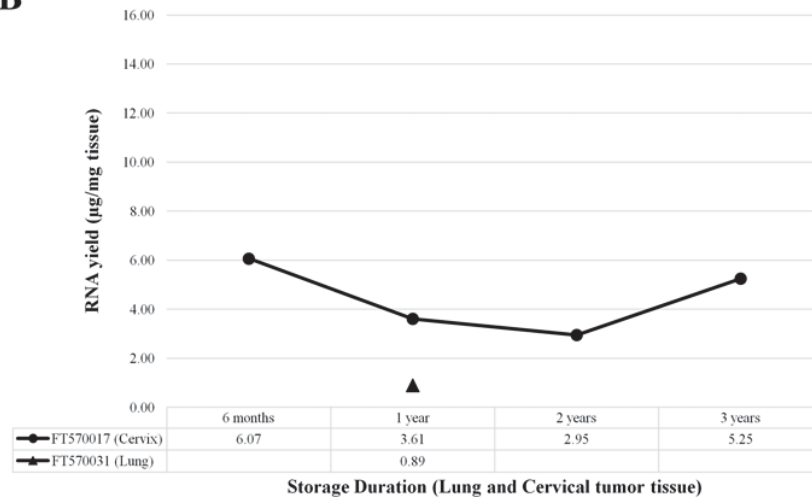
### What is already known on this topic?

Many biorepository units need to warrant their frozen specimens. Previous studies reported the RNA quality of frozen tissues preserved at  $-80^{\circ}\text{C}$  during a period of at least 5 years.

A



B



**Figure 3.** Effect of long-term storage on RNA purity and yield of cervical and lung tumor tissues. The fresh tissues were collected and stored at  $-80^{\circ}\text{C}$  until analysis using Nanodrop spectrophotometer from the sixth month to the third year after collection. A) The OD 260/280 ratio of RNA from one cervical cancer and two lung cancer samples. B) The RNA yield from one cervical cancer and one lung cancer sample.

#### What this study adds?

The Biorepository Unit, Chulabhorn Royal Academy has examined RNA purity and yield from the preserved frozen tissues, including colorectal, liver, lung, and cervical cancers. These tissues retained their RNA purity. A range of RNA yield from each cancer type varied between of 0.89 to 15.10  $\mu\text{g}/\text{mg}$ .

#### Acknowledgements

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#### Potential conflicts of interest

The authors declare no conflict of interest.

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