Storage of cancer tissue at room temperature using TissueReady™ PLUS

The sourcing of primary tissue is vital for many fields of research and is becoming increasingly important for use in advanced screening techniques/diagnosis, drug development and patient-derived model generation as healthcare moves towards personalised medicine. Tissue quality deteriorates rapidly after retrieval, and therefore tissues need to be used as close to the retrieval time as possible to ensure the best quality outcomes. Unfortunately, the sites of tissue retrieval are not always close to sites requiring the tissue, resulting in extended distribution times and a reduced quality of tissue.

TissueReady™ PLUS offers a simple solution that preserves the viability and quality of fresh tissue at ambient temperatures, increasing shelf life, enabling shipment, enhancing the flexibility of workflows and improving the quality of screening and model generation from these tissues.

Overview

Cancer tissue retrieved from patients is important for cancer diagnosis, research and model generation. This study found that cancer tissue stored for 2 days at room temperature in TissueReady™ PLUS retained histological integrity and viable cells could be isolated and expanded from cancer tissue preserved for 4-5 days and maintain cancer phenotype.

Cancer tissue retains histological integrity and marker expression when stored using TissueReady™ PLUS

An effective way to determine the integrity of tissue is to assess the tissue using histological techniques such as H&E staining and immunohistochemical staining. Cancer tissue was retrieved from patients and processed by a clinical pathology laboratory, stored in TissueReady™ PLUS for 2 days at room temperature and processed for H&E, IHC (AE1/AE3 and Ki67) and *in situ* hybridisation (Chromosome 17/HER2). The slides were then examined and stored by a clinical pathologist who was blinded to the tissue conditions. The observations were then collated and compared to non-stored tissue and tissue stored in medium alone.

Data and histological evaluation of cancer tissue preserved for 2 days at room temperature using TissueReady™ PLUS concluded that:

· Histological integrity was maintained · Markers for AE1/AE3 and Ki67 were retained · RNA could successfully be extracted

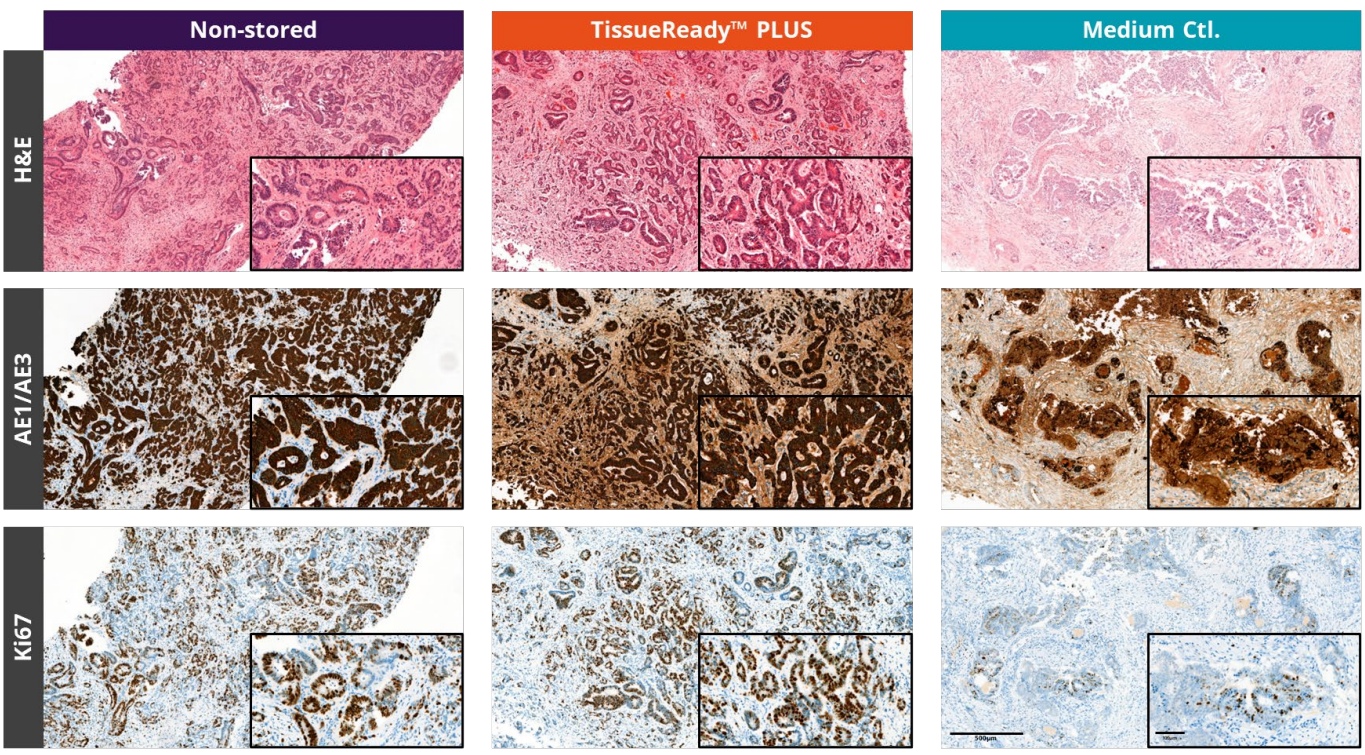

Figure 1: The histological integrity and histopathological markers of liver cancer tissue are retained in tissue preserved for 2 days at room temperature using TissueReady™ PLUS. Liver cancer tissue was retrieved from patients and sent to a clinical pathology laboratory where it was either processed immediately (non-stored) or stored in TissueReady™ PLUS or DMEM medium. Samples were stored for 2 days at room temperature before processing. Samples were fixed, paraffin embedded and sectioned before being stained for H&E, immunohistochemical markers - AE1/AE3 (cytokeratin cocktail) Ki67 (proliferation maker). Main image scale bar = 500µm, imbedded image scale bar = 100µm.

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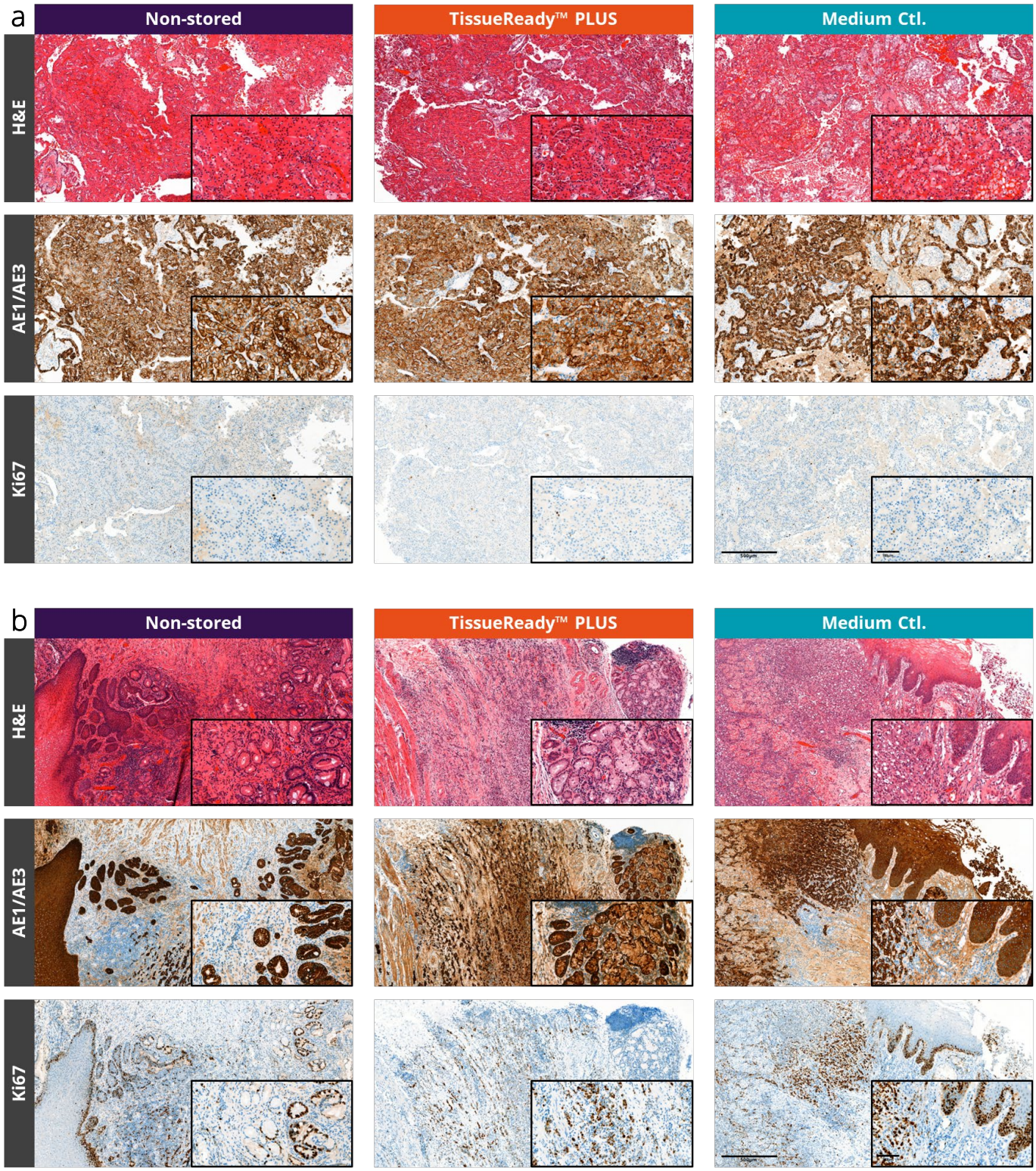

Figure 2: The histological integrity and histopathological markers of kidney and oesophagus cancer tissue are retained in tis sue preserved for 2 days at room temperature using TissueReady™ PLUS. Kidney **(a)** and oesophagus **(b)** cancer tissue was retrieved from patients and sent to a clinical pathology laboratory where it was either processed immediately (non-stored) or stored in TissueReady™ PLUS or DMEM medium. Samples were stored for 2 days at room temperature before processing. Samples were fixed, paraffin embedded and sectioned before being stained for H&E, immunohistochemical markers - AE1/AE3 (cytokeratin cocktail) Ki67 (proliferation maker). Main image scale bar = 500µm, imbedded image scale bar = 100µm.

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Cancer tissues stored using TissueReady™ PLUS maintained a good evaluable quality for pathological diagnosis with identification of cancer cell populations and morphological changes to tissue.

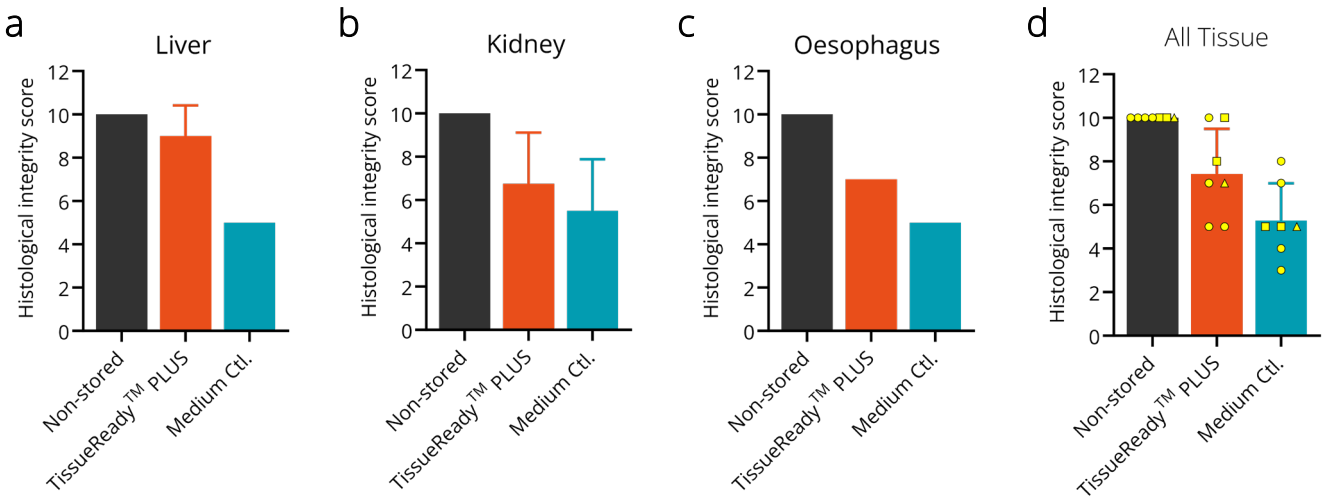

*Figure 3: Histological integrity of cancer tissue is good quality in tissue preserved for 2 days at room temperature using TissueReady™ PLUS. Liver **(a)**, Kidney **(b)** and Oesophagus **(c)** cancer tissue was retrieved from patients sent to a clinical pathology laboratory where it was either processed immediately (non-stored) or stored in TissueReady™ PLUS or DMEM medium. Samples were stored for 2 days at room temperature before processing. Samples were fixed, paraffin embedded and sectioned before being stained for H&E. H&E slides were then assessed by a clinical pathologist and given a histological integrity score. Histological integrity score is a score out of 10. 10 – Perfect, 9 to 5 – good quality, 4-0 – Poor quality (unusable). All Tissue **(d)** is a combination of the 3 tissues, point shapes indicate source tissue, Square – Liver, Circle – Kidney, Triangle – Oesophagus.*

Tissues stored in TissueReady™ PLUS are suitable for molecular biology assessment

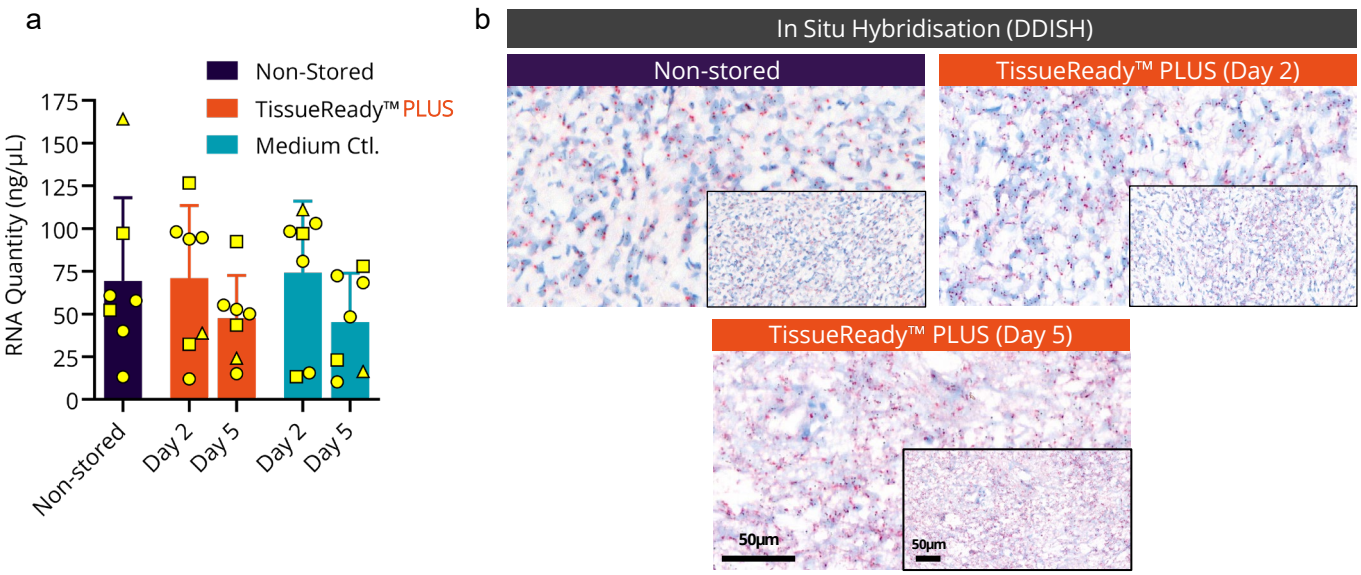

*Figure 4: RNA can be isolated and in situ hybridisation can be performed on tissue preserved using TissueReady™ PLUS. Cancer tissue was retrieved from patients and sent to a clinical pathology laboratory where it was either processed immediately (non-stored) or stored in TissueReady™ PLUS or DMEM medium. Samples were stored for 2 days and 5 days at room temperature before processing. **a:** RNA was isolated using an RNA extraction kit and RNA quantity was assessed using a nanodrop spectrophotometer. Datapoint shapes indicate source tissue, Square – Liver, Circle – Kidney, Triangle – Oesophagus. **b:** Dual DNA In Situ Hybridisation (DDISH) against the HER2 gene and chromosome 17 was performed on stored tissue to interrogate cancer status in oesophageal tissue.*

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Cells can be isolated, cultured and expanded for multiple passages from tissue stored using TissueReady™ PLUS

The capability of making cell models from cancer tissue is important in cancer research, drug discovery and personalised medicine. To assess cell isolation from cancer tissue, cells were isolated by tissue dissociation, expanded and stained for a cancer marker following storage in TissueReady™ PLUS for 4-5 days at room temperature and compared to cells isolated from fresh, non-stored cancer tissue.

Cells from tissue preserved using TissueReady™ PLUS was comparable to non-stored, fresh tissue for:

- Cell viability
- Cell yield
- Ratio of cytokeratin-7 positive (cancer cells) and cytokeratin-7 negative (normal cells)
- Continued expansion/passaging (passaged 6 times, frozen and revived)

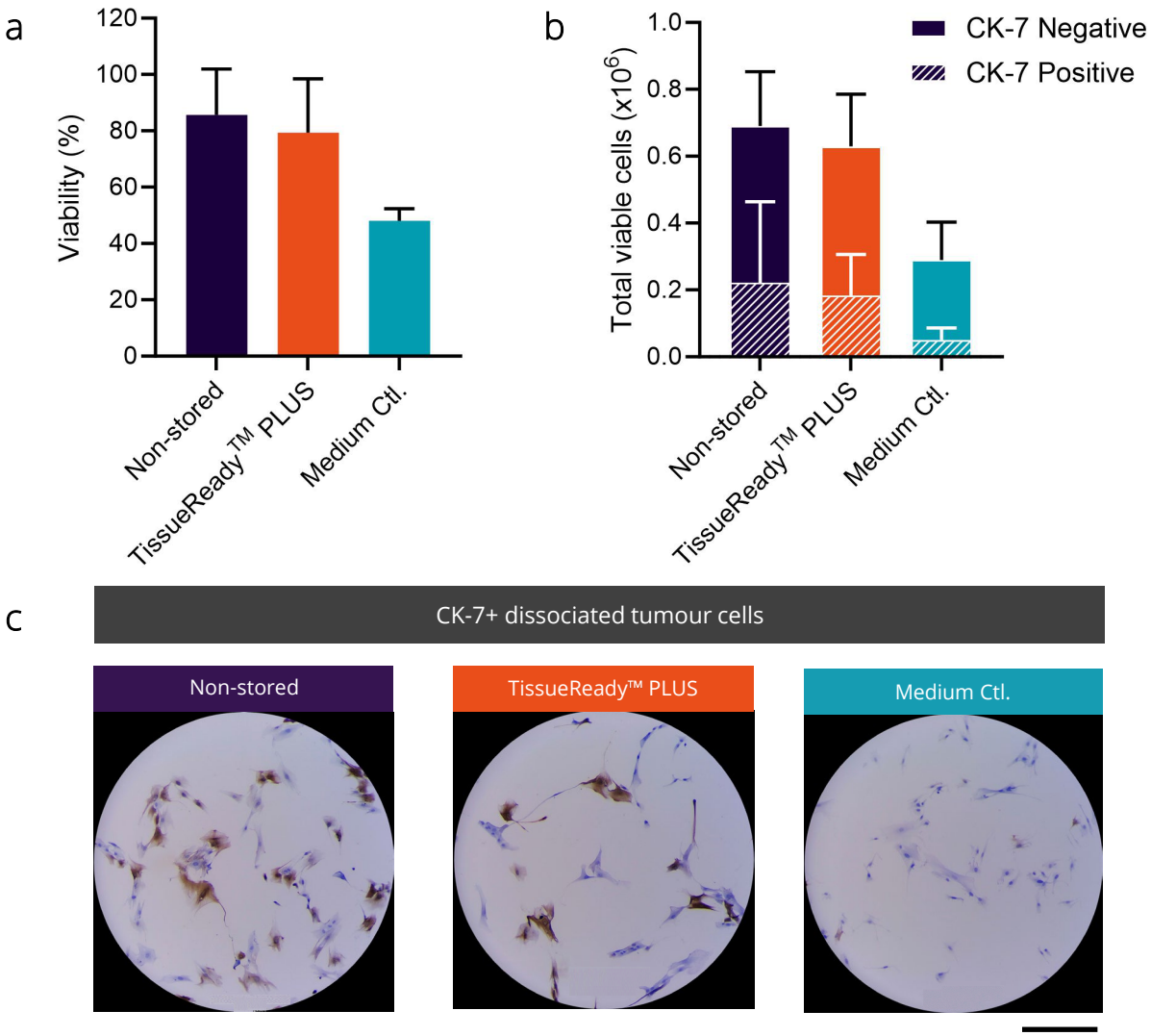

Figure 5: Cell viability and cell yield was comparable between cells isolated from fresh cancer tissue and cancer tissue preserved in TissueReady™ PLUS for 4-5 days at room temperature. *Cancer tissue was retrieved from patients and sent to a clinical pathology laboratory where it was stored in TissueReady™ PLUS or medium before being shipped to an oncology research facility. The tissue was preserved for 4-5 days (from encapsulation) at room temperature before cells were isolated by tissue dissociation. Isolated cells were expanded before being fixed and stained for Cytokeratin-7 to differentiate cancer cells from non-cancer cells.* ***(a)*** *Cell viability as determined by brightfield microscopy and trypan blue exclusion.* ***(b)*** *Viable cell yield calculated by counting the number of live cells using brightfield microscopy and trypan blue exclusion. Cells were fixed on slides were immunohistochemically stained for cytokeratin-7 using recombinant monoclonal rabbit anti-human cytokeratin 7 primary antibody and a biotinylated goat anti-rabbit polyclonal secondary antibody. Staining was visualised using Vectastain ABC and DAB substrate kits. . The percentage of positive and negatively stained cells was calculated, and these percentages applied to the viable cell yield.* ***(c)*** *Brightfield images of fixed cells stained for cytokeratin-7, Positive cells (Brown) and negative cells (Blue) were used to calculate the percentage of cancer cells present. Scale bar represent 360µm.*