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A comprehensive preanalytical protocol for fresh solid tumor biospecimens

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ABSTRACT

Nearly seventy percent of diagnostic lab test errors occur due to variability in preanalytical factors. These are the parameters involved with all aspects of tissue processing, starting from the time tissue is collected from the patient in the operating room, until it is received and tested in the laboratory. While there are several protocols for transporting fixed tissue, organs, and liquid biopsies, such protocols are lacking for transport and handling of live solid tumor tissue specimens. There is a critical need to establish preanalytical protocols to reduce variability in biospecimen integrity and improve diagnostics for personalized medicine. Here, we provide a comprehensive protocol for the standard collection, handling, packaging, cold-chain logistics, and receipt of solid tumor tissue biospecimens to preserve tissue viability.

1. Introduction

Cancer is a paradox in that it is simultaneously pervasive, with nearly half the world [1] projected to be diagnosed with cancer, and uniquely personal, such that every patient's tumor is unique. This makes cancer diagnosis and treatment, particularly for solid tumors, incredibly complex and, in many cases, intractable. Exacerbating this further, the incidence of cancer continues to rise with nearly 2 million new cases diagnosed every year just in the USA [2,3]. Although every patient's tumor is distinct, clinical teams are often forced to use a generalized approach to treat a disease that is uniquely personal. Consequently, according to US Food and Drug Administration (FDA), systemic therapy turns out to be ineffective for nearly 3 out of 4 patients [4]. A precision personalized medicine approach based on *ex vivo*, *in silico*, or combined evaluation [5] of each patient's tumor on an individualized basis, could result in considerable improvements across the cancer care continuum – from drug discovery to diagnosis, treatment, and surveillance.

However, reliability of live tissue analyses using fresh tumor biospecimens depends on how biospecimens are obtained from patient tumors, processed, and transported [6,7]. This is pronounced further in the context of solid tumors, where heterogeneity and biological complexity pose significant challenges for accurate characterization and subsequent clinical interpretation. Preanalytical processing encompasses factors starting from the time of tissue extraction until analytical testing, including collection, handling, and transport of biospecimens [8]. Variability during preanalytical processing increases the potential for degradation of biospecimens and diminished repeatability in subsequent downstream analyses. Thus, preanalytical processing is a significant contributor to the quality and reliability of analytical testing and cellular pathology readouts [6–9]. For increased reliability, tissue biospecimens must be maintained in a viable state for the entire duration, from collection to analysis, without significant apoptosis, necrosis, or drifting. However, due to lack of standardized live solid tumor tissue preanalytical protocols, integrity of biospecimens collected from patients is often compromised. Consequently, nearly 70% of lab test errors [6,8–10] occur due to variability in preanalytical factors. Variations in biospecimen collection, processing, and logistics can lead to discrepancies in results, thereby confounding interpretation of pathophysiological mechanisms, biomarker discovery, and therapeutic responses.

Recent advances in *ex vivo* cancer models [5,11–13] further underscore the need for standard preanalytical protocols to preserve, insofar as possible, fidelity of extracted biospecimens to the original patient tumor tissue. Such standardization is paramount for enhancing the reproducibility of tests, facilitating meaningful parity across sites, and ultimately supporting the development of more effective diagnosis and treatment strategies. Stemming from a commitment to reducing preanalytical variability and furthering a viable pathway to a precision personalized approach to cancer treatment, we present a preanalytical protocol for fresh solid tumor biospecimens that was developed over a multi-year collaborative effort between CerFlux and the Ohio State

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University Wexner Medical Center and The James Cancer Hospital [7]. Our protocol is informed by best practices in biospecimen handling and empirical evidence from our own research endeavors.

By addressing key preanalytical factors such as post-collection processing, transport media, and logistics, we aim to reduce preanalytical variability thereby enhancing rigor, reproducibility, and biological relevance of specimens. Further, the detailed, step-by-step approach adopted in the Materials and Methods section of this paper aims to facilitate implementation across diverse collection and analytical settings. It is our hope that adoption of this and other standardized protocols will further enhance accuracy of assays based on fresh solid tumor biospecimens, and pave the way for precision personalized cancer treatment strategies.

2. Materials and methods

Research manuscripts typically opt for brevity in this section to accommodate word and page length constraints. However, a detailed delineation of materials and methods is vital for rigor and reproducibility. This is of particular importance to cellular pathology and lab tests associated with live solid tumor tissue. Thus, this protocol paper is designed to instead focus on a detailed description of the materials and procedures involved in the collection, handling, and transport of live solid tumor tissues. It addresses the critical need for reducing variability in lab tests, which can compromise reliability and readout of the tests. By delineating each step and associated materials and supplies, we provide a blueprint for standardization that can be adapted across varied clinical and lab settings.

This section first lists the materials and procedures involved in reagent preparation and tissue transport kit assembly followed by a detailed protocol for collection, handling, packaging, shipping, and receiving live solid tumor tissue for lab tests. We encourage evaluation and adoption of this protocol to facilitate more reliable and standardized preanalytical processing of live solid tissue for cellular pathology and other lab tests.

2.1. Before you begin

Permissions: Patient permission for the tumor tissue study should be obtained. Tumor tissue specimens were collected from patients who underwent surgery at the James Cancer Hospital of the Ohio State University Wexner Medical Center, located in Columbus, Ohio. Biospecimens were reviewed internally to ensure de-identification. All patients provided informed consent for the collection and donation of the specimens.

Note: In order to distinguish laboratory at the collection site from the laboratory at the analysis site, in this protocol, the laboratory at the collection site will be referred to as OSUCCC-Lab and the laboratory at the analysis site will be referred to as CerFlux-Lab. Note that this convention is adopted for reader convenience and clarity only. The protocol is designed for use between any two sites.

2.2. Key resources

Key equipment and resources needed for this protocol are listed in Table 1.

2.3. Preparing reagents and supplies

2.3.1. Preparing transport media

Strict aseptic techniques must be followed when preparing and handling transport media. At a minimum, the biosafety hood that will be used to prepare media must be sterilized by exposure to UV for about 15 minutes followed by wiping down with 70% ethanol. Caution: Ensure biologic and reagent substances are not present in the biosafety cabinet during sterilization. In a sterile biosafety cabinet, combine specified

Table 1
Key equipment and resources table.

Reagent or Resource	Source	Identifier
Chemicals, peptides, and recombinant proteins		
ddH2O		
DMEM/F-12 50/50, with L-glutamine and without phenol red (1X)	Corning	REF: 16–405-CV
DMEM/F-12 50/50, with L-glutamine and phenol red (1X)	Corning	REF: 10–090-CV
Fetal Bovine Serum (FBS)	Corning	REF: 35–015-CF
GlutaMAX (100X)	Gibco	REF: 35050–061
Paraformaldehyde (PFA), 4 %	Thermo Scientific	CAT#: J61899
Penicillin-Streptomycin (100X)	Corning	REF: 30–002-CI
Phosphate-Buffered Saline, USP sterile (10X)	VWR	CAT#: 97063–660
Polyamine supplement (1000X)	Sigma-Aldrich	SKU: P8483
Primocin	InvivoGen	CAT#: ant-pm-1
Reagent alcohol, v/v, 70 %	RICCA	CAT#: 2546.70–5
Trace element A	Corning	REF: 25–021-CI
Trace element B	Corning	REF: 25–022-CI
Software and algorithms		
Govee Home application	Shenzhen Intellirocks Tech Co. Ltd.	Version: 6.0.02
Microsoft Office 365	Microsoft Corporation	
Other		
Analytical balance	U.S. Solid	
Biohazard specimen bags	VWR	CAT#: 11215–684
Biosafety cabinet	Baker Co.	
Bottle-top vacuum filter (500 mL)	VWR	CAT#: 10040–436
Centrifuge tubes (1.5 mL)	VWR	CAT#: 16466–046
ClipTip tips (1000 uL)	Thermo Scientific	CAT#: 14–488-010
Conical tubes (50 mL)	VWR	CAT#: 21008–178
Conical tubes (5 mL)	Globe Scientific	CAT#: 111580BS
Electronic single channel pipette (15–1250 uL)	Thermo Scientific	CAT#: 14–3879-53BT
Foam container	Polar Tech	CAT#: 204/T12C
Forceps		
Freezer (−20 °C)	Whirlpool	
Fridge (4 °C)	Whirlpool	
Glass beads	VWR	CAT#: 75999–332
Glass bead sterilizer	VWR	CAT#: 75999–324
Glass Petri dish	VWR	CAT#: 470313–346
Ice packs	Nordic Ice	CAT#: NB15
Kimwipes	Kimberly-Clark	
Label maker	Brother	CAT#: PT-D210
Packaging tape	UPS	
Parafilm “M”	Sigma-Aldrich	SKU: P7668
Pipette aid	Drummond	
Plastic Petri dish	VWR	
Ruler scale		
Serological pipette (10 mL)	VWR	CAT#: 75816–100
Serological pipette (50 mL)	VWR	CAT#: 75816–088
Scalpel		
Scalpel blade (No. 10)	VWR	CAT#: 76457–442
Transportation boxes	Polar Tech	CAT#: 204/T12C
UV sterilizer		
Vacuum filter (150 mL)	VWR	CAT#: 10040–460
Wi-Fi Thermo-Hygrometer	Govee	REF: 210–166043

amounts of each item from Table 2 to prepare 536.50 mL of transport media. Sterilize the solution using a 500 mL capacity vacuum filter. Store 50 mL aliquots at 4°C so that the prepared transport media is stable for up to 12 months.

2.3.2. Preparing washing media

Strict aseptic techniques must also be followed (Described in Section 2.3.1) when preparing washing media. In a sterile biosafety cabinet, combine specified amounts of each item from Table 3 to prepare 561 mL of washing media. Sterilize the solution using a 500 mL capacity vacuum filter. Store 50 mL aliquots at 4°C so that the prepared transport media is stable for up to 12 months.

2.3.3. Preparing buffer solution

Strict aseptic techniques must also be followed (Described in Section 2.3.1) when preparing buffer solution. In a sterile biosafety cabinet, combine specified amounts of each item from Table 4 to prepare 50 mL of buffer solution. Sterilize the solution using a 150 mL capacity vacuum filter. Store 50 mL aliquots at 4°C so that the prepared buffer solution remains stable for up to 12 months.

2.3.4. Assembling tissue procurement kits

Items listed in Table 5 will be used to assemble a tissue procurement kit.

2.4. Step-By-Step method details

2.4.1. Packaging and shipping tissue procurement kits to collection facility

This segment of the protocol describes preanalytical parameters involved in sending tissue procurement kits to collection facilities. To ensure consistency in packaging materials and procedures, fully assembled tissue procurement kits containing all materials needed for biospecimen storage and transportation are shipped from the CerFlux-Lab to the tissue collection facility. Strict aseptic techniques must be implemented when preparing and handling media and other reagents. Prepare and package tissue procurement kits for shipment to collection facility in a sterile environment as follows:

1. Transfer 3.5 mL transport media (See Section 2.3.1) into a sterile, labeled, 5 mL conical tube using a sterile 10 mL serological pipette in a sterile biosafety cabinet.
2. Use the 1250 uL electronic single channel ClipTip pipette with sterile 1000 uL ClipTip tips to dispense 1 mL of sterile 4% PFA into a sterile, labeled 1.5 mL centrifuge tube.
3. Spray exteriors of both the 5 mL conical tube, containing transport media, and the 1.5 mL centrifuge tube, containing PFA, with 70% ethanol; wipe down with Kimwipes.
4. Place both tubes in a sterile biohazard specimen bag and seal shut.
5. Prepare tissue procurement kit in a sterile environment.
   a. Open the foam container and spray the interior with 70% ethanol.
   b. Spray one reusable ice pack with 70% ethanol and place it on the bottom of the box.
   c. Spray the biohazard specimen bag containing both tubes and Govee Wi-Fi Thermo-Hygrometer with 70% ethanol; place them side-by-side onto ice pack.
   d. Spray two reusable ice packs with 70% ethanol and insert them on either side of the biohazard bag and Govee; angle ice packs to cover the top.
   e. Close the lid of the foam container and spray the exterior with 70% ethanol.
   f. Seal foam container using clear packaging tape.
   g. Spray interior of transport box with 70% ethanol and place foam container inside.
   h. Insert the tissue specimen collection form inside a biohazard bag and place on top of the foam container.
   i. Seal transport box using clear packaging tape and attach shipping label to the outside.
6. Notify the collection facility of package shipment.
7. Send return shipping label to collection facility electronically.

Table 2
Materials and supplies for preparing transport media.

Item	Amount
DMEM/F-12 with L-glutamine and phenol red	500 mL
FBS	25 mL
GlutaMAX 100X	5 mL
Pen-Strep	5 mL
Primocin	1 mL
Polyamine supplement 1000X	125 µL
Trace element A	125 µL
Trace element B	250 µL
Total	**536.50 mL**

Table 3
Materials and supplies for preparing washing media.

Item	Amount
DMEM/F-12 with L-glutamine, without phenol red	500 mL
FBS	50 mL
GlutaMAX 100X	5 mL
Pen-Strep	5 mL
Primocin	1 mL
Total	**561 mL**

Table 4
Materials and supplies for preparing buffer solution.

Item	Amount
ddH2O	45 mL
PBS (10X)	5 mL
Total	**50 mL**

Table 5
Materials and supplies needed to assemble one tissue procurement kit.

Item	Amount
1.5 mL centrifuge tube with 4 % PFA	1 mL
5 mL conical tube with transport media	3.5 mL
Biohazard specimen bag	2
Foam container	1
Govee Wi-Fi Thermo-Hygrometer	1
Reusable ice pack	3
Tissue specimen collection form	1
Transport box	1

2.4.2. Receiving tissue procurement kit at collection facility

This step of the protocol focuses on preanalytical parameters involved in receiving and storing contents of the tissue procurement kits. Strict aseptic techniques must be implemented when preparing and handling media and other reagents. Tissue procurement kit must be processed after receipt in a sterile environment as follows:

1. Spray exterior of transport box with 70% ethanol and open it in a sterile environment.
2. Retrieve biohazard bag containing tissue specimen collection form and spray exterior with 70% ethanol before opening. Note: This form should be completed at the time of biospecimen collection.
3. Remove the foam container and spray its exterior with 70% ethanol before opening.

4. Remove ice packs and sterilize with 70% ethanol before storing at –20°C for reuse.
5. Retrieve biohazard specimen bag containing media and PFA tubes; spray exterior of bag with 70% ethanol before unsealing.
6. Spray exteriors of both tubes with 70% ethanol before examining for damage or contamination.
7. Store 5 mL conical tube containing transport media in a sterile environment at 4°C until use.
8. Store 1.5 mL centrifuge tube containing 4% PFA in a sterile environment at ambient room temperature until use.
9. Sterilize transport box and Govee Wi-Fi Thermo-Hygrometer with 70% ethanol and retain for shipping back to CerFlux-Lab.
10. Notify CerFlux-Lab of package receipt.
11. Ensure return shipping label has been received electronically.

2.4.3. Collecting and handling biospecimens at collection facility.

Aseptic techniques must be implemented for all surgical procedures and handling of biospecimens in the operating room.

1. Under IRB approved protocol, consent and enroll patients diagnosed with gastrointestinal cancers who are undergoing standard of care surgery.
2. Prep patients and their operative site under established protocol.
3. Following entry into abdominal cavity, a portion of the resected tumor specimen being submitted for biobanking was immediately placed into a biospecimen container.
4. Within 30 minutes of specimen resection, research personnel should transport the biospecimens (on ice) to OSUCCC-Lab.

Additional considerations when tissue biospecimen must be cleaned after resection:

1. Immediately after resection, gently rinse resected tissue using sterile 1X PBS to remove blood, debris, and potential contaminants. Avoid harsh rinsing to prevent tissue damage.
2. In some cases, washing media (which contains antibiotic Pen-Strep solution) may be used to rinse tissue specimen to reduce the risk of bacterial contamination.

Additional considerations in cases of prolonged surgical procedure:

1. Immediately after resection and cleaning, carefully place tissue biospecimen into a pre-labeled sterile vial containing transport media. Ensure tissue is fully submerged in media.
2. Securely close the vial with a sterile lid and place the vial on ice in a covered sterile container until it can be transported to OSUCCC-Lab.
3. Ideally, assign dedicated personnel to transport tissue within 30 minutes of resection to OSUCCC-Lab.

2.4.3. Packaging and shipping of biospecimens from the collection facility

This step of the protocol focuses on preanalytical parameters involved in shipping fresh solid tumor tissue from OSUCCC-Lab. All specimens should be shipped to CerFlux-Lab within 12 hours of collection; all specimens must be received at CerFlux-Lab within 24 hours of shipment. Aseptic techniques must be implemented when handling biospecimens. Preparing and packaging the tissue procurement kit for shipment must be done in a sterile environment.

1. In OSUCCC-Lab, biospecimens must undergo appropriate de-identification, labeling, and processing.
2. Place fresh solid tumor tissue biospecimens in a glass Petri dish and wash with 1X PBS.
3. Cut a 2 mm x 2 mm piece from a tumor specimen and immediately place into the 1.5 mL centrifuge tube containing 1 mL of 4% PFA. Note: This fixed specimen will be used for histological and correlative studies.
4. Immediately place all remaining tumor specimens into the 5 mL conical tube containing 3.5 mL of transport media.
5. Label both tubes with biospecimen identification details. Note: These labels should only identify the specimen and should not contain any patient identification information.
6. Seal both tubes with Parafilm and spray exterior of tubes with 70% ethanol and wipe down.
7. Place tubes into the provided biohazard specimen bag; spray exterior of bag with 70% ethanol and wipe down.
8. Prepare transportation box as detailed under *Packaging and shipping tissue procurement kits to collection facility*, steps 5a-i. Ensure that ice packs are tightly packaged but not to the extent that tissue or other contents are strained or otherwise damaged.
9. Send package to appropriate delivery provider for overnight shipping.
10. Notify CerFlux-Lab of shipment.

2.4.4. Receiving and handling biospecimens at analysis facility

This final step of the protocol describes preanalytical parameters involved with receiving, storing, and handling tumor tissue after it is received at CerFlux-Lab. Aseptic techniques must be implemented when handling biospecimens in the laboratory. All procedures must be done in a sterile environment.

1. Spray exterior of transport box with 70% ethanol and open it in a sterile environment.
2. Retrieve biohazard bag containing completed tissue specimen collection form and spray exterior before opening. Confirm all information on the form and document into laboratory records.
3. Remove the foam container and spray the exterior with 70% ethanol before opening.
4. Remove ice packs and sterilize with 70% ethanol before storing at –20°C for reuse.
5. Retrieve biohazard specimen bag and spray with 70% ethanol before unsealing.
6. Spray exteriors of both tubes with 70% ethanol before examining for damage or contamination. Ensure tubes are properly labeled.
7. Confirm all items were maintained at the proper transport temperature and humidity by the Govee Wi-Fi Thermo-Hygrometer data.
8. Sterilize the thermo-hygrometer with 70% ethanol and retain for future use.
9. Handle fixed solid tissue biospecimens in a sterile biosafety cabinet. Note: At a minimum, biosafety hood must be sterilized by UV exposure for 15 minutes followed by wiping down with 70% ethanol. Ensure biologic and reagent substances are not present in the biosafety cabinet during sterilization.
   a. Complete tissue fixation by storing the 1.5 mL centrifuge tube, containing fixed tissue specimen in 4% PFA, in a sterile environment at ambient room temperature for 24 hours.
   b. Sterilize a glass Petri dish with 70% ethanol; once dry, sterilize in a UV hood for 10 minutes. Bring the dish into the biosafety cabinet.
   c. Place an aliquot of buffer solution (See Section 2.3.3) in biosafety cabinet.
   d. Using forceps, remove the fixed biospecimen from the tube and place it into the glass Petri dish. Note: Forceps should be sterilized in a glass bead sterilizer at 260°C then acclimated to ambient temperatures before use.
   e. Use a pipette aid and sterile 10 mL serological pipettes to wash biospecimen three times with 3 mL of buffer solution.
   f. Place biospecimen into a labeled, sterile 1.5 mL centrifuge tube containing 1 mL of buffer solution.
   g. Store tube in a sterile environment at ambient room temperature until histological and correlative analyses.

10. Handle fresh solid tissue biospecimens in a sterile biosafety cabinet. Note: At a minimum, biosafety hood must be sterilized by exposure to UV for about 15 minutes followed by wiping down with 70% ethanol. Caution: Ensure biologic and reagent substances are not present in the biosafety cabinet during sterilization.
    a. Sterilize glass Petri dish with 70% ethanol; once dry, sterilize in UV hood for 10 minutes. Bring dish into biosafety cabinet and keep cold using sterile ice packs.
    b. Place an aliquot of buffer solution (See Section 2.3.3) in biosafety cabinet.
    c. Place an aliquot of washing media (See Section 2.3.2) in biosafety cabinet.
    d. Remove live biospecimen from the 5 mL tube using a pipette aid and sterile 10 mL serological pipette.
    e. Use a pipette aid and sterile 10 mL serological pipettes to thoroughly wash biospecimen five times with 5 mL of ice-cold buffer solution to remove debris.
    f. Transfer specimen into a sterile, plastic, lidded Petri dish.
    g. Place Petri dish with biospecimen on a sterile analytical balance to accurately determine initial biospecimen weight. Note: Tare balance to 0.000 g using an empty Petri dish prior to weighing sample. Compare data provided in tissue specimen collection form and document for laboratory records.
    h. Move Petri dish with biospecimen on a millimeter precision ruler to image and record tissue dimensions. Note: Compare data provided in the tissue specimen collection form and document for laboratory records.
    i. Use forceps to transfer biospecimen back into cold glass Petri dish. Note: Forceps should be sterilized in a glass bead sterilizer at 260°C then acclimated to ambient temperatures before use.
    j. Add 6 mL of washing media into the glass Petri dish with biospecimen.
    k. Hold biospecimen in place using forceps; section off a sample from the outside of the specimen using a 1 mm biopsy punch, 3 mm biopsy punch, or scalpel. Note: Forceps, scalpel, and biopsy punch should be sterilized in a glass bead sterilizer at 260°C then acclimated to ambient temperatures before use.
    l. Place sectioned sample into a sterile, labeled 1.5 mL centrifuge tube containing 1 mL of 4% PFA. Note: This will be fixed for histological and correlative studies.
    m. Follow steps 9a-g for this fixed specimen as well.
11. The remainder of the fresh solid tumor tissue biospecimen can be processed for analytical laboratory testing.

3. Results and discussion

The temperature of the tissue procurement kit during transportation was monitored using Govee Wi-Fi Thermo-Hygrometer data; it maintained a consistent and stable temperature range of –5°C to 5°C throughout transport (Fig. 1). The box plot of temperatures recorded by Govee loggers during transport of tissue containers provides a representative summary of temperature measurements from the time that the tissue is packaged to the time when the container is received.

Overall, median temperatures within all containers were consistently recorded between 2°C and 3°C. The interquartile range (IQR), indicative of data dispersion, was narrowly confined to a 1°C interval, between 2°C and 3°C, suggesting that a strong degree of temperature control was maintained during transit. Notably, the majority of temperature readings during overnight transit were tightly clustered near and just above 0°C. Outlier temperatures, which extended beyond 1.5 times the IQR, occurred only around packaging and unpackaging. However, even these outliers remained within the acceptable predefined range of −5°C and 5°C. Moreover, these were observed only briefly at the start and end of the transit process, with no evidence of extreme outliers. These observations demonstrate that the packaging protocol is capable of tightly maintaining the internal temperatures of tissue procurement kits, which is a critical factor for integrity of biospecimens during transit.

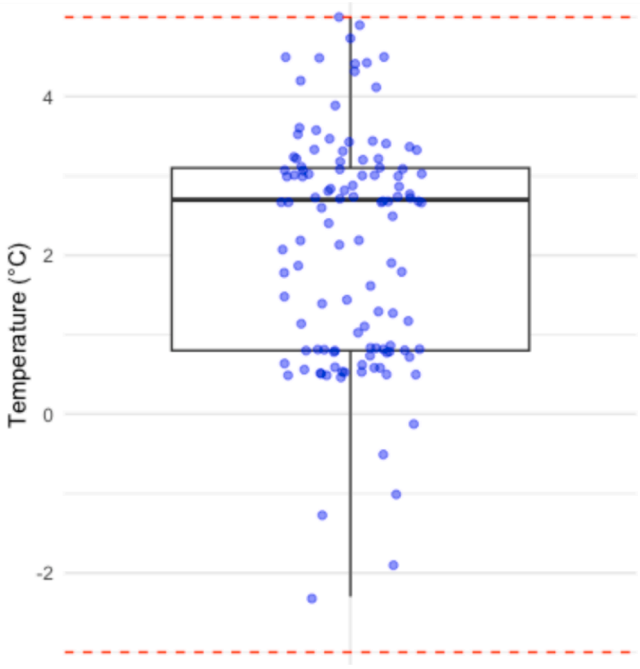

Fig. 1. Box plot of internal container temperatures recorded during transit. Median temperature is represented by the bold line within the box that demarcates the interquartile range. Jitter points represent dispersion of temperature measurements showing distribution within acceptable range.

Furthermore, to analyze the integrity of fresh solid tumor biospecimens, histological studies were conducted on live specimens that were collected, packaged, and shipped, as described in the protocol. Packages were shipped via standard UPS overnight shipping from OSUCCC-Lab and received at CerFlux-Lab within 24 hours of shipment. Hematoxylin and Eosin (H&E) staining of the tissue demonstrated well-preserved adenocarcinoma characterized by infiltrating irregular glandular structures with preserved cytoarchitectural features in the background of desmoplastic stroma (Fig. 2A and 2B). Features of tissue ischemia or autolysis are not identified. Ki-67 immunohistochemical stain highlighted frequent tumor cells and background inflammatory cells, indicating preserved proliferative activity (Fig. 2C). Cleaved caspase 3 (CC3) immunohistochemical stain is performed to assess apoptosis. Only rare apoptotic bodies (<1/100 tumor cells) are identified on CC3 immunohistochemical stain (Fig. 2D).

Moreover, received specimens continued to grow in culture thereby suggesting that tissue biospecimens not only remained viable after collection, handling, packaging, shipping, and receipt 24 hours later, they also maintained proliferation potential.

4. Conclusion

Advances in cancer treatment, from the bench to the bedside, are poised to be driven by precision personalized medicine, which in turn will be contingent upon the ability to accurately analyze fresh tumor tissue from patients. Preanalytical protocols that safeguard biospecimen integrity and viability are therefore critical, as they can minimize variability across sites and enhance reliability and reproducibility of readouts. In addition to precision personalized medicine applications, preanalytical protocols are also vital for conducting a broad array of quantitative cell activity analysis tests and experiments on cell suspensions generated from lysed tissue. Whether the objective is to devise personalized treatment plans, optimize subject segmentation in clinical trials, develop novel anticancer drugs, or enhance our understanding of

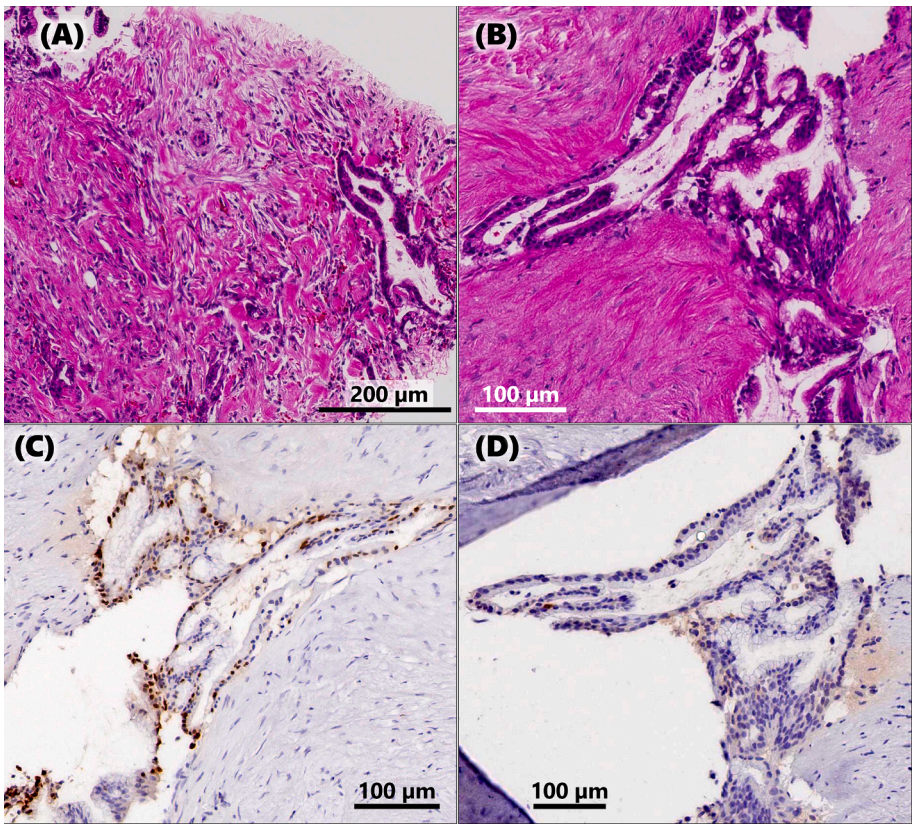

Fig. 2. (A, B) Histologic sections demonstrating adenocarcinoma with well-preserved cytoarchitectural features. (C) Ki-67 immunohistochemical stain highlights frequent tumor nuclei, indicating preserved proliferative activity. (D) CC3 immunohistochemical stain demonstrates only rare apoptotic bodies.

the disease, implementation of robust preanalytical protocols will be paramount.

The protocol delineated herein has been designed to support the evolution towards precision personalized medicine in oncology. Looking ahead, our continued efforts will focus on refining and expanding this protocol across a wider array of tissue types and transport conditions. It is through such concerted and collaborative efforts that we can collectively shape the future of precision personalized medicine in improving outcomes for cancer patients.

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6. Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki, under approved IRB 2019C0139, "*A banking facility for biological samples and clinical data to study gastrointestinal tumors*" at The Ohio State University Wexner Medical Center and James Comprehensive Cancer Center.

7. Informed consent statement

Informed consent was obtained from all subjects involved.

CRediT authorship contribution statement

Areesha A. Charania: Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Aman G. Pokal:** Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Dana R. Zuaiter:** Investigation, Data curation, Writing – review & editing. **Chelsea L. Crawford:** Writing – review & editing. **Ashwini K. Esnakula:** Validation, Resources, Writing – review & editing. **Mozaffarul Islam:** Investigation, Writing – review & editing. **Alex C. Kim:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Supervision. **Karim I. Budhwani:** Conceptualization, Methodology, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Budhwani reports financial support was provided by National Science Foundation. Dr. Budhwani reports financial support was provided by National Cancer Institute. Ms. Charania, Mr. Pokal, Ms. Zuaiter reports financial support was provided by CerFlux. Dr. Budhwani reports a relationship with Breast Cancer Research Foundation of Alabama that includes: board membership and funding grants. Dr. Budhwani reports a relationship with BIO Alabama that includes: board membership. Dr. Budhwani reports a relationship with American Cancer Society that includes: board membership. Dr. Budhwani reports a relationship with The University of Alabama at Birmingham O'Neal Comprehensive Cancer Center that includes: board membership. Dr. Budhwani has multiple patents pertaining to *in vitro*, *ex vivo*, and cancer supermodel technologies. issued to CerFlux. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper..

Data availability

Data will be made available on request.

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