Translational Research in Pediatrics IV: Solid Tissue Collection and Processing

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abstract

Solid tissues are critical for child-health research. Specimens are commonly obtained at the time of biopsy/surgery or postmortem. Research tissues can also be obtained at the time of organ retrieval for donation or from tissue that would otherwise have been discarded. Navigating the ethics of solid tissue collection from children is challenging, and optimal handling practices are imperative to maximize tissue quality. Fresh biopsy/surgical specimens can be affected by a variety of factors, including age, gender, BMI, relative humidity, freeze/thaw steps, and tissue fixation solutions. Postmortem tissues are also vulnerable to agonal factors, body storage temperature, and postmortem intervals. Nonoptimal tissue handling practices result in nucleotide degradation, decreased protein stability, artificial posttranslational protein modifications, and altered lipid concentrations. Tissue pH and tryptophan levels are 2 methods to judge the quality of solid tissue collected for research purposes; however, the RNA integrity number, together with analyses of housekeeping genes, is the new standard. A comprehensive clinical data set accompanying all tissue samples is imperative. In this review, we examined: the ethical standards relating to solid tissue procurement from children; potential sources of solid tissues; optimal practices for solid tissue processing, handling, and storage; and reliable markers of solid tissue quality.

Human tissue experiments are imperative for health research. Solid tissue is an invaluable substrate to understand the molecular and cellular mechanisms underlying childhood diseases. Tissue samples can be collected from subjects across the developmental spectrum, from fetal to late adolescent stages. Children with diseases can also live into adulthood, which may necessitate collection of tissue in later life. In the present review, which is the fourth in a series on tissue sampling and biobanking for child-health studies,[1–3] we describe issues that arise from the collection, processing, and storage of solid tissues. Our goals were to highlight the ethical standards for solid tissue collection from children and the various factors affecting tissue integrity. In addition, we reviewed newer methods for determining tissue quality.

ETHICS STANDARDS FOR SOLID TISSUE RESEARCH

Tissue retrieval for research purposes can be complicated by concerns for the individual's legitimate ethical rights, religious beliefs, and emotional well-being. Biobanks typically have a well-organized informed consent system, whereas the consent standards for archival tissues can be variable. Published ethical guidelines for biobanks suggest the following: (1) that children, as soon as mentally capable, should be asked to assent to research; (2) that biobanks collecting non-postmortem tissue should

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make an effort to recontact young adults for re-consent once they are capable of providing it; and (3) that minors, when permitted by law, withdraw their samples and clinical data regardless of their parents' consent.[4–6] Broad consent to cover unexpected studies is ideal, but if the research changes significantly from the original protocol, ethics committees should decide whether re-consent is necessary.[4] Archived tissues should be anonymized and consented for by the patients and/or their legal guardian. Internal resource committees should approve access to specimens.[7]

Acquiring parental/guardian consent for tissues is often difficult due to fear and/or grieving, but many still consent because they believe that tissue research may be useful for the development of new drugs and treatments[8] and/or because of a wish to prevent others from similar painful situations.[9] Obtaining consent from relatives after a child dies requires biobank staff to have sensitivity and training to avoid coercion and exacerbating distress. Tissue procurement via percutaneous needle biopsy, as opposed to full open autopsy, may be of greater acceptability to parents/guardians, but this approach can introduce sampling errors.[10] In addition, obtaining consent for tissues to be used in research must be coordinated with organ procurement organizations.

Although predeath autopsy permission for research tissues may be obtained during the informed consent process,[11,12] the institutional review board in accordance with law requires postmortem confirmation of the permission from the research participant's legally authorized representative.[13–15]

Additional issues to be considered by biobanks include the following: recruiting properly qualified professionals with religious and cultural knowledge to participate on research ethics committees (ie, pediatricians, lawyers, community members); access to larger numbers of shared control cases; standardized protocols between laboratories; and procedures for handling genetic information, incidental findings in DNA studies, and ownership rights.

Recent advances in human tissue engineering and organoids require unique guidelines and procedures.[16] The moral status of interfering with the beginning of life when using human embryonic stem and fetal cells is always questioned. Acquired tissues should only be used for the informed research purpose, and parents often consent only to autologous use with respect to a child's tissues. Researchers are encouraged to educate the public about their work, although it is challenging to provide details about the tissue engineering process to a lay audience.

Internationally, human tissue research is controlled by federal regulations, and studies require approval by research ethics committees or institutions' review boards.[12–14,17–23] Different approaches for informed consent and/or assent should be implemented in child research according to the targeted age (from fetal to adolescent stages).[6] Several guidelines are available for access, use, legal rights, handling genetic information, and the sharing of biological samples for research purposes.[24–28] In addition, the Genetic Privacy Act and Commentary,[29,30] the Genetic Information Nondiscrimination Act,[31] and the Health Insurance Portability and Accountability Act (termed the Privacy Rule)[32] were created to regulate genetic information and individual identifiable health information release. Commercial biobanks have raised significant ethical and legal issues, prompting several countries to create comprehensive regulations through mechanisms such as licensure.[33]

SOURCES AND TYPES OF SOLID TISSUES COLLECTED

Sources of solid tissue for child-health research include local collections, biobanks, and archived tissues collected as normal clinical practice by pathology laboratories. With the development of newer technologies to identify and quantify molecular biomarkers, including DNA microarrays, quantitative polymerase chain reaction (PCR), and mass spectrometry, biobanking is critical for many disease investigations, whereas archived tissue has been mainly used to detect rare diseases or to investigate specific populations.[34]

A variety of solid tissues have been collected for health research (Table 1). Biopsy/surgical specimens are collected to test histopathologic features[35–38]; to determine pathogenic mediators[36,39–41]; to obtain a definitive diagnosis of different syndromes, disorders, or diseases[37,42,43]; to test the safety of different techniques[44,45]; to study the role of toxic elements[46]; and to diagnosis new or rare diseases.[47] Biopsy/surgical specimens reflect the tissue in vivo state, but collection is hampered by accessibility, limited volume, or because the sampled area may not fully represent the disease.[10,48] Pediatric tissue is also required in studies on the normal development of the human.[49–51]

Fresh tissues can be used for engineering and regenerative medicine.[165] Donor somatic cells (fibroblasts from skin, adipose cells, and bone marrow; blood mononuclear cells from peripheral blood), term placenta cells, fetal and neonatal cells, and umbilical cord cells are all excellent sources of embryonic and adult stem cells. These cells can potentially be used in tissue replacement therapies, human in vitro models of disease,

screening of therapeutic and toxic effects of chemicals, and personalized medicine.[166]

Lack of accessibility of fresh tissue makes postmortem tissue an important alternative. Postmortem tissue has been used for studies on morphology[167–171]; to study the mechanical properties of different organs[167,168]; to validate new techniques[172–174]; to correlate morphology with microbiology findings[103]; to report new or rare diseases[175]; to study the molecular mechanisms[95,123] to determine toxic element exposure[87]; to determine a definitive disease diagnosis[88,96,104,176]; and to detect infectious agents.[10,68] The use of postmortem tissue is often hampered, however, by agonal factors, postmortem intervals (PMIs), and storage conditions that contribute to tissue degradation.[177,178]

Solid tissues can be derived from living patient organ donors, as well as from deceased patients, resulting from neurologic or cardiac death.[179–183] Recovery of donor medical information before the donor tissue procurement is ideal,[184] particularly in preregistered cases, and it can be supplemented with archived medical records. Importantly, the case must meet the collection criteria of the tissue bank. Exclusion criteria will vary according to project and may include drug usage, cancer, psychiatric illnesses, and extended PMI. Obtaining consent for tissue procurement is often the initial focus (if not acquired in advance), followed by tissue retrieval.

Tissue quality, physiology, and function are critical for tissue donation, as is the patient's medical condition at the time of death and the patient's location (termed geographical limitation).[179] The viability of the tissues is measured in hours from the moment of retrieval, which is a time critical for successful transplantation or research.[20] For example, ocular tissue must be recovered within 12 hours of cardiocirculatory death for transplantation, but it can be used for research within 15 to 24 hours of cardiocirculatory death.[179] Tissue banks often interact permanently with universities, hospitals, medical centers, and support foundations, and they offer a variety of organs/tissues for child-health research.[180–183]

Tissue that would otherwise have been discarded, including placentas and umbilical cords, can be obtained for research purposes because their collection poses little risk to the patients.[185–187] The placenta has been used in child-health research to investigate preeclampsia,[188] maternal obesity,[63] endocrine disruptors,[189] attention-deficit/hyperactivity disorder,[64] Smith-Lemli-Opitz syndrome,[190] and vertical virus transmissions.[65,66] Blood drawn from umbilical cord veins has been used for regenerative medicine[72] and stem cell research.[191,192] Other discarded tissues include the aborted fetus,[193,194] bone,[117] spine,[121] ovary,[140,141] and skin,[195] and extra tissue fragments from surgical removal/biopsies of neoplasms that are not appropriate for diagnostic procedures.[8,196]

Future tissue sources may include donor tissue not suitable for transplantation due to an extended postrecovery interval, age, abnormal macroscopic results, long ICU stay, or a positive result on the donor's serologic test (eg, HIV, hepatitis B

TABLE 1 Examples of Solid Tissues Used in Health Research

Biopsy/Surgical	Postmortem	Organ/or Tissue Donor	Otherwise Discarded
• Brain[41,52–54a]	• Brain[41,55–61]	• Cornea[62a]	• Placenta[63–67]
• Heart[35,44]	• Cardiac/skeletal muscle[68,69]	• Retina[70,71a]	• Umbilical cord[65,72–75]
• Liver[47,76,77]	• Heart[60,67,68,78,79]	• Posterior pole/eye[70a]	• Fetal tissues:
• Kidney[39,80,81]	• Pituitary gland[82]	• Whole eye [83,84b]	-Kidney[67,85,86a]
• Intestine[38,76]	• Liver[45,57,60,67,87,88]	• Lens[89a]	-Liver[67,85a]
• Bladder[90]	• Intestine[91,92]	• Skin[85a]	-Skeletal muscle[67,85a]
• Lung[93]	• Kidney[57,60,67]	• Heart valves, pulmonary artery, and aorta[94c]	-Heart[67,85a]
• Placenta[45,95]	• Hippocampus[96]	• Bone marrow[97–100]	-Pancreas[85a]
• Oral mucosa/ maxilla[101,102]	• Lung[10,57,103–105]	• Musculoskeletal tissue[106a]	-Brain[85a]
• Knee joint tissues[107]	• Bone marrow[108,109]	• Brain[106,110,111a 59,112–114]	-Skin[85a]
• Adipose tissue[115]	• Muscle[67]	• Lymph nodes[116c]	-Bone[117]
• Muscle[37]	• Cornea[118]	• Liver[106,119,a 120b]	-Spine tissue[121]
• Gastric mucosa[76,122]	• Placenta[67,95,123,124]	• Lung[106a, 125b]	• Cornea[126a]
• Bone marrow[39,109,127]	• Knee joint tissues[128]	• Trachea[129c]	• Kidney[130c]
• Bone[42]	• Spleen[57,104]	• Heart[106a, 131b]	• Pancreas[132a]
• Pancreas[76]	• Peripheral nerves[133]	• Pancreas and pancreatic islets[106,120,134–136a]	• Heart[137]
• Ovarian tissue[138]	• Bile duct[40]	• Stomach and esophagus[139b]	• Ovarian tissue[140,141]
• Thyroid gland[38,142]	• Gastric mucosa[143,144]	• Kidney[106a, 145b]	• Tumors:
• Adrenal glands[38,39]	• Bone[146]	• Bladder[147b]	-Skeletal muscle tissue[148]
• Soft tissue[36]	• Pancreas[149]	• Intestine[106a]	-Bladder[150]
• Nerve tissue[39]		• Spleen[106a]	-Brain[151]
• Esophagus[152]		• Bone:	-Kidney[153]
• Musculoskeletal tissue[42]		-Femur[154,155b]	-Liver[156]
• Bile duct[40]		-Tibia[157b]	-Soft tissue[158]
• Skin[159]		• Liver/living donors[160]	-Extremities (adipose tissue origin)[161]
• Sinus tissue[162]			-Thyroid[163]
• Lens tissue/eye[164]			

[a] References in which research was conducted by using organs/tissues from children/fetus and adults.
[b] References in which research was conducted by using adult organs/tissues only.
[c] Tissue specimens for no age of donor is indicated in the reference article.

or C). These latter tissues include cornea,[126] kidney,[130] and pancreas.[132] Table 2 summarizes examples of pediatric tissues/organs and the analytical techniques used in research studies.

FRESH, FRESH FROZEN, AND FIXED TISSUES

Fresh, fresh frozen, and fixed tissues are amenable to several investigative methods, including tissue engineering, microarray analysis, PCR, receptor autoradiography, proteomics, chromatography, immunochemistry, and protein sequencing (Table 3).[207–209]

Fresh tissue is used for tissue engineering research and organoid formation, following digestion of extracellular matrix and cell isolation in a sterile environment.[166] When isolated cells are reprogrammed to stem cells and the end point is differentiation to specific cell lineages, specific media conditions must be used. Because tissue function often relies on several cell types in a specific 3-dimensional order, the development of novel culture environments using biomaterials and bioreactors is imperative to maintain selected aspects of native tissue.[166] Mechanical stimuli, extracellular matrix components, or even long-term cell culture conditions are extremely important for the development of an organoid.[166]

Fresh frozen tissue preserves biomolecules in an intact state and provides a high yield of quality DNA and RNA.[225,226] Due to the small sample size of fresh frozen tissue, however, it is often difficult to correlate biochemical experiments with histologic/pathologic examinations.[225] Regardless of the tissue origin (either biopsy/surgical or postmortem), snap-freezing with storage at –80°C or below are ideal to maintain the integrity of biochemical molecules.[207,210,227]

Tissue samples are often fixed in formaldehyde or formalin before being embedded in paraffin wax for sectioning and subsequent histologic examination. Fixed tissue is most commonly used for protein localization and morphology studies. Paraffin blocks have been successfully used to isolate both DNA and RNA when optimal fixation and extraction methods were used.[228] However, fixation of solid tissue can degrade and damage DNA and RNA,[225,229] thus interfering with gene expression, mutation, and polymorphism studies. For better quality tissue, fixation should be conducted as soon as possible after collection,[209] and the samples should be immediately embedded in paraffin blocks.[229] DNA can be isolated from fixed tissue that has not yet been embedded,[225] but the DNA is of less quality compared with DNA isolated from frozen tissue.

FACTORS INFLUENCING SOLID TISSUE QUALITY

Many factors affect the quality of tissue directly or indirectly by interfering with protein stability and nucleotide yield or quality.[67,177,230–234] The parameters affecting solid tissue qualities are summarized in Table 4.

Gender and Age

Female gender and increased age at death are 2 factors that influence total tissue messenger RNA levels[256] and alter specific gene expression profiles.[258] Although both factors are reportedly important determinants of tissue quality,[232,258] other research has shown that brain RNA integrity is not influenced by gender or age <50 years (H.R.Z., unpublished observations).

Agonal Factors

Agonal factors include coma, pyrexia, hypoxia, seizures, dehydration, hypoglycemia, multiple organ failure, prolonged death, head injury, respiratory arrest, and neurotoxic substance ingestion; these factors can change global gene expression via signaling pathways (ie, the stress response and apoptosis) with activation of acid ribonucleases.[232,258] Consequently, there is variability of RNA integrity and divergence in gene expression profiles.[232,258] Individuals who suffered prolonged agonal states (ie, respiratory arrest, multiorgan failure, coma) have decreased gene expression for energy metabolism proteins and proteolytic activities, and increased expression of genes encoding stress response proteins and transcription factors. In contrast, those who experienced rapid deaths (ie, trauma, cardiac events, asphyxia) have limited alterations in gene expression.[236] Agonal factors, such as coma and hypoxia, can markedly affect the RNA quality and have a major impact on gene expression profiles in microarray analyses.[232] The activation of ribonucleases during the terminal phase may introduce large divergences of global gene expression profiles in microarrays compared with gender, age, postmortem delays, or diagnoses of psychiatric disorders.[232]

A sudden or prolonged death can affect tissue quality, resulting in the creation of the agonal factor score (AFS).[232] Each agonal condition is scored 1 if present or 0 if absent. Agonal duration is also rated.[261] Individual scores of the agonal conditions and agonal duration are summed to provide the final AFS for each sample. A lower AFS is associated with high-quality tissue.[233] The AFS has the practical limitation of its inability to assess each anatomic region separately, resulting in the development of an agonal stress rating system that evaluates the degree of stress based on gene expression data.[237] The agonal stress rating can reduce the number of false-positive findings by allowing a quantitative

TABLE 2 Examples of Pediatric Tissues/Organs Used in Specific Disease Research Studies

Tissue/Organ	Investigational Technique	Disease or Process Investigated	Molecules and/or Parameters Investigated
Brain	Western blot,[112,113] IHC,[53,111–114] IF, FISH,[53] FACS,[53,114] transmission electron microscopy,[113] lipid content analysis, gene expression analysis,[112] RT-PCR,[112,114] cell culture,[53,114] ICC, confocal microscopy, generation of lentiviral-based Gli binding site-RFP reporters and cell transfection,[114] Proton density-weighted MRI scan,[110] light and electron microscopy[111]	• Adrenoleukodystrophy[112]	• VLC fatty acid, cholesterol ester, cholesterol, sphingomyelin, cytokines (IL-1α, IL-2, IL-3, IL-6, TNF-α, and GM-CSF), chemokines (CCL2, -4, -7, -11, -16, -21, -22, CXCL1, CX3CL1, and SDF2) and iNOS[112]
		• Diffuse intrinsic pontine gliomas[114]	• Hh signaling pathway[114]
		• Infantile neuronal ceroid lipofuscinosis[113]	• PPT1, caspase-3, cleaved-PARP, caspase-9, ROS, and SOD-2[113]
		• Infiltrative glioma and gliosis[53]	• EGFR[53]
		• Autism[197–199]	• Neuronal microexons,[197] molecular networks,[198] microRNAs[199]
		• Acute myelogenous leukemia or medulloblastoma[111]	• Dcx, CD20, CD68, Ki67, CD3[111]
Heart/vessels	Light and electron microscopy,[167,200] fatty acid analysis by gas chromatography,[200] PCR,[137] histopathologic examination, IHC, confocal microscopy,[35] general histochemistry (H&E, Weigert's fuchsin-resorcin)[168]	• Infantile X-linked dilated cardiomyopathy[200]	• Saturated fatty acids, mitochondria structure[200]
		• Dilated cardiomyopathy and congenital heart disease[137]	• Levels of HHV-6[137]
		• Chagas disease[35]	• Quilty effect, rejection, C myocarditis reactivation, fibrosis, hypertrophy, and ischemia; as well as lymphocytic cellular infiltration of myocarditis[35]
		• Cardiovascular development[167,168]	• Morphology and mechanical properties of the atrial intima[167] • Normal histologic growth of ascending aorta, aortic isthmus, and descending aorta[168]
Muscle	General histochemistry (H&E, Gomori trichrome, detection of ragged-red fibers and nemaline rods, oil red detection of lipids, and periodic acid-Schiff detection of glycogen), electron microscopy, IHC[37]; Histologic examination[42]	• Neuromuscular disorders (including muscular dystrophy, neurogenic atrophy, and congenital and metabolic disorders)[37]	• Ultrastructural pathology of mitochondria, general and enzyme histochemistry and IHC of the muscle[37]
		• Musculoskeletal lesions (bone and soft tissue) in children[42]	• Test the accuracy and safety of image-guided PCNB for the detection of benign or malignant lesions[42]
Lung	Microbiologic analysis, bacteria and fungi analysis[103,104]	• Respiratory diseases[103]	• Morphology/anatomy of the lungs; levels of aerobic and anaerobic microorganisms; and fungi[103]
		• Sudden unexpected death in infancy[104]	• Postmortem translocation (the migration of bacteria from mucosal surfaces into the body after death, which occurs as part of the normal putrefaction process) and the association with PMI[104]
Spleen	CT scan,[201] histologic evaluation (H&E)[57,201]	• Splenic epidermoid cysts[201]	• Examination of the wall of the cyst for ultimate diagnosis[201]
		• Fetal malformations[57]	• Comparison of needle autopsy specimens and complete conventional autopsy specimens[57]
Pancreas, pancreatic islets, bile duct	DNA sequencing, chip assays, cell culture and transfections[136]	• Type 2 diabetes and other islet disorders[136]	• Epigenomic profile of unstimulated human pancreatic islets, including DNase I hypersensitive sites that mark regions of open chromatin, loci enriched for active histone H3 lysine methylation modifications (H3K4me1, H3K4me3, and H3K79me2), and binding sites for the insulator CTCF[136]

TABLE 2 Continued

Tissue/Organ	Investigational Technique	Disease or Process Investigated	Molecules and/or Parameters Investigated
		• Type 2 diabetes[106]	• Multiple TCF7L2 splicing forms[106]
	5′ RACE and analysis of splicing forms, RT-PCR[106] IHC, western blot[40]	• Pancreaticobiliary maljunction and bile duct dilatation[40]	• P-MLC20 and MLCK pathway[40]
Liver	Genotyping, QuantiGene Plex 2.0 assay (Affymetrix Ltd, High Wycombe, UK)[119]; Q-FISH telomere quantification method[160]	• Hyperbilirubinemia and prediction of UGT1A1-mediated drug metabolism and toxicity[119]	• Genetic polymorphisms on UGT1A1 transcription; enhanced transcription by CAR, PXR, GR, HNF1α, and HNF4α[119]
		• Pediatric living donor[160]	• Telomere length[160]
Intestine	RT-PCR[202]	• Oral drug absorption and hepatic drug excretion[202]	• Hepatic and intestinal drug transporters MRP2, OATP1B1, OATP1B3, OATP2B1, and MDR1[202]
Stomach	Histologic evaluation (H&E, and Giemsa staining),[13] C-UBT, RUT, fqPCR[122]	• Gastritis[122]	• Levels of *Helicobacter pylori*[122]
Cornea	Graft failure[62a]	• Endothelial disease[62]	• Success rate of penetrating keratoplasty and corneal graft survival[62b]
Retina, posterior pole	HPLC[70];	• Macular degeneration[70]	• CoQ10 levels[70]
	IHC[71]	• Retinopathy and choroidopathy[71]	• Choroidal endothelial Tbdn and choroid vascular Tbdn[71]
Lens	IHC, slit lamp biomicroscopy, tryptic digest tandem mass spectrometry protein sequencing, Aβ fluoro-ELISA and Aβ immunoblotting, light and electron microscopy, immunogold electron microscopy[89]	• Down syndrome and Alzheimer's disease[89]	• Aβ accumulation, amyloid formation, and fiber cell cytoplasmic Aβ aggregates[89]
Kidney	Doppler ultrasonography, histopathologic diagnosis[203]; IHC[39]; Histologic evaluation (H&E)[57]	• Kidney disease[203]	• Comparison of outpatient and inpatient renal biopsies: accuracy, safety, and risks of complications[203]
		• Pediatric solid tumors[39]	• HA117, P-gp[39]
		• Fetal malformations[57]	• Limitations of needle autopsies from a complete conventional autopsy[57]
Bladder	Histologic evaluation (H&E, and Masson's trichrome staining), ultrasonography[150]	• Bladder plate polyposis[150]	• Examination of novel technique of SUPER and UAAC. Evaluation on polyp recurrence, bladder tumor, urinary continence, and social dryness[150]
Lymph nodes	Node weight, ultrasound, pathologic examination[204]	• Acquired immune deficiency syndrome[204]	• CD4+ T-cell[2] count and HIV viral load, fecal calprotectin, and bowel ultrasound, with the latter evaluating bowel wall thickness and mesenteric lymph nodes[204]
Bone marrow	PCR, PCR-RFLP, histologic evaluation (Giemsa staining), bone marrow culture[205]	• Visceral leishmaniasis[205]	• Leishmania DNA[205]
Skin	Histologic evaluation (H&E), immunoperoxidase[206]	• Primary cutaneous plasmacytosis[206]	• Search for cell infiltration of dermis, clusters of B-lymphocytes CD20 reactive, and expression of κ light chains and λ light chains to diagnosis rare disease[206]
Placenta	Placental weights, pathologic examination (thrombosis present, leukocytes infiltration, infarction)[95]	• Stillbirth[95]	• Placental abnormalities including thrombosis present, placental infarction, leukocyte infiltration, and chorioamnionitis[95]
	MeDIP, human CpG island plus promoter microarray analysis, bisulfite sequencing PCR[188]	• Preeclampsia[188]	• Aberrant or altered patterns of DNA methylation and the distribution of CpG nucleotides in genes such as CAPN2, EPHX2, ADORA2B, SOX7, CXCL1, and CDX1[188]
Umbilical cord blood	Generation of iPSCs, transfection, RT-PCR, IF flow cytometry[73]	• Acquired immune deficiency syndrome[73]	• Anti-HIV genes including a CCR5 short hairpin RNA and human/rhesus chimeric TRIM5α gene; also, pluripotency markers OCT4, SOX2, NANOG, and SSEA4[73]
	HPLC and detection by an API 2000 (American Petroleum Institute [API], Washington, DC) electrospray triple quadrupole mass spectrometer, a gel permeation chromatography column for lipid content analysis[189]	• Exposure to endocrine disruptors during pregnancy and infancy[189]	• Levels of non-POPs (including MEHP, OP, and 4-NP), and PBDEs[189]

TABLE 2 Continued

Tissue/Organ	Investigational Technique	Disease or Process Investigated	Molecules and/or Parameters Investigated
Fetal tissues: Kidney, Heart, Liver, Muscle, Brain, Skin, Pancreas	Locked nucleic acid probe-Southern, CpG methylation analysis[85]	• Congenital myotonic dystrophy type 1[85]	• Expanded CTG repeat and methylation at the CTCF-binding sites[85]
		• Polycystic kidney disease[86]	• Structural proteins vinculin, α-actinin, and β_1-integrin; focal adhesion proteins including FAK, c-Src, paxillin, and p130cas; and fibrocystin-1[86]
	Cell adhesion assays, migration assays, western blots, IHC[86c]; MRI,[172,173] ultrasound[172]	• Prenatal ventriculomegaly[172]	• Images to compare postmortem examination, both by traditional neuropathologic examination and postmortem MRI, with antenatal MRI for diagnosis[172]
		• Major pathologic lesions associated with death[173]	• Images to compare minimally invasive autopsy (a postmortem investigation with no incisions or dissection, with MRI and postmortem blood sampling via needle puncture) with conventional autopsy for detection of cause of death[173]

ADORA2B, adenosine A2B receptor; CAPN2, calpain-2 catalytic subunit 2; CAR, androstane receptor; CCL, chemokine (C-C motif) ligand; CD3, cluster of differentiation 3; CD4, cluster of differentiation 4; CD20, cluster of differentiation 20; CD68, cluster of differentiation 68; CoQ10, coenzyme Q10; CpG, C–phosphate–G (cytosine nucleotide sites); ^{13}C-UBT, ^{13}C-urea breath test; c-Src, Proto-oncogene tyrosine-protein kinase Src; CTCF, CCCTC-binding factor; CXCL1, the chemokine (C-X-C motif) ligand 1; DCX, doublecortin; EGFR, epidermal growth factor receptor; EPHX2, epoxide hydrolase 2; FACS, fluorescence-activated cell sorting; FAK, focal adhesion kinase; FISH, fluorescence in situ hybridization; fqPCR, fluorescent quantitative PCR; Gli-RFP reporter, Gli luciferase reporter; GM-CSF, granulocyte-macrophage colony-stimulating factor; GR, glucocorticoid receptor; ICC, immunocytochemistry; IF, immunofluorescence; IHC, immunohistochemistry; IL, interleukin; iNOS, inducible nitric oxide synthase; HA117, a novel multidrug resistance gene; H&E, hematoxylin and eosin; Hh, Hedgehog; HHV-6, human herpesvirus 6; HIV-1, HIV type 1; HNF1α, hepatocyte nuclear factor 1α; HNF4α, hepatocyte nuclear factor 4α; HPLC, high-performance liquid chromatography; iPSCs, induced pluripotent stem cells; Ki67, antigen identified by monoclonal antibody; MDR1, multidrug resistance 1; MeDIP, methylated DNA immunoprecipitation; MEHP, mono-2-ethylhexylphthalate; MLCK, myosin light-chain kinase; MRP2, multidrug resistance protein 2; NANOG, homeobox protein NANOG; non-POPs, non–persistent organic pollutants; 4-NP, 4-nonylphenol; OATP1B1, organic anion transporting polypeptide 1B1; OATP1B3, organic anion transporting polypeptide 1B3; OATP2B1, organic anion transporting polypeptide 2B1; OCT4, octamer-binding transcription factor 4; OP, octylphenol; p130Cas, Crk-associated substrate; PARP, poly (ADP-ribose) polymerase; PBDEs, polybrominated diphenyl ethers; PCNB, percutaneous core needle biopsy; P-gp, permeability glycoprotein; P-MLC20, phosphorylated myosin regulatory light chain; PPT1, palmitoyl protein thioesterase-1; PXR, pregnane X receptor; Q-FISH, quantitative fluorescence in situ hybridization; ROS, reactive oxygen species; SDF2, stromal cell–derived factor 2; SOD-2, superoxide dismutase-2; SOX2, sex determining region Y-box 2; SOX7, SRY-related HMG-box 7; SSEA4, stage-specific embryonic antigen-4; SUPER, sub-urothelial polyp enucleation resection; RT-PCR, reverse transcription PCR; RUT, rapid urease test; Tbdn, Tubedown; TCF7L2, transcription factor 7-like 2; TNF, tumor necrosis factor; UAAC, urothelial auto-augmentation cystoplasty; UGT1A1, UDP-glucuronosyltransferase 1A1; VLC, very long chain fatty acids.

[a] Defined as regraft or, in the absence of regraft, a cloudy cornea in which there was loss of central graft clarity sufficient to compromise vision for a minimum of 3 consecutive months.

[b] $CD4^+$ T cells refer to cells that express the surface protein CD4.

[c] For kidney tissue only.

assessment of tissue quality and is useful for identifying anatomic regions that exhibit different stress outcomes.[237]

Postmortem Intervals

A PMI is defined as the difference between the time of death and the time of tissue preservation (eg, frozen, fixed).[184] After death, bodies are placed at 4°C for variable times before sample collection. Traditionally, PMIs can range from 6 to 72 hours,[262] depending on the period of time that parents wish to remain with their deceased child, with 1 to 6 hours as the minimum time to access the body for tissue collection.[184] High-quality tissue is generally associated with low PMIs.[233,263,264] Longer PMIs alter the tissue quality at the molecular level. Postmortem tissue can maintain gene expression profiles in heart tissue for at least 24 hours,[78] whereas longer PMIs result in greater epithelial cell loss in cornea tissue.[245] Delays in PMI can also affect protein stability, particularly receptors and cytosolic proteins.[251,253,265] PMI delays >40 hours result in posttranslational modifications and decrease protein immunoreactivity in human degenerative diseases.[241,254,266,267] Longer PMIs are associated with oxidation/nitration events.[240]

Other Factors Affecting Solid Tissue Quality

After death, the time to tissue collection, extraction, and processing, as well as tissue storage protocols and temperatures, are critical factors affecting tissue quality (Table 5). For example, brain RNA and protein degradation associated with postmortem delay is dependent on the storage temperature.[210,234,268]

INDICATORS OF TISSUE QUALITY

Solid tissue quality control is imperative for child-health research studies. Several methods have been used to ensure solid tissue quality. Some factors (ie, gender) that lead to bias can be statistically controlled by using matched designs, match-pairing of patients with disease and control subjects with the same values of factors, and/or by adding the factors as covariates in the analysis.[256]

RNA Integrity

RNA integrity is an important indicator of tissue quality. The most common and sensitive method used for determination of total RNA quality, with high reproducibility and with impartial measurements, is microfluidic chip-based capillary electrophoresis[270,271] using a Bioanalyzer (Agilent Technologies, Santa Clara, CA) or Experion (Bio-Rad Laboratories, Munich, Germany) instrument. Using electrophoretic data, an RNA integrity number (RIN) or RNA quality index is generated, taking into account the 18S/28S ribosomal RNA peaks, as well as background and degradation products. The RIN can range from undetectable to 10, with undetectable being completely degraded and 10 being mostly intact RNA[233]; RIN values ≤6 are usually considered insufficient for RNA studies.[210]

RNA integrity can be influenced by degradation, tissue type, BMI, and by other tissue quality markers, including pH, PMI, agonal factors, and relative humidity of the processing laboratory.[230–233] In some tissues, RNA degradation increases with longer PMIs.[177,230] Prolonged agonal factors, such as coma and hypoxia, together with a pH ≤5.9, can also affect the RNA integrity and have a major impact on gene expression.[232] RNA quality is reduced in a time-dependent manner at a relative humidity ≥31%[231] and with freeze/thaw cycles.[233]

RNA quality control is imperative when gene expression analysis is performed by using postmortem tissues, with quantitative data checked for the influence of antemortem and postmortem parameters.[230] The RIN values are used to identify samples that should be excluded from analyses and to distinguish similar biological replicates.[230] RNA preservation may be useful in RNA expression studies of individual subjects due to possible divergence of RIN and PMI values, as well as regional tissue differences in RIN.[210] Housekeeping genes must be carefully chosen and monitored to obtain normalization.

Importantly, RIN values should serve only as a guide to determine if the tissue appears of sufficient quality to commence actual studies of gene expression. In addition to the RIN, RNA integrity has been previously tested by using northern blot hybridization,[272–275] quantitative in situ hybridization,[274–276] and quantitative reverse transcription PCR.[273] For microarrays, several RNA integrity indicators, as well as the percent present call (percentage of the total number of probe sets detected as present on the array), have been used.[232]

TABLE 3 Types of Analyses Conducted With Biopsy/Surgical and Postmortem Tissues

Molecules	Analyses	Tissue	Studies
DNA	Immunoblotting, IHC, immunoprecipitation assay	Fresh or frozen	Nucleosome preservation and histone modifications[210,211]
	DNA sequencing	Frozen or formalin-fixed	Detection of mutations and CAG repeats[210,212]
	DNA microarrays	Frozen	DNA polymorphisms[59,210]
RNA	PCR	Fresh or frozen	Differential gene expression studies,[56]
	Microarrays	Formalin-fixed	Large-scale profiling of transcriptional activity,[213] Transcriptome expression patterns[214]
	In situ hybridization	Formalin-fixed	
Proteins	Western blot analysis, 2-dimensional gel electrophoresis, 2-dimensional DIGE using a range of pH, ELISA, mass spectrometry	Fresh or frozen	Differential protein expression,[215,216] Protein–protein interactions,[217] Transcription factor translocations[216]
	Protein sequencing	Fresh or frozen	Toponomics[218]
	IHC	Formalin-fixed	Protein localization[216]
	Receptor autoradiography	Fresh or frozen	Receptor detection[207,219]
Lipids	HPLC	Frozen	Detection of glucosylceramide and other lipids storage[220]
	Metal concentrations (ICPMS)		Heavy metals detection[221]
	Thin layer chromatography		Determination of lipid classes,[222] separation of phospholipids and neutral lipid subclasses, and unesterified and esterified fatty acids from total lipid extract[223]
	Gas chromatography/gas-liquid chromatography		For fatty acid methyl ester detection[108,223]
	Iodine uptake method		Plasmalogens, plasmenyethanolamine, and plasmenylcholine detection[224]

DIGE, polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; HPLC, high-pressure liquid chromatography; ICPMS, inductively coupled plasma mass spectrometry; IHC, immunohistochemistry.

Protein Integrity

Tryptophan, an essential amino acid in protein biosynthesis and a biochemical precursor for the biogenic amines, increases with longer PMI and is considered a good indicator of tissue quality.[55] Elevated tryptophan levels indicate protein degradation and alterations in enzyme activity. Tissue tryptophan levels also reflect freezing/packaging methods, in which lower levels are associated with dry ice storage temperatures and aluminum packing methods.[55] Although the reason for selective preservation is unknown, protein integrity may vary depending on the specific protein, cellular localization, protein posttranslational modification stage, and protein function.[210]

Tissue pH

Tissue pH values ≥6.4 are associated with higher quality samples.[178,233,236,277] The pH of the tissue can be directly correlated with

TABLE 4 Parameters Affecting Tissue Quality of Biopsy/Surgical and Postmortem Tissues

Parameter	Molecule(s)	Tissue	Effect(s)
Tissue pH	Nucleic acids: Gene expression	Brain	• pH is a good marker of perimortem tissue quality,[178,233] but pH is insensitive to freeze/thaw degradation[178,233,235] • pH is constant across all brain regions, and it is well represented by the cerebellum pH[233,235] • pH and RIN are highly correlated when pH <7[233] • pH is not correlated with either postmortem delays or time in storage[178] • pH is not correlated with freezing method or tissue packing[178] • Gender has a significant influence on pH (male subjects more than female subjects), even across different ages[178] • pH of postmortem brain tissue is most dependent on agonal factors and ischemia before death[178,236] • Lower tissue pH is associated with a decrease in gene expression involved in energy metabolism and proteolytic activities, and a consistent increase of genes encoding stress response proteins and transcription factors[236]
Agonal factors	Nucleic acids: RNA integrity; Gene expression profiles	Brain	• Predeath medical conditions correlate with tissue pH[236] • Agonal factors decrease brain tissue pH and facilitate RNA degradation[232,235] • Prolonged agonal states are associated with lower tissue pH and changes in gene expression patterns involved in mitochondria respiratory function and stress response[236] • Brief PMIs are associated with normal tissue pH and stable gene expression[236] • ASR-based matching provides better results of agonal effect than using tissue pH[237] • Agonal factors affect RNA integrity and divergent global gene expression profiles in microarrays[232] • The brain has the lowest RNA integrity, which may be due to antemortem brain acidosis by prolonged agony[232,238,239] • The ACI is a reliable indicator of agonal factor influence on brain acidosis and RNA integrity[232] • mRNA degradation and altered gene expression profiles occur in cell types more vulnerable to agonal stressors[232]
Histology	Nucleic acids: RNA integrity, Protein degradation	Brain	• DNA/RNA stability in histologic samples depends on the fixative solution used and on the time of tissue storage in the fixative before paraffin embedding[229] • DNA preservation in paraffin blocks is variable. Tissue quality increases with a short buffered formalin fixation time[229] • Generally, DNA degrades in fixation solutions and embedding processing[225,229] • Increased PMIs (from 4 to 18 h) did not decrease protein signals by immunohistochemistry[240] • Protein immunoreactivity is generally stable when tissue is stored as paraffin-embedded blocks for extended periods[241]
		Intestine	• Use a new formalin-free fixative called FineFIX (Milestone, Bergamo, Italy) that allows for stable DNA amplicons of 2400 bp, RNA fragments of 600 bp, and proteins comparable in quality with those obtained from fresh frozen tissues[242]
BMI	RNA integrity	Skeletal muscle	• BMI influences RNA integrity[230]
Autolysis (self-digestion)		Heart	• RNA integrity is reduced up to 24 h autolysis[78] • >24 h at RT autolysis maintains gene expression profile[78] • Multiple probe-based exon arrays reliably determine gene expression profiles in tissue with extended autolysis[78] • Protein expression, as detected by IHC, remains intact up to 24 h after autolysis, but degradation is noticeable at 48 h[78]

agonal states (prolonged versus brief death) and with differential gene expression patterns. A prolonged terminal phase is associated with a significantly lower brain pH compared with that of rapid death.[178] Subjects with pH ≤5.9 exhibit specific changes in gene expression.[236] Tissue pH does not correlate with PMI, time in storage, freezing method, or tissue packing, but it can be influenced by gender.[178] Brain tissue pH has been shown to be remarkably consistent across different anatomic regions.[178,233,235] In animals, there is a rapid drop of brain tissue pH within 10 minutes after death,[261] followed by a period of pH stability for 24 to 36 hours.[235] Because pH is not a reliable indicator of storage delays and temperature fluctuations that directly affect tissue quality, pH is a better indicator of premortem events than postmortem events.

NUCLEOTIDE ISOLATION

Fresh or frozen tissues are an ideal source for DNA and RNA (Table 6). When RNA isolation is the end point, working in a ribonuclease-free environment should be done to avoid introducing ribonucleases into the sample.[18] Fresh solid tissues are best placed in liquid nitrogen or dry ice. Tissue samples can also be stored at 21°C in an RNA stabilization solution (ie, RNAlater solution, Thermo Fisher Scientific, Waltham, MA).[278]

DNA is relatively resistant to PMIs, but it is vulnerable to degradation in solutions, particularly fixatives used for tissue preservation.[229] Thus, better DNA yield is obtained from solid tissue stored at –80°C. Isolation of high-quality DNA can be obtained by using QIAamp Micro Qiagen (Qiagen Inc, Valencia, CA).[229]

Two methods to isolate tissue are laser cutting and laser capture microdissection. Laser cutting is used to isolate large areas, particularly from hard tissues. In contrast, laser capture microdissection is

TABLE 4 Continued

Parameter	Molecule(s)	Tissue	Effect(s)
PMIs	Nucleic acids: Gene expression; Protein degradation	Cardiac muscle	• RNA degradation is increased with longer PMI[230]
		Blood vessel	• IHC staining intensity is not influenced by a PMI of 24 h[243] • PMIs increase the levels of triglycerides and very-low-density lipoprotein cholesterol but not levels of total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol[244]
	Posttranslational modifications	Fetal tissues	• DNA yield and purity are not influenced by PMIs between 2 and 10 d[67]
	Lipids	Cornea	• Many structural changes including increased cell loss occur in the corneal epithelium with a PMI up to 7 d[245] • Epithelial regeneration and survival of epithelial cells in the cultured cornea organ can occur even with PMI up to 6 to 7 d[246] • The RIN values from corneal and trabecular meshwork tissue were significantly higher than those from the ciliary body at an early PMI (<6 h) but were similar after 8 h[247] • Rapid preservation and processing of postmortem human donor eye tissue (especially for vascularized ocular tissues) is imperative[247]
		Olfactory bulb/ tract	• PMI delay increases olfactory bulb fragility on dissection from postmortem brain, being more accessible in female subjects than male subjects[248,249]
		Brain	• RNA quality does not correlate with PMIs ranging from 6 to 40 h[233] or from 28 to 114 h[56] • PMI and pH do not correlate in brain[233] • PMI delays cause RNA degradation with a decreased gene expression (>3.5 h)[177] • IHC is used to detect protein signals in tissue with a PMI of 24 h[250] • PMIs >40 h result in protein degradation[233,241,251] • PMIs from 4 to 18 h increased protein oxidation/nitrate events and expression of glial fibrillary acidic protein, synaptophysin, and neurofilament[240] • Proteins that were frozen at 5, 8, 23, and 50 h after death showed more severe patterns of protein degradation compared with those stored at 2 h[234] • Delayed PMI results in alterations in expression levels of τ, MAP2, MAP1B, and MAP5[252] • PMI delays increase tryptophan levels[55] • PMI delays cause nucleoside degradation[253] • PMI duration is not associated with an increased risk of postmortem translocation of micro-organisms from mucosal surfaces into the body[104] • With the exception of Gαq and Gαo, which decrease with PMIs from 5 to 21 h, all other G proteins are stable during extended PMIs in brain[251] • PMI >50 h results in τ dephosphorylation[234,254] • Nucleosomal DNA remains attached to histones during the first 30 h after death, suggesting preservation of methylation histone residues with longer PMIs,[210] and is maintained within a range of autolysis and tissue pH[255]
Gender and age	Nucleic acids: RNA integrity; Protein degradation	Brain	• Gender and age at death influence measurement of mRNA levels[256] • Female subjects have lower mRNA levels[256] • Variable correlations between mRNA and age at death[256,257] • Gender and age affect gene expression in psychiatric disorders[232,258] • Decreased levels of the GTP-binding proteins Gαq and Gαo were significantly correlated with increased age (2%–4% loss per decade)[251] • Tryptophan levels are variable with age or gender[55,259,260] • Glutamate decarboxylase activity is higher in male subjects[55]

gentle, and it can isolate small areas or even single cells. The latter method does not alter or damage cellular morphology and preserves biomolecule integrity, making the technique useful for collecting RNA/DNA from small numbers of cells in tissue regions of interest.[225] When laser capture microdissection is combined with quantitative PCR, changes in region-specific gene expression can be detected.[231] The effect of environmental humidity on RNA quality requires that a relative humidity be maintained at or below 23% for high efficiency of capture and the collection of high-quality RNA.

Microarray analysis of postmortem tissue is a vital tool to investigate gene expression patterns. Microarrays such as GeneChip Human Exon 1.0 ST Array (Affymetrix Ltd, High Wycombe, UK) provide reliable results over a wide range of RINs (from 1–8.5) to help determine RNA quality.[56] A reliable and specific parameter used with microarrays is the present call or the percentage of probe sets with signal detection above background probe levels.[232] The average correlation index is another tool developed to evaluate agonal factors and RNA integrity on the basis of the similarity of gene expression profiles for each microarray among a total set of microarray data.[232]

PROTEIN ISOLATION

Protein levels derived from solid tissues (Table 7) can be affected by storage temperature >4°C and PMI delays >40 hours. Bidimensional gel electrophoresis, western blot analysis, and mass spectrometry are useful tools for identifying protein degradation in postmortem tissues.[234] Immunohistochemistry studies on postmortem tissue have shown stable signal intensity for several proteins during a PMI of 24 hours.[250]

TABLE 4 Continued

Parameter	Molecule(s)	Tissue	Effect(s)
Relative humidity	Nucleic acids: RNA quality	Brain	• RNA quality is reduced by the relative humidity at ≥31%[231] • RNA is stable with a low relative humidity (6%–23%) when extracted with LCM, stained, and fixed[231] • Relative humidity must be maintained at ≤23% to facilitate LCM high-efficiency capture[231]

ACI, the average correlation index; ASR, agonal stress rating; GTP, guanosine triphosphate; IHC, immunohistochemistry; LCM, laser microdissection; MAP, microtubule-associated proteins; mRNA, messenger RNA; RT, room temperature.

Tissue microarray can be a low-cost, high-throughput technique to investigate protein expression.[43,243,289,290] In addition, proteomic methods can be used to investigate tissue quality, and they include 2-dimensional polyacrylamide gel electrophoresis matrix-assisted laser desorption/ionization time-of-light[291,292] and surface-enhanced laser desorption ionization time-of-flight mass spectrometry. These latter methods can be used to detect different patterns of protein susceptibility to PMI and storage temperature.[293]

LIPID ISOLATION

Lipids can be isolated from fresh or frozen tissue (Table 7) by sample homogenization in an organic solvent at 4°C followed by an extraction protocol.[288,294] Extracts can be stored frozen at –80°C until analysis.[287]

TABLE 5 Solid Tissue Storage and Processing

Parameter	Molecule(s)	Tissues	Effect(s)
Temperature	Nucleic acids: RNA integrity; Protein stability; Posttranslational modifications	Brain	• PMI effects on RNA integrity are dependent on storage temperature[269] • Protein stability depends on storage temperature[234] • Reduced intensity of phosphorylated-τ occurs when samples were stored at RT for longer periods[234] • Dry ice freeze step significantly reduces the PMI effect on tryptophan levels[55]
		Heart	• Gene expression profile is maintained at RT for up 24 h[78]
Fixation	Nucleic acids: DNA degradation	Intestine	• Fixation of solid tissue causes DNA fragmentation and formation of covalent adducts[225] • Incomplete fixation produces greater loss of DNA than complete fixation due to autolytic or bacterial degradation, and may interfere with immunohistochemistry[225] • Fixation of solid tissue can be successfully done for pathologic examination and protein detection techniques[225] • The use of fixative containing mercury or picric acid, results in DNA that cannot be amplified by PCR[225]
		Brain	• Long-term formalin fixation at RT results in DNA degradation due to the progressive acidification of formalin to formic acid[229] • Periodical renewal of the buffered fixative solution improves DNA preservation[229]
		Intestine	• Long-term storage in formalin fixative alters DNA, thereby reducing the identification of point mutations, deletions, or triple expansions[229] • Buffered formalin (4%) is somewhat less DNA damaging than unbuffered (10% formalin)[225,229]
Freeze/thaw steps	Nucleic acids: RNA degradation	Brain	• Tissue thawed to RT and then refrozen is often usable for biochemical studies[234] • RNA quality, estimated by the RIN, is reduced by freeze/thaw, in which the tissue pH remains unchanged[233]

RT, room temperature.

Different lipid subtypes can be successfully quantified by using mass spectrometric detection coupled with either gas, liquid, or thin-layer chromatography.[108,222,223,295] Tandem mass spectrometry with a lipid database[296] can be used to discriminate among chemical lipid variants with identical masses due to the identical number of acylic carbons and double bonds.[287] Matrix-assisted laser desorption/ionization mass spectrometry permits the direct scanning of tissue slides, and it thus identifies the precise localization of different lipids in the tissue.[297]

STORAGE PROCEDURES

Tissue samples can be cryopreserved, in either a –80°C freezer or in liquid nitrogen (–170°C), or fixed in formalin and embedded in paraffin (Tables 6 and 7). Choosing the optimal storage protocol depends on the study end point. Embedding in paraffin is a relatively inexpensive technique with histologic morphology of high quality, but genetic material may be damaged.[18] Cryopreservation is ideal for extraction of high-quality DNA, RNA, and protein, and it is a good storage method for morphologic studies, but it is expensive.[18] Specimens should be quick-frozen either in a dry ice/isobutane mix, between chilled aluminum plates or in liquid nitrogen vapor to avoid ice crystal formation. The samples should be on a flat surface to avoid distortion. Frozen specimens are best stored in heat-sealed plastic. It is desirable to use bar coding to identify the case number and tissue sample, and tissue from multiple donors with a specific disorder should be stored in different freezers if >1 freezer is available. Freezers should be equipped with an alarm system to avoid catastrophic failure and nucleotide degradation. The inner-chamber temperature

TABLE 6 Sample Processing and Storage Tips for DNA/RNA

Parameter	DNA	RNA
Source	• The best quality and quantity of material are extracted from fresh or frozen solid tissue, followed by (in decreasing order) tissue preserved but not fixed, tissue fixed shortly after collection[209] but not embedded, and tissue fixed and quickly embedded[225]	• RNA should be extracted from fresh or snap frozen material at –70°C or below[225,226]
	• DNA can be obtained from sections stained with hematoxylin and eosin,[225] but toluidine blue is preferred if microdissection is required[225]	• RNA can be extracted from fixed embedded tissue, but it has usually suffered more from the treatment of the specimen before fixation than from the processing itself[225]
	• When DNA is isolated by LCM from frozen solid tissue, relative humidity in the laboratory must be controlled[231]	• When LCM is used, and to avoid RNA degradation, it is imperative to keep tissue sections as dry as possible after fixation[231] • Maintain tissue in desiccators after staining and during transport[231] • Maintain relative humidity near 20% during LCM[231]
Time to freeze	• Freeze immediately after collection	• Freeze immediately after collection
	• Solid tissues should not remain more than 30 min after death or surgery removal without optimal storage conditions to prevent degradation[18,210,229]	• Solid tissues should not remain more than 30 min after death or surgery removal without optimal storage conditions to prevent degradation[18,210,229]
Extraction protocol	• DNA can be extracted from frozen or formalin-fixed and paraffin-embedded tissues reliably with QIAmp Micro Qiagen (Qiagen Inc, Valencia, CA)[210]	• TRIzol reagent (Thermo Fisher Scientific, Waltham, MA) or traditional phenol-chloroform extraction methods[279] are often used for RNA isolation from fresh or frozen samples[56,210]
	• Several other methods have been also tested in postmortem tissue derived from brain, spleen, liver, heart, intestine, and oral tissue[101,280–282]	• RNeasy Mini Kit (Qiagen Inc) provides RIN values much higher that TRIzol reagent[177]
Tissue fixation and preservation	• If fixation is needed, 4% buffered formalin is less DNA damaging than the 10% nonbuffered formalin.[229] The shorter the time a tissue is stored in buffered formalin fixation, the better the DNA preservation[229]	• Immediate immersion of tissue sample in TRIzol reagent is currently used in many laboratories to improve RNA preservation[210]
	• Periodical renewal of the buffered fixative solution reduces the deleterious effects of old formalin[210]	• Hepes-Glutamic Acid Buffer-Mediated Organic Solvent Protection Effect fixation can be also successfully used[283]
Storage	• DNA can be successfully preserved for several years if stored at –80°C or below,[24,184,210] whereas some suggest that DNA is stable at 4°C for at least 3 y[284]	• RNA can be successfully preserved if stored at –80°C or below[24,184]
	• For optimal preservation, formalin-fixed, paraffin-embedded tissue should be stored as a block. Paraffin blocks should be stored at temperatures below 27°C in an area with pest and humidity control[24]	• For optimal preservation, formalin-fixed, paraffin-embedded tissue should be stored as a block. Paraffin blocks should be stored at temperatures below 27°C in an area with pest and humidity controls[24]
Freeze/thaw steps	• Unnecessary thawing and refreezing of frozen biospecimens or frozen samples of biomolecules extracted from the biospecimens should be avoided[24]	• RNA integrity is severely affected by freeze/thaw steps[24,233]
Detection technique	• PCR is commonly used to detect genomic or viral DNA from fresh tissue, or from LCM, formalin-fixed, and paraffin-embedded postmortem tissues[67,282,285,286]	• RNA quality can be detected with high reproducibility and sensitivity with the microfluidic chip-based capillary electrophoresis[270,271] using a Bioanalyzer (Agilent Technologies, Santa Clara, CA) or Experion (Bio-Rad Laboratories, Munich, Germany) instrument

LCM, laser microdissection.

of the freezer should be monitored at least weekly and recorded in a log.[18] The benefits of the freezer are the ability to store many samples in a relatively small storage space, with ease of access, and fewer infrastructure requirements. The disadvantages of freezers include high cost, fragility of the equipment, and the dependence on energy.[18] Liquid nitrogen storage provides better preservation of samples, independent of energy, but requires constant maintenance of the nitrogen level and is associated with a greater difficultly of accessing the samples.[18]

TISSUE DISTRIBUTION

A trained pathologist or technician may be necessary to identify and section tissue. For some specific conditions/diseases and tissues/organs, a systemic evaluation of the tissue by a pathologist might be necessary to confirm diagnosis before distribution for research. Efforts need to be taken to prevent thawing of the tissue during the sampling procedure. Different approaches will be necessary to section the frozen tissue, including chipping, sawing, or use of a dental drill to excise tissues from large sections (ie, coronal brain section). The sample should be heat-sealed under vacuum in plastic and immediately placed in a –80°C freezer until it is packaged for shipping. Tissues are packed in inner and outer pouches consisting of an amber translucent Teflon-Kapton (Dupont, Circleville, OH)material and then heat-sealed after packaging. The pack is vacuum-sealed within a plastic bag and placed in a disposable transport box containing dry ice.[298] Commercial companies now offer a large variety of containers for temperature-sensitive shipments.[299] β-ray sterilized plastic bags and vacuum sealing of fresh excised specimens at surgical theaters, followed by time-controlled

transferring at 4°C to the pathology laboratory, do not affect morphology, nucleic acids, proteins, or cell viability.[300–303] Antifreeze solution E/P20 (containing 20% ethylene glycol and propylene glycol) preserves cells and DNA integrity derived from human blood tissue and bone marrow.[304] Generic insulated shipping containers with appropriate grams of wet ice for the given time interval are used for cornea shipping (≤8°C).[305]

After death, RNA integrity is influenced by the PMI, which is enhanced by higher storage temperatures.[268] The levels of proteins and posttranslational modifications are also dramatically affected at higher temperatures such as 4°C to 21°C.[234] Preservation of protein samples at 21°C for >12 hours decreases biochemical reliability. In addition, thawed tissue stored at 21°C and then refrozen is no longer usable for biochemical studies.[234] Overall, optimal storage conditions should be obtained for solid tissues by 30 minutes (for DNA or RNA) or 2 hours (for protein) after death to prevent degradation.[18,234] PMIs are often longer for medical examiner cases or for those who die at home or in hospice care. Best practices for collection, transport, and processing of solid tissues for nucleotide and protein isolation have been provided by the International Society for Biological and Environmental Repositories[306] and the National Cancer Institute.[24,184]

TISSUE LINKAGES TO CLINICAL DATA

Research using solid tissues must be linked back to the patient and his or her disease. Thus, accurate recording of clinical data and the specifics of tissue collection are essential. Table 8 outlines the recommended minimal clinical data set.

TRANSMISSION OF GENETIC TESTING RESULTS TO FAMILIES

Thresholds have been established for reporting genetic testing results to study participants, with 3 key criteria: (1) the risk for the disease should be significant (variants with greater penetrance or associated with younger age of onset should receive priority); (2) the disease should have important health implications (ie, fatal or substantial morbidity); and (3) proven therapeutic or preventive interventions should be available. In addition, the genetic results should come only from certified laboratories.[307,308]

TABLE 7 Sample Processing and Storage Tips for Protein and Lipids

Parameter	Proteins	Lipids
Source of material	• Proteins should be extracted from fresh or snap frozen material at –70°C or below[184,210,234]	• Lipids should be extracted from fresh or snap frozen material at –20°C[108] or –80°C[223,287]
Time to freeze	• Freeze immediately after isolation. Samples derived postmortem should be frozen (at –80°C) short after death (maximum 2 h) for optimal results in protein studies and to avoid protein degradation[210,234]	• Freeze immediately after isolation. Samples derived postmortem should be frozen (at –20°C to –80°C) shortly after death (maximum 6–8 h) for optimal results in lipids studies[108]
Extraction protocol	• General buffer for protein extraction and/or homogenization can be used with fresh or frozen samples[233,234,265,267]	• Lipids can be extracted with the method of Folch et al[108,223,288] • Lipid extracts are dried in a vacuum evaporator at ≤40°C and dissolved in solvent for later analysis or storage[108]
Tissue fixation and preservation	• For optimal preservation, formalin-fixed, paraffin-embedded tissue should be stored as a block[24]	• After extraction, lipids extracts are dissolved in solvent for storage[108]
Storage	• Protein can be preserved for several years for proteomics if samples are stored at –80°C or below[24,184] • Paraffin blocks should be stored at temperatures below 27°C in an area with pest and humidity controls[24]	• Lipids can be successfully preserved for several years if stored at –80°C or below[24,184,287]
Freeze/thaw steps	• Unnecessary thawing/refreezing of solid tissues or isolated biomolecules should be avoided[24]	• Unnecessary thawing and refreezing of frozen biospecimens or frozen samples of biomolecules extracted from the biospecimens should be avoided[24]
Detection technique	• When IHC is used to detect proteins in solid tissues, antigen retrieval may be required to unveil proteins[225]	• TLC and a specific solvent system are used to separate individual lipid classes[222] • Phosphorous assay is used to measure concentrations of phospholipids[223]

IHC, immunohistochemistry; TLC, thin-layer chromatography.

CONCLUSIONS

This review summarizes pertinent issues regarding the procurement and storage of solid tissues from children. Due to the variability in collection method, site, time, and storage conditions of tissues, it is imperative to understand the factors affecting tissue quality. Standardization of methods of collection and linked clinical information will aid reliable research results. Minimal quality control measures for solid tissue are required for each study,[184] which may include a search of donor information, record of agonal states, standardization of PMIs, standardization of sample collection and storage processes, histologic reviews of samples to assess proper biospecimen identification, and the use of best practices for molecule isolation. Translational research on solid tissues will contribute to new diagnostics and therapeutics in child diseases.

TABLE 8 Recommended Clinical Data Set to Accompany Solid Tissue

Clinical Data	Biopsy/Surgical	Postmortem	Organ or Tissue Donor	Otherwise Discarded
Ethics approval no.	Yes	Yes	Yes	Yes
Consent/assent on file	Yes	Yes	Yes	Yes
Authorization document for tissue transfer/disclosure of Information Form on file	Yes	Yes	Yes	Yes
Underlying disorder	Yes	Yes	Yes	Yes
Stage of disease/treatment status[a]	Yes	Yes	Yes[b]	Yes
Age	Yes	Yes	Yes	Yes
Gender	Yes	Yes	Yes	Yes
Ethnicity	Yes	Yes	Yes	Yes
Active infection[c]	Yes	Yes	Yes	Yes
Weight, height, and BMI[d]	Yes	Yes	Yes	Yes
Medical history	Yes	Yes	Yes	Yes
Comorbidities	Yes	Yes	Yes	Yes
Medications	Yes	Yes	Yes	Yes
Cause of death	NA	Yes	Yes	NA
Agonal stage/survival time	NA	Yes	Yes	NA
Autopsy findings details[e]	NA	Yes	Yes	Yes
Family history[f]	Yes	Yes	Yes	Yes
Age of mother/gestational age of fetus	NA	Yes	Yes	Yes
Access to other tissues from same patient	NA	Yes	Yes	Yes
Access to patient-matched healthy control tissue/ or patient-matched nonmalignant tissue	Yes	Yes	Yes	Yes
Reason for not suitable for transplantation	NA	Yes	Yes	Yes
Isolation site	Yes	Yes	Yes	Yes
PMI	NA	Yes	Yes[g]	NA
Processing protocol	Yes	Yes	Yes	Yes
Storage protocol	Yes	Yes	Yes	Yes
Shipping system available	Yes	Yes	Yes	Yes
Storage time[h]	Yes	Yes	Yes	Yes
Tissue integrity marker[i]	NA	Yes	Yes[b]	NA
Freeze/thaw steps[j]	NA	Yes	Yes	NA
Purity of the tissue[k]	Yes	Yes	Yes	Yes
Warm ischemia time[l]	NA	Yes	Yes	Yes
Duration of cold ischemia time[m]	NA	Yes	Yes	Yes
Assessment of ECD[n]	NA	Yes	Yes	Yes

ECD, endothelial cell density; NA, not applicable.

[a] Especially for samples derived from patients with cancer.

[b] Very important also for tissues derived from living donors.

[c] Bacterial, fungal, and viral (HIV, hepatitis B and C, cytomegalovirus).

[d] Especially required for bioengineering research studies.

[e] Including the presence of alcohol or drugs in the body at time of death, macroscopic changes in the organs at time of autopsy, and evidence of hypoxia/ischemia. Blood test results from autopsy are also included in these details.

[f] Especially for studies involving several generations within the same family.

[g] NA for fresh resection after neurologic death or cardiac death.

[h] Updated regularly.

[i] RIN, tissue pH, and/or protein assay.

[j] Record of thaw/freeze cycles before it is used for research.

[k] During dissection some tissues cannot be separated properly from others and must be specified before research is conducted.

[l] The time during which a tissue/organ remains at physiologic temperature during decreased blood perfusion or in the absence of blood supply.

[m] The time during which a tissue/organ remains cold during decreased blood perfusion or in the absence of blood supply. Also the time between tissue removal and cell/tissue preparation; 1 example is islet preparation from the pancreas.

[n] Specific for research studies of eye tissue.

ABBREVIATIONS

AFS: agonal factor score
PCR: polymerase chain reaction
PMI: postmortem interval
RIN: RNA integrity number

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