

Optimizing Pathogen-Based Sampling Methods for Vertebrate Field Research

Table 1: Comparison of preservation methods by vertebrate sample types and cold-chain requirements for pathobiology studies.

Strong cold chain: immediate access to LN2 or dry ice to flash freeze samples in the field and subsequently store samples in LN2 or at ULT for long term storage.

Weak cold chain: Access to store samples at ambient temperature (21°C) or 4–8°C (via fridge, freezer blocks or cooler,) and subsequently at (at least) -20°C within 7–14 days for long term storage.

Useable RNA defined as yielding RNA with RIN >7. See Section "Storage Buffers". Pathobiology studies referring generally to sample use for ELISA, Luminex, PhIP-Seq, RT-PCR, etc. as discussed in text.,

Sample type	Buffer	Limitation	Yields useable RNA? (Y/N)	Appropriate for weak cold chain use for pathobiology (RNA/antibody) studies (Y/N)	Notes	Storage temperature and duration limits for pathobiology uses	References
Whole Blood	No buffer	Liable to freezer failure	Unknown	N	Despite being the quoted gold-standard, we are unable to confirm the RIN obtained from RNA in flash frozen, unbuffered whole blood.	Immediately flash-freeze and storage at -80 °C. Must maintain cold-chain at all times, necessitating back up generators or similar fail-safes.	[9, 42, 43, 52, 60, 85, 88–91]
	RNAprotect®	Expensive, typically does not inactivate pathogens	Y	Y (4-8°C for up to 2 weeks)	RNAprotect® Animal Blood is a strong buffer candidate for field use due to its protection of DNA and RNA over long periods at room temperature [52, 53, 67, 70, 71]. This product yields RIN values of 7 or more [52, cold-chain unknown]; note: this study collected blood first in EDTA coated tube and then transferred the blood to RNAprotect buffer, this is not necessary per the manufacturer instructions. RNAprotect [68] is not recommended for inactivating or neutralizing pathogens. RNAprotect® allows for collection of whole blood volumes of either 100uL and 500 uL and is purchased in specialized tubes.	These samples should be stored upright and can be kept at 15–25 °C for up to 48h; at 2–8 °C for up to 14days; or at -15 to -30 °C or below for several months [63, 68]	[52, 53, 62–67, 70, 71]
	DNA/RNA Shield™	Expensive	Y	Y (4°C for up to 2 weeks)	DNA/RNA Shield™ preserves high-quality DNA and RNA in whole blood samples [70, 73]. It has been shown and is considered by bodies such as the Centers for Disease Control (CDC), to inactivate pathogens, or at least significantly reduce viral titers while preserving RNA integrity [69, 71, 72, 84]. Samples in DNA/RNA Shield can yield RIN of 7 or above after storage at 4 °C for up to 15days, or at 21 °C for up to 3days before loss of integrity. DNA/RNA Shield™ samples do not yield RIN values of 7 or above after storage at 37 °C for 1 day, suggesting these samples should be stored at 21 °C (room temperature) or lower as soon as possible when working in hot climates [70]. This study also found that DNA/RNA Shield™ preserved RNA in better (in infected-RBC culture) at 21 °C for up to 3days than TRIzol [70]	4°C for up to 15 days, or at 21°C for up to 3 days, followed by immediate freezing (ideally flash freezing) and storage at -20°C or -80°C for best results.	[56, 64, 69–73]
	TRIzol and other lysis buffers	Hazardous	Y (potentially unreliable, see note)	Y (4°C for up to 2 weeks, but results vary)	See [67] for a detailed analysis of TRIzol use in animal pathogen studies. Some studies cite lysis buffers such as TRIzol as good nucleic acid preservation [86] (blood), and [74] (blood) and some cite degradation or poor performance compared to other buffers [92] (blood) [93], (liver) [88], (blood). A lysis buffer used without an RNase inhibitor will drastically reduce RNA integrity [42, 43, 94]. Specifically for whole blood, Longmire's lysis buffer gave evidence of good RNA yield but DNA degradation [85, 86, 95]. TRIzol preserves high-quality DNA and RNA in whole blood samples [70, 73]. However, TRIzol is hazardous to handle and transport as it is a potential carcinogen/mutagen, it can cause contact burns and systemic poisoning, it must be handled in a ducted biosafety cabinet or fume hood to avoid exposure by inhalation, and it must be carefully discarded as a hazardous waste [96, 97]. In a study on infected-RBC cultures [70] preferred TRIzol to DNA/RNA Shield as it was as effective as well as cost-effective and practical. TRIzol could yield RIN of 7 or above after storage at 4 °C for up to 15days, or at 21 °C for up to 3days before loss of integrity, quality not maintained after more than 24 hours at 37°C [70]. Meanwhile, [52] showed that whole blood samples extracted with TRIzol® LS (12–24h at ambient temp, stored at -20°C) showed a broad range of results from well-preserved RNA (RIN and RNA yield) to very low preservation of RNA (RIN and RNA yield). This study suggests that TRIzol is not the most reliable RNA Preserving buffer. TRIzol [67, 98, 99] typically inactivates high-risk pathogens, rendering samples "non-infectious" by the CDC guidelines. ThermoFisher recommends freezing samples in TRIzol immediately at -80 °C [100]. The PREDICT handbook does not recommend TRIzol if samples cannot be refrigerated (2–4°C) within 24hours.	12-24h at ambient or 4°C, followed by immediately freezing at least -20°C, preferably -80°C. But note that this blood preservative has not been shown to be reliable.	[42, 43, 52, 67, 70, 73, 74, 85, 86, 88, 92–100]
	RNAlater®	Not recommended for whole blood	N	N	RNAlater® has been used to store anticoagulated whole blood (whole blood with EDTA, potassium, heparin or citrate added to it) and as a protectant it has the same temperature restrictions as RNAprotect®, but it is not recommended for whole blood samples [101, 105]. Whole blood collected in EDTA tubes and stored directly in RNAlater® yielded poor RNA integrity (RIN=4.6±2.3), whereas blood pre-filtered through the LeukoLOCK™ system (leukocyte capture with PBS flush) prior to RNAlater® saturation yielded high-integrity RNA (RIN=7.6±1.9), indicating that RNAlater® is appropriate for whole blood only when paired with upstream leukocyte filtration [52]. RNAlater® has been found to preserve RNA in avian blood samples collected in heparinized tubes, yielding RIN>7 [73] (after an unknown amount of time at ambient temperature, samples were kept at 4 °C refrigerator for 24hours, then moved to a -80 °C freezer) Ambion Inc. and ThermoFisher do not recommend storing whole blood in RNAlater® for RNA extraction [101]. RNAlater® is cheaper per unit than RNAprotect®, but it does not come in ready-made tubes as DNA/RNA Shield™ can and RNAprotect® does. It is best practice to add RNAlater® incrementally to blood to prevent the mixture from coagulating, which can negatively affect RNA preservation and it should be extracted using a filter system [52]. RNAlater® [102–104] is not recommended for inactivating or neutralizing pathogens	Not recommended by manufacturer for whole blood storage. However, in the case of avian blood, when whole blood is collected with heparinized tubes, vortexed, stored at 4°C for 24 hours and frozen at -80°C, results yield RIN>7 [73]	[17, 52, 64, 72, 73, 75, 85–87, 92, 101–106]
	PAXgene™	Expensive, limited inactivation of pathogens	Y	N	Whole blood stored in PAXgene™ blood buffer yields RIN values similar to RNAprotect® but can be expensive [52]. (This product has not been developed or tested on swab samples.)	Up to 3 days at 18- 25°C or for 5days at 2-8° C, but this likely needs further validation in real field studies	[52]
	NAP	Needs further validation	Unknown	Unknown	A non-commercial homemade NAP buffer has not yet been shown to preserve RNA integrity for whole blood samples as it has for tissue samples [85]. This method may be affordable and viable for RNA preservation, but needs further validation. See 85 and 60.	Unknown time-temperature breakdown. Needs further study.	[85, 60]
	PrimeStore® MTM	Expensive, needs further validation	Unknown	Unknown	Found to inactivate pathogens in virus-spiked human blood in lab setting, though RNA quality not evaluated in that study [83]. Larger natural infection field studies need to be conducted to confirm the reliability of this buffer for nucleic acid storage at room temperature for the purposes of wildlife studies.	The specific RIN of samples stored in PrimeStore® MTM at ambient or frozen temperatures needs further validation.	83; 243; 82; 244; 245; 84
	VTM/UTM	Potential live virus, must have strong cold chain	Y (according to Ct values, RIN not typically assessed)	N	Commonly used to allow for downstream virus isolation [75, 76, 78–81], particularly for influenza viruses [78, 87]. VTM/UTM can also be used for whole-blood preservation (used in 96), but DNA/RNA Shield™ may provide better nucleic acid preservation [72]. Commercial & homemade formulations vary, including viral-inactivating formulations that preclude downstream virus isolation and non-inactivating formulations. These differences, as well as the potential inclusion of RNA-degrading nucleases, should be considered based on the target species and pathogens and any travel or handling restrictions [76, 110]. VTM/UTM generally requires refrigerated storage before use [111], unlike buffers such as RNAprotect®, DNA/RNA Shield™, and RNAlater®, according to manufacturer handbooks. WHO recommends storing VTM/UTM-collected samples at 2–8 °C for no more than 5 days, compared with 4days by the CDC and 2days by PREDICT [96, 112]. PREDICT recommends an alternative buffer when 2–8 °C storage is unavailable within 24 hours, although some formulations may provide longer ambient-temperature stability [76]. Because VTM/UTM is primarily designed for virus isolation, often requires refrigeration, may provide less effective RNA preservation at ambient temperatures, and generally does not inactivate viruses, many field teams use alternative buffers and methods.	2-8°C for 24 hours [96], depending on type of VTM/UTM used.	[75, 76, 78–81, 87, 96, 110–112]
	DBS	Safety: if viral inactivation is necessary, there are some filter papers that are designed for viral inactivation	N	Y	DBS can be kept at ambient temperature for between 14 and 60days depending on analytes and assays, followed by freezing at -20 °C or ULT for longer term storage [113]. Not viable for RNA studies but can be used for Luminex and ELISA: varying between for no more than 14days for DNA SNP studies [114], for no more than 28days [115] or 30days [116] for ELISA assays (or up to 60days in the case of SARS-CoV-2 per 117). When detecting antibody titers via passive hemagglutination assay, [118] found DBS on Nobuto strips began to degrade at 6weeks. Proper desiccation procedures help retain viability, and short term storage at 4 °C or -20 °C extends the viability of DBS further, in some cases yielding viable antibody signal through ELISA for up to 2years [113]. For longer term storage, DBS must be kept at -20 °C or below [113].	For best results, keep at ambient temperatures or 4°C for up to 14 days, then freeze for long term storage at -20°C.	[113, 114, 116–118]

	EDTA/EtOH	Typically poor RNA preservatives. EtOH specifically: flammable, potentially travel restricted, may evaporate if not properly sealed, recommended to maintain a high ethanol to tissue volume ratio if used in collection [88, 106]	N (Typically, but refer to [51])	N	EDTA or a mixture of EtOH and EDTA are commonly used in sample storage and are typically effective for DNA but not RNA preservation [72, 85, 119] However, procedures have recently been developed that successfully extracted high-quality RNA (RIN> 7) from frozen EDTA blood frozen immediately at -80 °C [51]. 95% EtOH can preserve DNA at ambient temperatures, but struggles to maintain RNA [72, 85, 119]. While samples collected into EtOH are considered shelf stable for DNA analyses indefinitely, it is always good practice to freeze samples as soon as possible following collection and store at -80 °C (ideally) or -20 °C if resource-constrained [72, 93].	EDTA: frozen immediately at -80°C (See [51])	[51, 72, 85, 88, 93, 106, 119]
	DMSO + nutrient-containing cell medium	Unreliable RNA preservative. Must be frozen in a step-down fashion.	N	N	The gold standard buffer for freeze medium for downstream cell culture is a mixture of 10% DMSO with 90% of some type of nutrient-containing cell medium buffer like Dulbecco's modified eagle's medium (DMEM) or fetal bovine serum (FBS) [120, 121]. These can be used for RNA analysis when frozen slowly in a Mr. Frosty container or a step down freezer [122–125], which is not common in field work, though some studies have recovered cells after tissues were flash-frozen in freeze media rapidly following collection [161]. Because DMSO degrades methylated DNA, it is suggested that, if tissue samples are collected into DMSO with the aim of cell culture, a second standard tissue sample should be collected into a non-DMSO buffer to allow for other analyses (refer to the supplemental material 1). Alternatively, XT-Thrive-A, XT-Thrive-B and CryoSOfree [®] are said to perform similarly without DMSO for samples with the ultimate aim of cell culture [124].	RNA not reliably high enough for pathobiology studies, but if goals include cell culture, researchers should consider this buffer and its restrictions.	[120–125]
Serum	No buffer, flash frozen	Liable to freezer failure	Y	N	There is evidence that storage time and temperature have a large effect on results obtained from serum samples, and more studies are needed to confidently provide serum storage guidelines [126]. There is evidence that serum samples change significantly at both room temperature and refrigeration temperature (4°C) after 6 hours [127], but other studies found serum stable at 4 °C for 72hours [128], 120 hours [126] or 7days [129, 130] depending on the analyte and proteins measured. There is evidence that serum samples are stable at -20 °C for 3 to 4months [126, 127, 130]. Storage at -70 °C or -80 °C is preferred to -20 °C [130–132]. When stored at -70 °C serum samples can be stored for 1–5years [129, 130]. Over decadal time scales, serum has been successfully used for retrospective antibody detection in canid samples stored for a mean of 22years at -20 °C [133]. Similarly, human serum stored for 18–29years at -20 °C to -25 °C has remained suitable for several epidemiological and immunoassay applications, although analyte stability varies by biomarker [134–136]. Best RNA integrity results are obtained when these samples are processed within 3years when stored at -70 °C or colder [137].	Serum should stay at 4°C for no more than 6 hours, if possible, before freezing (ideally flash freezing) and storing at -20°C or -70°C.	[126–137]
	DSS	Safety: select viral inactivating card if necessary	N	Y	RIN> 7 not met, but DSS can be eluted for ELISA and Luminex. Hepatitis A antibody detection on DSS (Whatman paper) was 100% sensitive and specific after up to 4 weeks at 4 °C, room temperature, or 37 °C [218]. Although this is a single pathogen, these results suggest DSS may perform comparably to DBS for other serological and molecular targets – which can be kept at ambient temperature for between 14 and 60days depending on analytes and assays, followed by freezing at -20 °C or ULT for longer term storage.	For best results, keep at ambient temperatures or 4°C for up to 14 days, then freeze for long term storage at -20°C	[113–118, 218, 219]
RBC	No buffer, flash frozen	Liable to freezer failure	Unknown	N	RNA is typically not extracted from RBC samples, RIN has been shown to increase with red blood cell lysis [138]	Ambient temperature resilience unknown. Ideally flash freeze and store at -20°C or -70 °C.	[138]
Plasma	EDTA, citrate, heparin	Not as well validated as serum for pathobiology studies	potentially	N	Plasma has been shown to perform as well as serum in ELISA for some pathogens, including dengue virus [139] and Influenza A [140]; EDTA is the best-performing coagulant for those assays [139, 140]. [139] recommends assaying plasma samples in duplicate for ELISA to offset variability. Plasma has not been as thoroughly validated on ELISA or Luminex; most ELISAs and serological tests are optimized for serum. These samples had been stored directly in -80 °C for 2–3months. For ELISA, EDTA blood samples should be stored at -20 °C immediately.	For downstream use in ELISA, EDTA blood samples should be stored at -20°C immediately.	[139, 140]
	Filter paper (dried plasma spots)	Safety: select viral inactivating card if necessary	N	Y	Hepatitis A virus RNA spiked into plasma on FTA cards and stored at room temperature showed no loss of viral load after 5, 15, or 30 days at ambient temperature [218]. Although this is a single pathogen, these results suggest plasma on filter paper, like DSS, may perform comparably to DBS for other serological and molecular targets – which can be kept at ambient temperature for between 14 and 60days depending on analytes and assays, followed by freezing at -20 °C or ULT for longer term storage.	For best results, keep at ambient temperatures or 4°C for up to 14 days, then freeze for long term storage at -20°C	[218]
Blood smears	Methanol fixed	Hazardous	N	see note	Methanol is hazardous, but only used for fixation, not for storage. Take care to pack slides carefully to avoid breakage. Not suitable for RNA or serology, but used for microscopy in pathogen and immune cell studies.	No cold chain needed. Stable at ambient temperature for microscopy use.	[9, 141]
Swabs	DNA/RNA Shield™	Expensive	Y	Y (ambient or 4°C for 1 month)	DNA/RNA Shield™ can preserve both DNA and RNA well in swab samples under field conditions [56, 69, 71, 72]. As stated in Section “Storage buffers”, typically inactivates pathogens.	DNA/RNA Shield™ samples do not have to be frozen immediately. According to Zymo Research, samples in DNA/RNA Shield™ can remain at 35 to 40°C for up to 7 days, 5°C to 30°C (ambient) for 30 days, and at -20°C indefinitely.	[42, 56, 69, 71, 72, 75]
	RNAlater®	limited inactivation of pathogens	Y	Y nblent for 1 week or 4°C for 1 month	RNAlater® preserves DNA and RNA in samples [72, 75, 85, 87], but may not inactivate pathogens. DNA/RNA Shield™ and RNAlater® yield similar RNA viral detection results; however DNA/RNA Shield™ samples can last at ambient temperature for 1month as opposed to 1week for RNAlater® buffered samples [72, 75].	According to Sigma-Aldrich, samples collected into this buffer can be kept at 37 °C for 1 day, 25 °C for 1 week, 4 °C for up to 1 month and long term at -20 °C.	[17, 72, 75, 85–87]
	PrimeStore® MTM	Expensive, needs further validation	Unknown	Y (for some assays)	Sufficient RNA quality for RT–PCR after ambient storage for between 14 and 30 days [82, 107]. This buffer has been shown to preserve RNA at ambient temperatures for long periods of time making it appropriate for field use: in laboratory settings, influenza virus was detected by RT–PCR from virus-spiked swabs stored in PrimeStore® MTM at ambient temperature for 14days [82]. Enterovirus D68 was detected in swab samples after 30days at ambient temperature [107]. viral RNA has been detected from human nasopharyngeal samples stored in PrimeStore® MTM [108, 109] and finally, livestock and avian virus were detected by qRT-PCR from virus-spiked sera or virus-spiked cloacal swabs stored in PrimeStore® MTM at ambient temperature for 21days [84].	PrimeStore® MTM may be another strong buffer candidate if studies establish extracted RIN>7.	[82,84, 107, 108, 109]
	VTM/UTM NAP	Potential live virus, must have strong cold chain	Y (according to Ct values, RIN not typically assessed)	N	Used for viral isolation and viral culturing, RIN values not explicitly recorded and RNA quality/integrity varies on VTM/UTM formulation used. RNA quality in VTM/UTM-preserved samples is typically assessed using Ct values rather than RIN, although VTM/UTM has been used in numerous pathobiology studies, particularly for viral isolation [17, 72, 77]. DNA/RNA Shield™ has been shown to better preserve nucleic acid than VTM/UTM in the case of fecal samples [72].	2-8°C for 24 hours [96], depending on type of VTM/UTM used.	[17, 72, 77, 96]
		Needs further validation	N	Unknown	May be appropriate for RNA and weak cold chain use, but needs further study	Needs further study	
	EtOH/EDTA	Typically poor RNA preservative; EtOH specifically: flammable, travel restricted, monitor evaporation and volume	N	Y (for some assays)	Influenza and adenovirus have been identified using RT-PCR from human nasal swab samples stored in 100% EtOH at ambient temperatures for up to 6months [142], but the reliability of EtOH as a preservative would need to be verified with other pathogens, considering the evidence that EtOH does not maintain RNA [72].	Not typically useable for immune and RNA studies, but viable in some cases when kept at ambient temperature for 6 months. See Notes. While samples collected into EtOH are considered shelf stable for DNA analyses indefinitely, it is always good practice to freeze samples as soon as possible following collection and store at -80°C (ideally) or -20 °C if resource-constrained [72, 93]	[72, 93, 142]
	DMSO	Unreliable RNA preservative. May need to be frozen in step-down fashion. Damages methylome	N	N	DMSO is commonly used for DNA preservation [42, 93], but may impact nucleic acid acetylation [143] and methylation [144, 145], precluding downstream epigenetic analysis. DMSO may otherwise affect RNA structure [146], although it has also been investigated as part of the treatment or protocol for RNA sequencing and found to improve reads [147, 148]. Very high concentrations of DMSO will degrade all nucleic acids [149].	While samples collected into DMSO are considered shelf stable for DNA analyses for 2years, it is always good practice to freeze samples as soon as possible following collection and store at -80 °C (ideally) or -20 °C if resource-constrained [72, 93].	[42, 72, 93, 143–149]
Ectoparasites	DNA/RNA Shield™	Expensive	Y	Y	DNA/RNA Shield™ has been used to preserve ectoparasites for downstream pathogen surveillance, including PCR-based detection and RNA/DNA sequencing [150, 151]	DNA/RNA Shield™ samples do not have to be frozen immediately: according to Zymo Research, samples can remain at 35 to 40 °C for up to 7days, 5 °C to 30 °C (ambient) for 30 days, and at -20 °C indefinitely.	[150, 151]

Ectoparasites	EtOH	Typically poor RNA preservative. Flammable, travel restricted, monitor evaporation and volume	N	N	Can use EtOH if uninterested in viral RNA of ectoparasites. Store these ectoparasite samples in cryovials of >95% EtOH with at least a 5:1 buffer to ectoparasite volume to retain DNA data [28, 119, 152, 153]. In the case of preserving morphological features, teams may consider lower concentrations of EtOH which is preferred for morphological preservation of invertebrates.	No cold-chain needed. These samples are shelf stable indefinitely for DNA studies, but it is best practice to freeze as soon as possible at -80°C, ideally, or at -20°C.	[28, 119, 152, 153]
Hair/Feather/Fur	Not suitable for RNA or serology	N/A	N	see note	Hair samples can typically be stored in a coin envelope or similar package and do not have to be frozen. Not suitable for ELISA, Luminex, PhiP-Seq or RT-PCR. Useful for some DNA analysis, stable isotope analysis, and hormone analysis. Keep dry to prevent mold.	No cold-chain needed	[23]
Organ samples	No buffer	Liable to freezer failure	Y	N	Although collecting tissues into tubes without buffers poses a degradation risk in the presence of cold chain failure or repeated freeze-thaw cycles [154], tissues preserved without buffer and immediately flash frozen in LN2 remain the gold standard for whole genome sequencing [155, 156]	Immediate flash-freezing and storage at -80 °C. Must maintain cold-chain at all times, necessitating back up generators or similar fail-safes.	[85, 154–157]
	DNA/RNA Shield™	Expensive	Y	Y	More widely validated for blood and swab samples, needs further organ sample validation. As stated in Section "Storage buffers", typically inactivates pathogens.	DNA/RNA Shield™ samples do not have to be frozen immediately. According to Zymo Research, samples can remain at 35 to 40°C for up to 7 days, 5°C to 30°C (ambient) for 30 days, and at -20°C indefinitely.	[158]
	RNAprotect®	Expensive, typically does not inactivate pathogens	Y	Y	Has been used in organ studies, appropriate for weak cold chain according to manufacturer.	These samples should be stored upright and can be kept at 15–25°C for up to 48h; at 2–8° C for up to 14 days; or at –15 to –30°C or below for several months	[63, 68, 159]
	RNAlater®	Expensive, limited pathogen inactivation	Y	Y	Necessitates time at 4 °C for the buffer to penetrate the tissue before freezing or decanting the buffer after absorption prior to freezing. DNA/RNA Shield™ does not need to be decanted and inactivates pathogens. [85] found low RIN values for RNAlater® preserved tissues, but these tissues were kept at ambient temperature longer than manufacturer recommended (8weeks)	According to Sigma-Aldrich, samples collected into this buffer can be kept at 37 °C for 1 day, 25 °C for 1 week, 4 °C for up to 1 month and long term at -20 °C.	[85, 95]
	NAP	Needs further validation	unknown	unknown	Needs further validation	Unvalidated	[85, 60]
	EDTA/EtOH	Typically poor RNA preservative. Flammable, travel restricted, monitor evaporation and volume	N	N	shelf stable for DNA for 2 years (EDTA) or indefinitely (EtOH), not validated for RNA or antibody assays	No cold-chain needed. Shelf stable for DNA, not validated for RNA or antibody studies.	[42, 72, 93, 160]
	DMSO	Unreliable RNA preservative. May need to be frozen in step-down fashion. Damages methylome	N	N	shelf stable for DNA for 2 years, not validated for RNA or antibody assays	No cold-chain needed. Shelf stable for DNA for 2 years, not validated for RNA or antibody studies.	[42, 93, 160]
Epigenetic aging tissue sample	DNA/RNA Shield™	Expensive	N/A	Y (ambient or 4°C for 1 month)	EtOH commonly used. It is not known exactly how long these buffers can maintain methylation quality at certain temperatures. Follow established epigenetic clock protocol for your SOI, if possible. In general, these samples can likely be stored at ambient temperature for 7–14days in 95% EtOH according to [85] and [268], but the exact time of storage prior to freezing –20 °C or –80 °C before methylome quality degradation is not known. N/A in RNA column as this epigenetic aging sample is not meant to maintain RNA stability.	Same restrictions for these buffers listed above.	[85, 268]
	RNAlater®	Expensive, may not inactivate some viruses	N/A	Y mbient for 1 week or 4°C for 1 mon			
	NAP	Untested	N/A	Unknown			
	EtOH	Flammable, travel restricted, monitor evaporation and volume	N/A	Y (likely viable at ambient temp for 7-14 days according to [85] and [268])			