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Nomenclatural, Curatorial, and Archival Best Practices for Symbiotypes and other Type Materials in Natural History Collections

Robert D. Bradley, Lisa C. Bradley, Rodney L. Honeycutt, Kathryn A. MacDonald, Heidi N. Amarilla-Stevens, and Richard D. Stevens

Abstract

The emergence of several zoonotically-driven pandemics and near-pandemics (MERS, SARS, H1N1, Ebola, COVID-19, among others) over the last 20 years has placed an increasing emphasis on the importance of symbiotypes and the role of natural history collections, as virologic and human health studies are incorporating symbiotypes and host specimens as part of their research response. Because designation of symbiotypes and host specimens involves natural history collections, there is a growing need for a discussion pertaining to the archival integration of information on symbiotypes, host specimens, and associated data in natural history collections. Furthermore, due to the involvement of specialized researchers, such as virologists and epidemiologists, there is an expanding portion of the zoonotic research community that is further removed from research practices involving the principles of nomenclature and the rules and standards governing the description of taxa new to science. Finally, as the number of traditional field biologists and natural history museum curators retire and are not replaced, there is an increasing urgency to develop protocols and procedures for the establishment of best practices and to standardize methodologies in zoonotic research for all participants. In this paper, the authors: 1) outline a number of nomenclatural, curatorial, and archival best practices for the treatment of symbiotypes in natural history collections; 2) introduce the concept of the symbioparatype and symbiotopotype, which represents a compromise between the conflicting mission of natural history museums to protect archivally important specimens and the ever-growing demand for critical research material; and 3) comment on multiple standards and guidelines to assist a broad array of researchers ranging from field biologists and systematists to virologists and public health scientists.

Key words: archival preservation, genetic resource collections, protocols, symbioparatypes, symbiotopotypes, symbiotypes, zoonoses

INTRODUCTION

Several authors (e.g., Frey et al. 1992; Brooks 1993) have discussed the importance of symbiotypes, their utility to research, and archival considerations. A recent manuscript by Dunnum et al. (2017) further serves as an excellent model for museums and biorepositories to follow in developing procedures to best support and serve zoonotic research as it relates to the standards of both systematic biology and systematic collections. Herein, the curators, staff, and colleagues of the Natural Science Research Laboratory (NSRL) at the Museum of Texas Tech University expand upon these principles as they address nomenclatural, curatorial, and archival best practices for both standard collecting and archiving of specimens and tissues and the treatment of symbiotype materials. This discussion is intended to not only serve the museum and biorepository community but also to stimulate collaborative interactions between human health scientists, virologists, cellular and molecular biologists, and the more traditional biological researchers associated with natural history collections.

Our focus is primarily on discussing standards pertaining to viral, bacterial, and in some cases, intra- or intercellular endoparasite symbiotypes (e.g., protozoans located within host tissues or bacteria found within the blood plasma). Although our discussion of symbiotypes does not necessarily pertain directly to traditional ectoparasites (e.g., fleas, ticks), the standards and ideas developed herein could be applicable to ectoparasites in many cases. Further, discussions are restricted to our experiences with mammals; however, these protocols presumably could be extended to other vertebrates, invertebrates, and plants. Finally, we discuss recent and ongoing studies at the NSRL pertaining to degradation of sample quality (DNA and RNA) both in the field (time elapsed from death of the specimen to cryopreservation of the tissues) and in natural history collections (various freezing temperatures and storage periods).

STANDARD COLLECTION AND ARCHIVAL PROTOCOLS

Collection of Specimens and Tissues

We begin this discussion by outlining recommended standards that should apply to all specimen and tissue collection activities, including the recommended tissues to be collected, field collection and field storage methodologies, archival storage methods, subsampling and loaning of tissues, and investigator safety protocols. These standards apply regardless of whether the specimens and tissues are being collected specifically for virological research or if they are part of a general research project with varied goals, including ecological, systematic, genetic, or other types of study. However, any specimen or tissue that is collected, regardless of the original research purpose, could potentially later be determined to be the host of a new virus and thus be designated as a symbiotype. Therefore, all collection activities should assume that this is the case and should follow collection and sampling protocols to best preserve potential viruses and all relevant data.

Below, we discuss several specific activities and concepts associated with obtaining and archiving biological materials. Before discussing specifics, we would like to make the reader aware of several guidelines for these diverse activities. Because several of these guidelines extend across the specific categories discussed below, we present them here as a generalized resource. For example, there are several guidelines and standards for collecting mammalian biological material (e.g., Mills et al. 1995; Sikes et al. 2016). Also, there are citations for guidelines for PI safety during collecting activities (e.g., Mills et al. 2007; Kelt et al. 2010). Finally, we encourage the reader to become familiar with guidelines and regulations that govern collecting, importation, transfer and shipping, and research pertaining to biological specimens that may harbor potential zoonotic pathogens. In the U.S., for example, such regulations are set forth under the auspices of and enforcement by the U.S. Centers for Disease Control and Prevention, U.S. Department of Agriculture Animal

and Plant Health Inspection Service, U.S. Customs and Border Protection, and others, as well as state agencies and institutional committees, such as Animal Care and Use Committees and Environmental Health and Safety departments.

Tissues to be collected.—It is our opinion that the collection of any specimen for any research project should maximize the potential utility of that specimen and its parts. In other words, a researcher should be ethically bound to preserve as many samples, tissue types, and data as possible under reasonable time and monetary constraints (assuming the species or population is not of conservation concern) to maximize the research potential of each specimen. For example, approximately 20 years ago, the NSRL expanded our "standard" tissue collecting protocols to include lung and blood (in addition to heart, kidney, liver, muscle, spleen, and others), and more recently, feces and colons were included (see TK sheet, Fig. 1). Our efforts were to "be prepared" for any potential zoonoses and any other viral or bacterial species that might manifest itself in the future.

Field collection and temporary storage procedures.—Collecting tissues in the field should follow appropriate sterile protocols, to the extent that this is possible under field conditions. Cryotubes should remain sterile; if tubes from previously opened packages or previously handled tubes are used, they should first be autoclaved. The investigator should be cautious when obtaining tissue samples that they do not transfer human RNases or DNases to the tissue sample and to avoid cross-contamination of samples, utensils, and surfaces. For example, we recommend that the researcher use sterile forceps or hemostats to place tissue samples within a cryovial; if samples must be handled, then gloved hands should be used and gloves should be changed between each specimen. Care should be taken to clean surface areas and utensils between individual specimens. Cleaning solutions may include a disinfectant such as a weak bleach solution or alcohol wipes. In some cases, it may be wise to use a DNase or RNase inhibitor to pretreat utensils and surface areas. There are numerous protocols and reagents that can be used for decontamination (e.g., Champlot et al. 2010; Fischer et al. 2016), and we recommend that researchers establish a protocol that works best for the area in which handling of samples is occurring.

To fully preserve genetic information contained in a sample, we recommend that all tissue samples taken in the field be placed in liquid nitrogen (LN2) or otherwise stabilized with an appropriate preservative as soon as possible (preferably within five minutes) after death of the specimen. This protocol requires that: 1) the obtainment of tissues becomes the top priority for the specimen (i.e., the skin and skull are prepared only after the tissues are taken and properly preserved); 2) specimens are not euthanized until the preparator(s) are set up to obtain the tissues (i.e., work station is clean and prepared with labels, tubes, instruments, etc.); and 3) specimens are euthanized one at a time and not before the person collecting the tissues is ready for the next specimen. If several individuals are to be processed during a single prepping session, it is advisable to dedicate one or more experienced workers solely to obtaining tissues, after which the specimen is passed to other individual(s) to complete the preparation process. A researcher should be able to obtain all tissues from a specimen within a three-to-five-minute window of time if this "assembly line" approach is utilized. In circumstances where a large field party is prepping greater than 50 individuals per day, it may be prudent to select representative individual specimens for this type of handling so that the time standard can be met; other specimens for which time is less sensitive then can be prioritized at a lesser standard. An alternative approach to taking a complete set of tissues, if this is deemed to be too time consuming, would be to immediately sample a tissue type designated specifically for virology research; this approach would ensure that at least one tissue type is archived at a sufficient level for subsequent virological research.

The time elapsed from death to preservation of samples should be noted on the field data pages, as this information may determine the later utility of the samples for specific types of research. The NSRL, for example, requires preparators to complete the query "Tissues placed in liquid nitrogen ___ minutes after death" on the TK page for each specimen (Fig. 1). In situations where LN2 is not available, field preparators should preserve samples by other methods, such as RNA stabilizers (e.g., RNAlater®, Ambion, Inc., Austin, Texas), dry shippers, dry ice, wet ice, 70% ethanol, or, only as a last resort, a lysis buffer (such as that referenced by Longmire et al. 1997). Based on our experiences, lysis buffer such as that discussed

TK

SPECIES _____

Country _____ **State** _____ **County** _____

Specific Locality _____

_____

UTM or **Lat/Long** _____

Elevation _____ (Locality same as: TK _____)

Collector _____ **Coll Date** _____

Preparator _____ **No.** _____ **Prep Date** _____

VOUCHER: **____Skin** **____Skull** **____Post-cranial Skeleton**
____Alcoholic **____Other**_____

Measurements _____-_____-_____-_____-_____≡_____
total tail hind foot ear tragus weight

____Male____Female Reproductive Condition _____

TISSUES: *(indicate # of tissue tubes taken; fill in tissue type for Other, Ethanol, or Lysis)*

____Heart/Kidney	____Brain	____Submandibular Gland
____Heart	____Embryo	____Entire Specimen
____Kidney	____Gonad	____Other_____
____Liver	____Karyotype	____Other_____
____Muscle	____Colon	____Ethanol (%)_____
____Spleen	____Urine	____Lysis Buffer_____
____Lung	____Feces	____Lysis Buffer_____
____Blood	____Saliva	____No tissues taken

Tissues placed in liquid nitrogen _____ minutes after death.

MISCELLANEOUS:
Age: ☐ Juvenile ☐ Subadult ☐ Adult Molting: ☐ Yes ☐ No

Comments _____

_____

_____

_____

_____

Please fill in above form completely. Items in bold are mandatory.

Special #s _____ Accession #_____

Museum Collection _____ Catalog #_____

Figure 1. Image of a standard data entry page, or 'TK page', used by the Natural Science Research Laboratory, Museum of Texas Tech University. This data page allows the recording of several fields of data pertaining to collection, preparation, and archival information for an individual specimen. Each individual is assigned a unique TK number, which is used for internal tracking of each specimen and its parts and data. The range of tissue samples that are obtained for each specimen should be noted. The NSRL also asks preparators to note the time elapsed from death of the individual to the tissue samples being placed in liquid nitrogen.

in Longmire et al. (1997) does not preserve the RNAs critical to examinations of virology and other research topics. Therefore, NSRL-affiliated staff no longer collect tissues in lysis buffer, and we strongly discourage depositors from doing so unless there is no other option for tissue preservation.

Given concerns pertaining to the safety of collection personnel and researchers, several methodologies are available for the stabilization, storage, and transfer of zoonotic pathogens via tissue samples. With various purposes, these include RNAlater®, RNAprotect Tissue Reagent (QIAGEN, Hilden, Germany), DNA/RNA Shield™ (Zymo Research, Irvine, California), and Whatman® FTA® cards (GE Healthcare, Piscataway, New Jersey). Some of these are designed to deactivate (in part or fully) RNA viruses that might present a risk to personnel, but questions remain about long-term deactivation of mRNAs and other RNAs that might be the focus of research conducted decades in the future (see Greenwood et al. 2001). We recommend that PIs consider these alternative storage and transfer methods, but we cannot fully endorse their usage without additional data because, at this time, it is not clear whether these methods are in the best interest of long-term storage and future research.

For various reasons, it may not be ideal to freeze blood samples (e.g., due to fracturing of blood cells). Under those cases, alternative methods should be used. For example, blood can be collected utilizing absorbent filter strips, such as Nobuto strips. These paper strips can be stored at room temperature and still provide utility for certain types of studies such as antibody assays; however, even at -20°C, their viability decreases after a year (Bevins et al. 2016). In terms of genetic studies involving microsatellites and DNA barcoding, blood sample strips have proven effective, but such samples are not useful for genomic studies that require longer strands of DNA (Stowel et al. 2018). Whole blood collected for serological studies should be centrifuged and preserved in the field by placing the serum samples on ice or light freezing (-20°C). Whole blood containing EDTA and maintained at -80°C has been shown to yield high-quality DNA (Bulla et al. 2016), although the quality of RNA appears to decline (Huang et al. 2017). Therefore, it is critical to consider the intended use when collecting blood samples.

Archiving specimens and samples in perpetuity.—Samples collected from the field should be deposited in an accredited natural history museum, where they will be properly managed for the long term and will be available for subsampling and loaning to researchers, as appropriate. It is crucial that both the specimens and the genetic materials be properly curated and archived under long-term storage standards following the best practices available. The American Society of Mammalogists has adopted a dual accreditation process that ensures that both the mammal voucher collection and genetic resources collection of an institution meet established standards (see ASM guidelines at http://www.mammalsociety.org/uploads/committee_files/curatorial%20standards%202004.pdf; and see Phillips et al. 2019). Accredited collections maintain the highest standards of collections care and provide loans of tissue subsamples to qualified researchers, which maximize the scientific value of each sample.

Specimens and samples collected under permit typically are the legal property of a state or agency (especially if collected by university or agency researchers), and therefore, they should be stored in a public repository for the benefit of society. In most circumstances, samples should be immediately placed in an accredited natural history museum and should not be taken directly from the field to a researcher's laboratory for storage and research purposes. There are multiple justifications for this requirement. For example, outside of an accredited environment, samples and data are less likely to be properly managed; samples may be stored under subpar conditions; individual researcher laboratories typically are not equipped with alarms to notify a researcher of freezer failures; and research lab "collections" typically are not digitized, which hinders retrievability of samples. Further, if sequestered in a private collection, samples cannot be accessed by other researchers as they would be through an accredited natural history collection. We realize that the protection of samples and associated data collected by an independent researcher may be a sensitive topic. To address this concern, the NSRL routinely enters into agreements with depositors in placing a temporary loan moratorium (if requested) on such samples. This moratorium enforces a "do not loan" protocol (negotiated between the NSRL and the depositor) for a specified

time-period, thus allowing for a first-right-of-refusal process in regards to loan requests.

Phillips et al. (2019) discuss a series of standards and guidelines relative to long-term care and storage of genetic resource collections. Ideally, all tissues (with the exception of whole blood) should be stored in vapor-phase LN2 freezers to ensure the preservation of all genetic components of a sample for future research (DNA, RNA, viruses, bacteria, etc.). If a genetic resources collection does not have a LN2-based preservation system, samples may be stored in -80°C freezers, although long-term storage at -80°C results in degradation of genetic data contained within the samples (Soniat et al., in prep.).

Several other alternatives (chemical additives) have been shown to help preserve DNA and RNA samples. For example, some studies have shown that preservation of tissue samples stabilized with RNAlater is comparable to samples maintained in liquid nitrogen (Keating et al. 2008; Arnold et al. 2016). According to the technical note for RNAlater (https://www.thermofisher.com/us/en/home/references/ambion-tech-support/rna-isolation/tech-notes/rna-remains-stable-during-long-term-tissue-storage.html), samples preserved in RNAlater and kept at -20°C or -80°C maintain RNA integrity "indefinitely". The mission of natural history museums is to maintain the research utility of samples for multiple decades or "in perpetuity"; however, there are very few studies that have examined samples over such periods of time, particularly for advanced genomic studies. One of the few longer-term studies did reveal that microdiversity of five-year old human stool samples could be identified by 16S rRNA sequencing (Tap et al. 2019). Based on the current evidence, we suggest that samples be archived in liquid nitrogen or in RNAlater to optimize the long-term molecular integrity of samples.

Loaning of genetic samples for research.—At the NSRL, the loan process is a four-step endeavor as follows: 1) evaluation and possible approval of the borrower for the loan, 2) consideration of the status of the samples, 3) physical preparation of the loan, and 4) shipping.

Step 1 – Loans of any tissue to qualified researchers must be approved by the appropriate individual responsible for the natural history collection, such as the curator of the genetic resources collection, depending on the collection administration hierarchy. The natural history collection's loan policy provides the basis for this approval process. A typical natural history museum's loan policy will include much of the same information as the NSRL's loan policy, available at https://www.depts.ttu.edu/nsrl/collections/loans.php. The first step of the loan process is to approve the credentials of the borrower. This generally requires a written statement (short proposal) in which the borrower states their methodology, experience, likelihood of success, prior successes, etc., all of which demonstrate a level of competence that can be used to justify a destructive loan. Second, we subjectively examine the philosophical contributions of the borrower to natural history collections. For example, are they solely a user and do they ultimately contribute to the depletion of this scientific research material without replacement, or are they actively working to increase these kinds of scientific holdings through their own collection and research activities? Considerable time, effort, and funding are devoted by researchers to obtain specimens (Bradley et al. 2012) and by natural history collections in curating and archiving specimens (Baker et al. 2014; Bradley et al. 2014). Solving this dilemma is relatively easy; in the NSRL's destructive loan application form, we have proposed four user criteria whereby reciprocation is an option. Option 1 - Deposit research material (specimens, tissues, etc.) deemed of equal scientific value in an accessible, preferably accredited, museum collection. Option 2 - Provide research material (of equal value) to the NSRL for material borrowed from the NSRL collections. These may include vouchered samples for permanent deposition in the NSRL or loans of tissues to our university researchers. Option 3 - Provide funding towards NSRL fieldwork to replace material associated with the Destructive Loan. Option 4 - For applicants who cannot fulfill one of the above options, they may be charged a replacement and service fee to help defray the cost of acquiring and maintaining the collection, as well as the handling, subsampling, and packaging of the loan. This fee is determined on a case-by-case basis (and based on the value of specimen replacement; see Bradley et al. 2012, 2014 and Baker et al. 2014) to be commensurate with the rarity of the specimen and the extent of the services provided. We encourage all researchers to include reimbursement costs for such Destructive Loan service fees when

they are preparing the budgets for their research grant proposals.

Finally, the history of the researcher in providing feedback information on the loan (i.e., researcher dependability in providing GenBank numbers, holotype designations, taxonomic revisions, publication copies, etc.) also is considered when evaluating a loan request. It is an unfortunate reality that this feedback rarely occurs easily, despite the efforts of curators, collection managers, or registrars to follow up and obtain these data. Studies resulting from borrowed tissues may be years in development before a tissue sample is determined to be associated with a new species, or that a new virus, bacterium, etc., is identified in a sample, and the results are published. Therefore, biorepositories often are not aware of the existence of symbiotypes or other type specimens and tissues in their care. This is a definite roadblock in properly identifying and archiving types within a collection and extending the appropriate level of protection.

Step 2 – Concerns when approving a loan for genetic resource samples can be different from traditional loans of voucher specimens. The primary consideration is that genetic resource loans (i.e., tissues) fall under Destructive Sampling governance. Even though we follow subsampling logic, this means that genetic samples from an individual eventually could be exhausted and, consequently, studies of that individual would be unrepeatable. Thus, before any loan is approved, the rarity of the requested material within the biorepository also should be considered. Subsampling, at the smallest quantity likely to give adequate results for the researcher, should be standard protocol for any requested tissue. In some cases, a request may be denied if a paucity of material is available and the justification provided by the requestor does not meet a collection's standards for loans of rare material; thus, this becomes a highly subjective process. In addition, there may be moratoria or other research agreements that preclude the loan of individual samples.

The NSRL's policy is not to loan material from threatened or endangered species or the last of a sample for a species in our collection, unless the justification is deemed appropriate and no other reasonable solution or compromise exists. For example, under an extreme justification, we might loan a subsample from a threatened or endangered species if it is only one of a handful of samples known to science and the research proposal of the borrower justifies the granting of the loan.

Step 3 – When preparing material for a loan, there are two considerations. One pertains to preserving the physical integrity of the tissue. The second is ensuring safety of museum personnel (see Investigator Safety, below). With respect to the sample, sterile procedures should be used for utensils and surface areas to avoid cross contamination, and efforts should be made to minimize thawing of frozen samples by subsampling over a freezer or dry ice, on a cryotable, or by other appropriate means.

Step 4 – There are several considerations for shipping of biological samples. Preserving the physical integrity of the tissue during shipping is a priority. The gold standard typically has been shipping on dry ice. At times this can be expensive to either the natural history collection or the borrower; it is strongly recommended that the payment of such costs be determined early in the loan process. Other methods, such as placing samples in 70% ethanol or dessicating onto filter paper, may suffice if dry ice cannot be used. A second consideration involves shipping of known virus-positive samples, which best practices require utilization of a cold-chain process (i.e., must be maintained at the appropriate chilled temperature throughout all stages of the shipping process). This can be quite expensive, particularly for international shipments, thus placing a priority on the question of who is responsible for payment; again, this should be determined during the loan approval process and prior to loan processing. In addition, shipping of known virus-positive samples requires precautions and biosafety protocols. Given that these circumstances may involve international and interstate regulations, and these can be quite complex and ephemeral, it is beyond the scope of this paper to discuss this topic in detail. However, it is recommended that the natural history collections and borrowers work together in advance to ensure that guidelines, standards, and laws governing such shipments are strictly followed. Finally, shipping of biological material may involve the presentation of various permits. These permits may include collecting, import, biohazard, etc., and may involve adherence to international, national, and state laws. Therefore, the loaning institution and borrower must be informed and follow such regulations.

Investigator safety.—All samples in a genetic resources collection should be treated as if they contain a pathogen or some zoonotic agent and are therefore a potential risk to museum personnel. Thus, a BSL-2 or higher designation for the collection may be required relative to facility and handling standards. Precautions in handling and subsampling all tissues to protect the handler should include: do not open tubes unless absolutely necessary; perform subsampling of tissues within a biosafety cabinet; use an appropriate chemical, such as RNase AWAY™, that will remove all contaminants (DNA, RNA, bacteria, viruses, etc.) to clean instruments; and use a disinfectant such as CaviCide 1™ (Metrex Research, Orange, California) to clean work surfaces. Staff members must be trained in all appropriate safety protocols and utilize appropriate personal protective equipment (PPE). Given the variance and continual updating of such protocols, we cannot effectively present all that might apply to a given investigator or pathogen; however, it is incumbent upon the researcher to follow institutional, local, state and federal regulations governing biosafety. Not all pathogens are treated equally (see Select Agent lists at https://www.selectagents.gov/). As mentioned previously, we encourage museum personnel and PIs to be familiar with and follow institutional, state, and federal guidelines set forth by those appropriate agencies and committees. For example, institutional Environmental Health and Safety committees (or equivalent) may be the best source for developing a workable biosafety plan.

Data Protocols

All data must be linked to an archived specimen.—Ideally, all tissue samples in an accredited collection should be associated with an archived voucher specimen. There are exceptions, of course, including tissues taken from live animals that are released (e.g., wing punches, toe clips). Even under these conditions, it is recommended that a voucher collection be made to document the ability of the researcher to identify the species of an animal that has been released (see DuBois and Nemesio 2007; but also see Garraffoni et al. 2019). The voucher specimen allows verification of the field identification and the correction of identification data if necessary, as well as the updating of data due to subsequent taxonomic changes. For example, in a typical mark-recapture study, the investigator says that they collected species A, B, and C. Science must rely on the ability of that investigator to correctly identify those species. If the researcher designates a small number of individuals (perhaps three to five) to be prepared as voucher specimens with tissues to represent their proposed boundaries for species A, B, and C, the identification of the taxa can be confirmed (or refuted and corrected, as necessary). These animals can be taken adjacent to the study area so as not to compromise the study, or they can be animals resulting from mortality during the study. In the case in which voucher specimens cannot be deposited into an accredited natural history collection, the researcher should provide a genetic profile (DNA sequence) for each sample that demonstrates its authenticity (Hebert and Gregory 2005; Baker and Bradley 2006). As a last resort, a high-resolution photograph, scanning electron microscopy image, or other scientifically-generated image may suffice for authenticity (see DuBois and Nemesio 2007 and Garraffoni et al. 2019 for a discussion). As such, we are not soliciting for phone images and camera-trap images in lieu of voucher specimens and samples; instead, we are referring to images that can be used to document diagnostic phenotypic characteristics, such as numbers of scales, hairs, coloration, etc., that are accepted as sufficient standards in the identification of some species.

Archived specimens, parts, tissues, and data from each individual in a biorepository should be linked with a common identifying number to allow for retrieval and matching of specimens, specimen parts, and data (see Fig. 2). If possible, a barcode system (Monk 1998) should be used for tracking and retrievability. By assigning a unique barcode number to each voucher specimen and each part or item related to that individual, including each vial of tissue, the "parts of the whole" can be tracked throughout the various locations in the facility during normal curatorial and archival activities, as well as during the loan process, and thus permanently identified without doubt as to the unique individual from which it was obtained.

Data fields.—Core data (see Fig. 1) collected in the field should include the unique identifying number for that individual (TK#, in the case of the NSRL), species (field-identified, but updated as appropriate if a revised identification is determined through morphological or genetic study or a taxonomic revi-

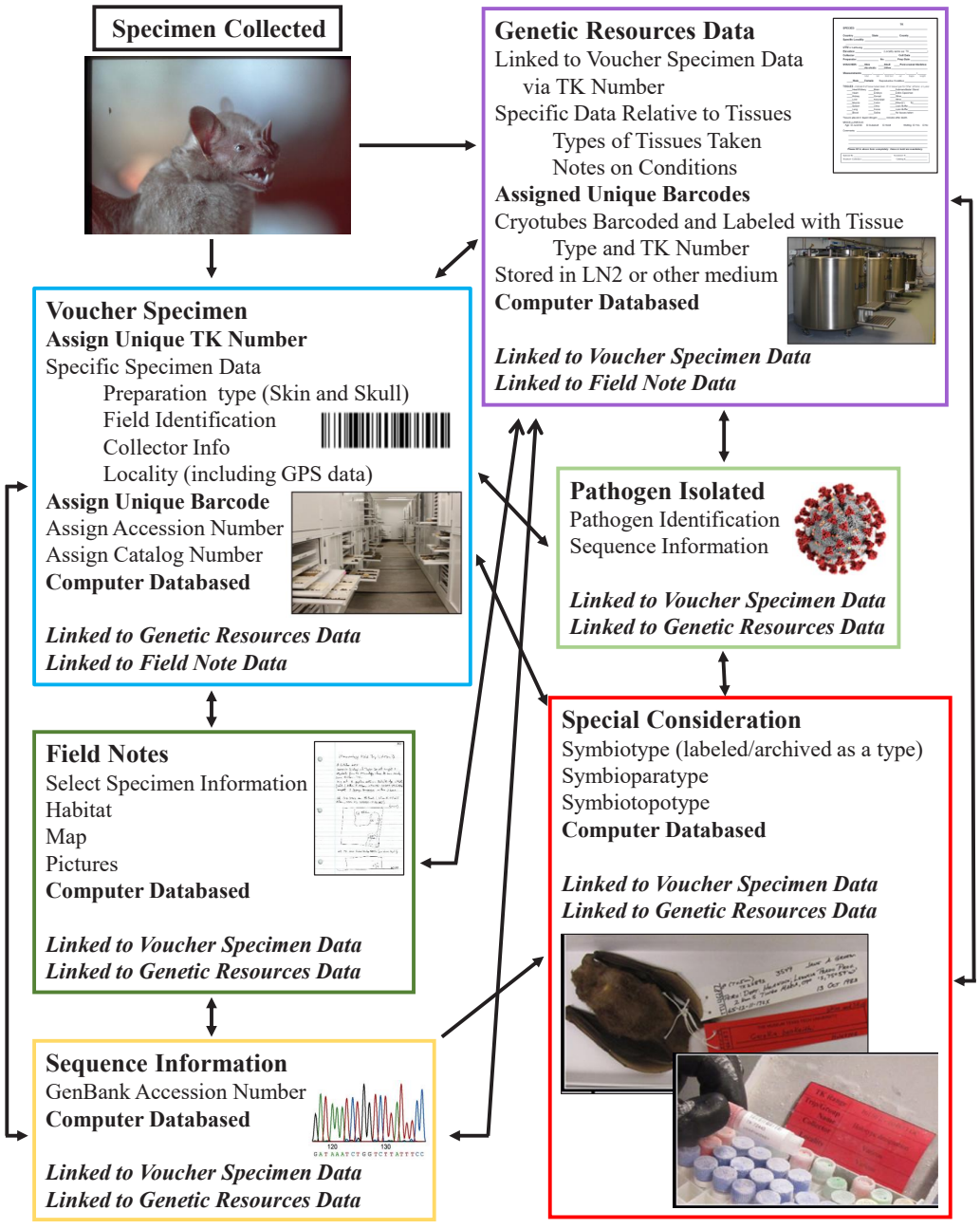

Figure 2. Flow diagram depicting the information pertaining to an individual voucher specimen at point of capture followed by inclusion of other biological information and samples retrieved from the specimen. The emphasis of this diagram pertains to integration and interconnectivity (see arrows) of information pertinent to the overall status of all data and samples obtained. This information includes: assignment of collectors number and a TK number and collection of materials used in genetic analyses; field notes pertaining to location and other conditions; proper curation of the original voucher specimen as well as other biological materials that are tracked by assignment of a unique barcode; inclusion of biologically relevant findings, such as sequences and pathogens identified; and treatment of symbiotypes associated with discovery of a pathogen. As illustrated by the boxes, each step requires inclusion of accurate, digitized information that can be tracked and updated. In this case, tracking is enhanced by assignment of a barcode, which can be used to obtain other information, including the location of biological samples and other types of data.

sion is made), detailed locality description (country, state, county, distance and direction to nearest town, GPS-derived UTM or lat/long coordinates, elevation), collection date, collector name, preparator name and preparation date, preparator number, type of voucher specimen, standard morphological measurements, sex, age, reproductive condition, tissue types collected, and time elapsed from death to placement of tissues in liquid nitrogen.

Specimen data should be entered into a standard online, public database; such data typically includes order, family, genus, species, subspecies, country, state, county, specific locality, elevation, latitude and longitude or UTM coordinates, collection date, collector name, preparation date, preparator name, preparator prep number, sex, age, standard measurements, type of specimen (skin and skull; skin, skull, skeleton; etc.), specimen parts (such as tissues), and type status (Fig. 2). Any ancillary data resulting from the specimen should be either provided at the time of accessioning and cataloging (by the natural history museum) or provided as an update if it results from later research efforts by borrowers. For example, if a DNA sequence is obtained and submitted to GenBank (see below), or if a sample is shown to possess a virus, bacterium, etc., that information should be provided to the natural history collection.

GenBank.—Sequences derived from archived tissues should be deposited in a publicly accessible genetic sequence database, such as GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/). Sequences should be linked to a unique number assigned by the biorepository that identifies the tissue sample and voucher specimen from which it was derived (e.g., the TK number assigned by the NSRL). This information should be entered in the GenBank field "Specimen Voucher" (current version of GenBank) or "Isolate" (older GenBank entries). In addition, researchers are strongly encouraged to update GenBank data, as new research results are obtained, and notify the biorepository of these updates, as appropriate. For example, if a species identification changes due to taxonomic updates or a catalog number has been assigned, the GenBank entry should be updated. Again, because researchers often do not notify natural history collections of new or updated GenBank entries related to their loans, registrars or collection managers should periodically perform searches of GenBank for newly accessioned data and publication references relating to their collections.

SUGGESTED COLLECTION AND ARCHIVAL PROTOCOLS FOR SYMBIOTYPES

After a virus, bacterium, etc. has been discovered, there are three major steps that should be followed with respect to the host type specimens. The first step involves a series of nomenclatural designations that serve to not only formally designate type host materials but to provide a unique level of protection. The second step involves procedures for archiving and storing symbiotype materials (see also Dunnum et al. 2017). Finally, the third step is a series of recommendations that natural history collections should follow relative to accessibility and loans of symbiotypes and other type materials.

Nomenclature and Other Procedural Protocols

Once a new virus, bacterium, endoparasite, etc., is identified and named following best taxonomic and nomenclatural practices (King et al. 2012; Janda 2016; Lefkowitz et al. 2018; Anusha Rai et al. 2019; International Committee on Taxonomy and Viruses 2018; and see https://talk.ictvonline.org/), a series of nomenclature designations, as defined below, should be attached to the host voucher specimen, tissues, and other archived materials as appropriate (Fig. 2). By assigning such nomenclatural designations, it affords the specimens and tissues special recognition and protection. Natural history collections should remove these voucher specimens and all associated archived body parts from general storage and circulation and follow systematic collection guidelines for treatment of type material (i.e., storage, labeling, etc.).

Symbiotype.—The term symbiotype (sym = shared) refers to both the tissue sample (or other body part) that was the source of the newly described virus, bacterium, etc., as well as the voucher specimen from which the tissue was obtained. The symbiotype functions in two ways. First, the voucher material

(traditional skin and skeleton) serves as the species designation for the species that is "hosting" the virus, bacterium, etc. (i.e., the type host). Second, the tissues or body parts serve as an indication of the source of the virus, bacterium, etc. Designation of as many body parts as appropriate may be crucial, especially if a virus is tissue-specific. For example, it might be critical to have designated a lung sample as the source of a respiratory virus.

Symbioparatype.—To our knowledge, the term symbioparatype does not exist in the literature. Herein, we propose that a symbioparatype should be defined as a voucher specimen and associated body parts that were collected as part of the original descriptive study and containing the same virus, bacterium, etc., as the symbiotype. It seems prudent to designate all of the material used in the initial description of a new virus, bacterium, etc., as symbioparatype material (there may be multiple symbioparatypes), following the International Code of Zoological Nomenclature for traditional paratypes (https://www.iczn.org/the-code/the-international-code-of-zoological-nomenclature/the-code-online/).

In some cases, a virus may be associated with hosts representing different species. This could lead, in certain circumstances, to a situation where a virus is isolated from individuals representing multiple host species. Whereas the symbiotype is associated with a single specimen regardless of its species designation, it would be allowable for symbioparatypes to be named from more than one host species, as long as they were part of the study in which the symbiotype was designated.

By designating one or more specimens as symbioparatypes, it allows natural history collections to: 1) either extend a level of protection to the specimens; or 2) designate a level of accessibility to researchers while protecting the symbiotype. See example under Loan section.

Symbiotopotype.—To our knowledge, the term symbiotopotype does not exist in the literature. However, it would seem prudent to designate any additional virus-positive specimens from the symbiotype locality as symbiotopotype material, following the International Code of Zoological Nomenclature for traditional topotype material.

In some cases, especially with viruses, it may be that a virus is associated with hosts of different species obtained from the same locality as the symbiotype. The symbiotype is associated with a specimen regardless of its species designation; therefore, it would be allowable for symbiotopotypes to be named based on more than one species, if they are part of the study in which the symbiotype was designated and if they originated from the same locality as the symbiotype.

As with symbioparatypes, by designating one or more specimens as symbiotopotypes, it allows natural history collections to 1) either extend a level of protection to the specimens, or 2) designate a level of accessibility to researchers while protecting the symbiotype. See example under Loan section.

Storage and Archival Procedures

Known symbiotype tissues should be isolated from the general collection and stored in designated symbiotype boxes. Vials should be identified with color-coded labels and/or the box should contain a color-coded box label. Color-coding makes explicit the special nature of the symbiotype, potentially enhancing care and protection over the long-term. For frozen tissues, symbiotype boxes should be stored in freezer racks designated and reserved specifically for type specimens. Symbiotype tissue samples should be noted as such (in the "type status" field) in the publicly available database of the biorepository. For each symbiotype tissue, the name of the virus, bacterium, etc., and the citation of the publication that named it also should be provided in the database.

Similarly, symbiotype vouchers should be isolated from the general collection and stored in a designated type cabinet. Such isolation provides an added level of care and protection over the long term. Symbiotype vouchers should be identified with color-coded tags and designated as such in the database of the biorepository as well as linked to the publication that established its type status. For added protection, it may be advisable to identify individuals in the database with a comment such as "do not loan".

Symbioparatype and symbiotopotype tissues and vouchers also should be designated by unique labeling and by referencing their type status in the biorepository database. However, whether it is appropriate to

physically remove these vouchers and tissues from general circulation and isolate them in type cabinets or freezer racks should be considered by each natural history collection.

If a researcher does not have these capabilities at their institution or agency and are unable to guarantee the archival-quality preservation and availability of specimens and tissues for future research, they should collaborate with an accredited natural history collection and deposit their samples at such an institution where the value of the specimens and tissues will be maintained for the long term.

Loans of Symbiotypes, Symbioparatypes, and Symbiotopotype Specimens

Consideration of who should be granted loans and how they are handled was discussed previously. Here, we are restricting our discussion only to loaning of symbiotypes, symbioparatypes, and symbiotopotypes.

Because viruses, bacteria, and some endoparasites are often associated with tissue samples and/or specimen body parts, they generally cannot be studied without destroying the sample, and thus symbiotype tissue samples or body parts should, ideally, never be loaned. Although symbiotype (host) voucher specimens can be visited at the holding institution and examined, only under an extremely rare situation should symbiotype tissue samples be subsampled and loaned. Under these rare circumstances, the research outcome should outweigh the irreversible damage to the sample (i.e., destructive loan). There are instances, however, where symbioparatypes and symbiotopotypes can be and perhaps should be accessible for loans; hence, our efforts to define symbioparatypes and symptopotypes, so they can be available to scientific research under justified circumstances. Below, we provide an example of how symbiotypes and other type material can be made available for research while offering a stratified level of protection.

In a study of North American arenaviruses, Cajimat et al. (2013) obtained an unknown arenavirus sequence from four specimens of *Neotoma micropus* collected from a locality near Flomot, Texas, as well as from seven individuals from a locality near Afton, Texas. Two of the 11 individuals (TK137081 and TK147378) were selected for more intense analyses and were instrumental in the designation of this unknown virus as the Middle Pease River Arenavirus. Given that TK137081 from Afton, Texas, appeared first in the Cajimat et al. (2013) paper, it serves as the symbiotype, having page priority over TK147378. Therefore, TK147378 and the remaining nine individuals obtained in that study should be designated as symbioparatypes. The six individuals from the Afton locality would be designated as symbiotopotypes as well as being symbioparatypes. By designating these 11 individuals as symbiotype, symbioparatype, and symbiotopotype specimens, the NSRL (repository for these specimens) can remove these individuals from general circulation and from being subjected to potential destructive loans for "ordinary" studies where any *Neotoma* tissue would suffice. If future arenavirus research dictates that an exemplar of the Middle Pease River Arenavirus is needed to establish the authenticity of that virus, the NSRL can designate select symbioparatypes as reference samples for the Middle Pease River Arenavirus without jeopardizing the symbiotype and symbiotopotype samples.

The recognition of symbioparatypes and symbiotopotypes may seem excessive, but official designation of this kind offers a level of formal recognition and by default extends a level of protection to archived specimens or, at minimum, provides natural history collections with a basis for decision-making. Further, it provides direction for the scientific community as to where the recognized exemplars are archived, and it allows researchers to request specific samples for authentication.

NSRL-Based Studies of Tissue Quality and Degradation

Many questions remain regarding best practices of tissue collection and preservation and their ramifications for quality into the future. In recent decades, -80°C storage has been the standard used for most

frozen tissue archives. A recent study (Soniat et al., in prep.) was conducted at the NSRL to determine the effects of long-term -80°C storage on DNA degradation. Tissue samples (muscle and liver) archived for 30, 20, 10, or one years were obtained from the Genetic Resources Collection. To control for the influence of body size variation and preparator experience, samples from cricetid rodents that had been prepped by a single individual were selected. The integrity of DNA extracted from samples was determined using an automated DNA isolation protocol followed by microfluidic DNA fragment size distribution measurement. Findings from this study indicate that the oldest samples were significantly more degraded than more recent samples, but more recent time points did not significantly differ from each other. Further, liver tissues displayed significantly lower quality of DNA in comparison to muscle tissues from the same time points. These results document for the first time trans-decadal degradation trends and indicate the long-term value of cryobanking at the coldest temperatures possible to mitigate degradation of biological samples of ever-increasing scientific value.

A follow-up study underway at the NSRL, being conducted by H. Amarilla-Stevens (in prep.), examines how quickly tissues need to be prepared and frozen, preferably in LN2, after death of an individual to minimize degradation of DNA. This parameter is poorly understood, and the rate of degradation with time elapsed since euthanasia to freezing has not been characterized. Indeed, DNA is quite stable and can be sequenced from samples even from subfossils (Orlando et al. 2008). Nonetheless, degraded DNA from such samples has sequence segments of short length that compromise utility for many types of analyses. Degradation leads to breakage of DNA strands and the more degraded, the fewer the number of base pairs in a segment. Many genetic analyses require long, intact nucleotide segments. Current best practices are to remove tissues as quickly as possible and to freeze them or place them in some sort of buffer immediately. Nonetheless, the veracity of these best practices is unclear. To examine effects of preparation time on DNA degradation, personnel at the NSRL prepared two series of tissues (muscle and liver) and placed them into LN2 after varying times postmortem: 2, 4, 8, 16, and 32 minutes, 1, 2, 4, 8, 16, and 24 hours, and 2, 4, 8, and 16 days. The integrity of DNA extracted from samples was determined using an automated DNA isolation protocol followed by microfluidic DNA fragment size distribution measurement. Analyses are ongoing, but it appears that muscle is more stable than liver across all time periods. For liver, there are substantive reductions in DNA integrity through time. Large decreases in the proportion of long segments (>35kb) occurs after 32 minutes postmortem, and at the longest time periods postmortem, intermediate (>20kb and <35kb) and long segments make up a very small proportion of preserved DNA. The exact characterization of the trajectory of decay is still under investigation.

Whereas the studies described above evaluated DNA degradation, a question of equal or greater importance relative to the preservation of symbiotypes, especially those containing RNA viruses, is the degradation rates of RNA associated with tissues. With respect to liquid nitrogen storage, we recommend as a best practice that tissues be immersed in LN2 within five minutes of death of the individual in order to preserve RNA. As in the DNA study, personnel from the NSRL (H. Amarilla-Stevens, in prep.) recently have initiated a study where they are preparing a series of tissues and placing them in LN2 after 2, 4, 8, 16, and 32 minutes, and 1-hour postmortem. These efforts will be followed by quantitative analyses to characterize the effects of degradation on RNA quality.

Historically, the dogma has been that vapor phase liquid nitrogen is the gold standard for preservation of nucleic acids. Although the study by Soniat et al. (in prep.) indicates that liquid nitrogen storage is superior to -80°C storage for DNA samples, there is some evidence that, for RNA preservation, liquid nitrogen storage is not a significant improvement over methods such as long-term storage at -80°C or the addition of RNA stabilizers such as RNAlater (e.g., see Mutter et al. 2004; Auer et al. 2014) . At the end of the day, it is necessary to determine whether storage reagents and other archival methods, such as those discussed herein, are a sound compromise for the storage of biological specimens, future research potential, and the safety of collection personnel and researchers. Consequently, there are several comparative studies that should be undertaken to determine the utility of various preservation and stabilization methods. For example, are there more effective preservatives or methods to deactivate viruses and bacteria and enhance the safety of the investigators for handling, shipping, and research? However, if these

methods negate any future studies of viruses, bacteria, etc., this may not be preferred. Similarly, if a method does retain the molecules for research, but only for a relatively short time period (i.e., degrades over time), the utility of those samples for examining any viruses, bacterium, etc., may be limited.

CONCLUDING STATEMENT

Genetic resource collections housed at natural history museums contain a wealth of biological information. Such collections are paramount to zoonotic and other human health related research (Tsangaras and Greenwood 2012; DiEuliis et al. 2017; Dunnum et al. 2017). These collections are maintained by professionals (curators and collection managers), who follow established procedures, protocols, and guidelines to maintain the safety, archivability, retrievability, and long-term care of such biological materials. It is our recommendation that procedures and protocols, such as those outlined in this document, as well as those from the traditional systematic collections community, serve as standards for future research as it relates to zoonotic disease outbreaks and human health. However, with respect to either short-term storage (e.g., in the field or shipping of samples) or the long-term storage of samples in genetic resource collections, we recognize the need for a better understanding of various archival and storage methods and their compatibility with future research and methods that have yet to be developed. It is unknown what method(s) may prove to be the new "gold standard" for natural history collections as they become increasingly important to the studies of zoonotic and infectious diseases.

As noted by DiEuliis et al. (2016), when an outbreak of a zoonoses occurs, questions about the primary host, type of agent, and location of the initial exposure to humans require answers. The chain of evidence begins with well-maintained information, and museums can provide that information. In the future, this means that collections and the appropriate public health agencies should collaborate in an effort to maintain a global database. Such organization would provide a database that could be accessed when the next emerging infectious disease arises.

In early 2020, in addition to a tragic loss of human lives, the COVID-19 virus brought the US economy, health infrastructure, and workforce to a standstill. Before any sense of normalcy returns, the US government will spend trillions (if not tens of trillions) of dollars to assist its citizens financially and to stimulate a struggling economy. All of this is because of a novel virus for which we had no apparent advanced warning (beyond a few months). The daunting fact is that the COVID-19 pandemic may not be unusual; certainly, it is not a one-off event. In fact, zoonotic experts believe that the World dodged potential pandemics with the MERs, SARS, Ebola, and other recent outbreaks. It is estimated that thousands, if not hundreds of thousands, of zoonoses remain unknown to science. Not all zoonoses are pathogenic to humans; however, it only takes one disease outbreak to generate chaos at a worldwide level. Although nobody can predict when the next pandemic will occur or which virus may be involved, it is certain that another pathogen will surface in the not too distant future. To protect humankind, we (the scientific and medical communities) need to have a documented and detailed catalog of the zoonoses that exist in wildlife reservoirs and an assessment of the potential risk of spillover into humans. It is certain that natural history collections, with their specimens and tissues, will play a major role in future zoonotic research. Consequently, the time is at hand, more urgently than ever, to support these collections and their research potential.

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Addresses of authors:

ROBERT D. BRADLEY

Department of Biological Sciences and the Museum
Texas Tech University
Lubbock, TX 79409-3131
robert.bradley@ttu.edu

LISA C. BRADLEY

The Museum
Texas Tech University
Lubbock, TX 79409-3191
lisa.bradley@ttu.edu

RODNEY L. HONEYCUTT

Pepperdine University
Natural Science Division
Malibu, CA 90263-4321
rodney.honeycutt@pepperdine.edu

KATHRYN A. MACDONALD

The Museum
Texas Tech University
Lubbock, TX 79409-3191
kathy.macdonald@ttu.edu

HEIDI N. AMARILLA-STEVENS

The Museum
Texas Tech University
Lubbock, TX 79409-3191
heidi.stevens@ttu.edu

RICHARD D. STEVENS

Department of Natural Resources Management
Texas Tech University
Lubbock, TX 79409-2125
richard.stevens@ttu.edu

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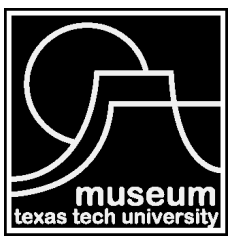

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