A New Quality Management Perspective for Biodiversity Conservation and Research: Investigating Biospecimen Reporting for Improved Study Quality (BRISQ) and the Standard PRE-analytical Code (SPREC) using Natural History Museum and Culture Collections as Case Studies

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Perspective

A New Quality Management Perspective for Biodiversity Conservation and Research: Investigating Biospecimen Reporting for Improved Study Quality (BRISQ) and the Standard PRE-analytical Code (SPREC) using Natural History Museum and Culture Collections as Case Studies

Abstract

The aims of this paper are to debate and raise awareness about the use of systematic, interconnected approaches for biodiversity collection curation by exploring the multi-disciplinary relevance of quality management tools developed by clinical biobanks. An appraisal of their best practices indicated the need for improved sample and process chain annotation as a significant number of historical collections used in medical research were of inadequate quality. This stimulated the creation of a new discipline, Biospecimen Science to develop quality management tools for clinical biobanks, two of which, Biospecimen Reporting for Improved Study Quality (BRISQ) and the Standard PRE-analytical Code (SPREC) report critical information about samples and process chain variables.

Unprecedented advances in molecular-genetic and *in silico* technologies applied across the tree of life require international conservation networks to generate and share knowledge. This is used in biodiversity and systematics research, and to address the accelerating loss of
species, including the sustainable use of bioresources. This review investigates the application of BRISQ and SPREC for biodiversity research and conservation using natural history, museum and living culture collections as case studies. The distinction between preservation and conservation is discussed with regard to process and storage treatments and how they impact on the usability of biospecimens and cultures. We conclude: (a) more rigorous approaches are needed for the quality management of biospecimens, bioresources and their associated sample and processing data to assure their fitness-for-purpose; (b) Biospecimen Science tools developed by clinical biobanks can be adapted to future-proof the quality of biodiversity collections and the reliability of molecular data generated from their use.

*Keywords*: biobanks, collections, genebanks, pre-analytical variables, quality management, standards

**Introduction**

The biodiversity sciences community comprises thematically diverse and geographically dispersed institutions and consortia, this presents the complicated task of continually evaluating how best to harmonize and validate methods to assure consistent and reliable preservation, conservation and research outcomes. Standardisation enables collections, museums, and Biological Resource Centres (BRCs) to share effectively and accurately knowledge about biodiversity specimens, bioresources and data, although in practice this is difficult to realize and remains aspirational in many cases (Gachon et al., 2013; Mackenzie-Dodds et al., 2013; Vogt, 2013). Nevertheless, there are tangible benefits in different biorepositories cooperating to develop Best Practices (BPs), quality standards and
guidelines (Benson et al., 2011a, 2011b, 2013; Field et al., 2008; Hanner & Gregory, 2007; ISBER, 2012; Mackenzie-Dodds et al., 2013). No one institution can answer the ‘big’ biodiversity conservation questions (Sutherland et al., 2009) by working alone, thus motivating the creation of international networks and research infrastructures that comprise different types of collections. Examples include: the Global Genome Biodiversity Network (GGBN), the Frozen Ark Project, Consortium for the Barcode of Life (CBOL), Synthesis of Systematic Resources (SYNTHESYS) and Scientific Collections International (SciColl) (Mackenzie-Dodds et al., 2013). Barriers to sharing biobanking information are well known across the clinical sector (Colledge et al., 2013) and the importance of molecular data standards is recognized in biodiversity research as molecular-genetic and in silico technologies become integrated with traditional conservation practices (Droege et al., 2014; Vogt, 2013). Detailed, consistent sample and process history annotation enables collaboration and is advantageous for individual institutions, especially those holding diverse collections of organisms used in systematics research that investigates evolutionary histories and environmental adaptations. Standardized formats report minimum information about genome sequence data (MIGS) and include environment, habitat and sample collection information (Field et al., 2008). There is a need for consistency in reporting environmental variables and molecular methods as noted in the metagenomics studies of Wooley et al. (2010), although documenting sample and process chain information within a formal quality management framework receives less consideration. Whilst comprehensive data sets are captured for genome sequence analysis they may be less complete for general acquisitions and pre-analytical variability can be poorly documented in legacy and archived collections. Standardization is problematic for collaborating networks comprising different types of biorepositories that hold diverse biospecimens and bioresources that have been exposed to different processes and storage regimes. This review addresses these challenging
issues by exploring quality management using two case studies representing opposite ends of the biodiversity collections spectrum.

(1) Dead collections preserved in natural history museums.
(2) Viable, replicable cultures maintained in ‘living’ collections.

**Figure 1** defines the spectrum of biodiversity collections in terms of three operational dimensions:

- **Dimension 1 Diversity**: from low, for single taxon culture collections, to multiple kingdoms representing biomes, ecosystems, environmental biobanks, natural history/museum collections.
- **Dimension 2 Functionality**: from abiotic/biotic to non-viable biospecimens, to cell derivatives or fully totipotent cells and replicable organisms.
- **Dimension 3 Time**: from deep (Millions of Years - MOY) geological time lines represented by natural history museum collections, to long-term ‘*ad infinitum*’ storage (decades – 100s years) of viable cells in cryobanks, medium-term storage for *in vitro* cultures (months - years); stabilization of labile samples in transit (hours - days).

These dimensions also relate to the use of biological collections in systematics research that focuses on the study of taxonomic diversification (Dimension 1) in the context of evolutionary time (Dimension 3). Molecular systematics research also provides the evidence base (Harding et al., 2013) for the conservation and curation of living resources (Dimension 2) in genebanks, culture collections and BRCs. Functional and temporal dimensions of biodiversity collections are represented by samples acquired for immediate study and
preserved in the longer-term for retrospective analyses to confirm future findings or to
establish Type and voucher specimens, reference strains and cultures (Gachon et al., 2013).

Natural history collections use time series in systematics, molecular ecology, and
conservation biology (Fig. 1). Habel et al. (2014) caution the problems of using degraded,
historic samples in biomarker analyses and finding specimens of suitable quality for
temporal studies. Future-proofing biodiversity samples thus becomes a quality assurance
exercise as they can be utilized in a different way from that anticipated at the time of
acquisition as technological advances generate new types of data from historical collections
(Allentoft et al., 2012; Austin & Melville, 2006; Holt et al., 2014; Staats et al., 2011; Welker
et al., 2015). Equivalent time series are represented by living (viable) active and base
genebanks and working and master culture collections which culture and maintain three
types of biorepository: (a) actively growing cell lines, (b) slow-grown cultures which have
reduced proliferation (termed medium-term storage) and (c) cryobanks which preserve
viable cells and organisms in the long-term at ultra-low (-196°C) temperatures in liquid
nitrogen (Benson 2008; Benson et al., 2011b, 2013).

A more overt approach to the quality management of different (Fig. 1) biological
collections is now required, particularly for users expecting high quality materials to be
supported by a comprehensive sample life history from acquisition to storage, including the
annotation of process chain variables. Pre-analytical variables can affect sensitive molecular
analyses that discriminate small differences in base sequences and they can influence
analytical outcomes. Whilst it is desirable to have biorepository standards for all relevant
sample data this is not always available or it is ambiguous and incomplete. Some
biorepository clients stipulate the formal accreditation of services and quality standards and
it is prudent to account for pre-analytical variability which has implications for the
commercial application of bioresources as demonstrated in algal chemo-diversity and
bioactivity studies (Stengel et al., 2011). Biodiversity researchers are more inclined to select higher quality samples that are metadata and specimen history rich because they are lower risk for molecular research (Fig. 2). Annotating sample and process chain history enables biodiversity collection curators to meet the existing requirements and future expectations of stakeholders and clients. This emphasises the value of Biospecimen Reporting for Improved Study Quality (BRISQ) and the Standard Pre-analytical Code (SPREC) as they report critical information about how samples are handled that may affect their quality, research reliability and fitness-for-purpose.

The science of collections: multiple quality management perspectives

Modern and historic collections are dependent upon the systematic reporting of critical information about samples and their acquisition, processing and storage. The need to improve biological collections through robust quality assurance (QA) and quality control (QC) was recognized by the clinical biobank community which launched Biospecimen Science as a new discipline dedicated to the ‘science of collections’. Clinical researchers apply this paradigm to raise awareness about biobank quality management and improve biospecimen quality (Riegman et al., 2008). This new discipline is now recognized by the wider biopreservation community because it addresses: (a) the pre-analytical variability attributed to the processes to which a sample is exposed during its collection, handling and storage; (b) the lack of accurate processing history and (c) encourages the use of evidence-based standards and BPs (Harding et al., 2013). Betsou et al. (2010) consider that a clinical biospecimen represents and contains implicit information about the ‘real world’ and that the more precise is the annotation and recording of processing variables during a sample's life history the more accurate and extensive will be the extraction of information when it is used. This ideology is pertinent to all biodiversity collections because it encapsulates two core
quality management principles: (1) the utility of biospecimens and bioresources depends not only on their intrinsic quality but also on the level and accuracy of the data associated with them and (2) accurate process chain reporting, annotation and records-keeping reduces the risks of ambiguity about sample quality.

**Biodiversity collections: integrating risk, regulatory and quality practices**

There are wider benefits in using quality management tools such as BRISQ and SPREC in biodiversity collections, as not only do they capture data generated by new technologies, they can support the preparation of regulatory specimen acquisition documents and help offset non-compliance risks (Stacey, 2004). The complexity of large-scale biodiversity projects requires risk and quality management provisions where biospecimens, bioresources and data are contributed across multiple disciplines spanning different sectors and national or international regulatory frameworks. Examples include, virus and parasitological research and the curation of collections involving inter-kingdom associations (symbioses, parasites, pathogens, pests, disease vectors, pollinators) that concern the safe and regulatory-compliant use and transfer of non-axenic and hazardous samples, particularly across international borders (Benson, 2008; Harding et al., 2013; Herniou et al., 1998, Williams, 2007).

Regulatory requirements range from collection permits to the various types of agreements that assure the safe deployment of biological materials, including the World Trade Organization Treaty and the Agreement on the Application of Sanitary and Phytosanitary Measures. New obligations, pertaining to the Convention on Biological Diversity (CBD) Nagoya Protocol (NP) on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (ABS) came into force in October 2014 (see Secretariat of the Convention on Biological Diversity, 2011). At the time of writing nearly 60 countries have ratified the NP and more are expected to do so over the
next few years. Countries Party to the Protocol and including all EU Member States (since the EU is already a Party) are likely to have laws and regulations governing monitoring compliance and requiring collection-holders and researchers using those collections to report on activities. The NP only covers countries which are Party to it however, compliance with and monitoring of any collection permit contract must be followed irrespective of the Protocol. Transparent contract management requires traceable sample use and agreed access benefit sharing and will largely comply with the NP. This will simplify the implementation of national and regional regulations, although care must be taken to satisfy local legal requirements (Lyal, 2014). Codes of Conduct, BPs and NP compliance tools are being developed by several consortia of museums, herbaria and other research bodies. Some countries, such as France are developing legislation for access that treats all specimens in their collections that were originally collected in France (or French Territories) as if they are newly acquired, meaning whenever they were collected they fall under the Protocol. Other countries, for example, some of those in the African Union, wish to assert sovereign rights over any specimen, wherever it is and whenever it was collected (Lyal, 2014). Currently, there is a risk of permit contract mis-management worldwide, which could be mitigated, at least in part, by the application of quality management tools that report relevant sample and pre-analytical information. How such data is managed and used within institutions or transferred to third parties demonstrates compliance and the systemization of sample process history in BRISQ and SPREC could help access buried data required to comply retrospectively or ‘in the spirit of CBD’.

Is Biospecimen Science relevant for biodiversity collections management and research?
The accelerating loss of biodiversity has stimulated the mining of previously untapped resources and specimens especially from museum specimens and Types where taxa are now
extinct, endangered or protected in the wild (Särkinen et al., 2012). Progressively more museum and herbaria collections are used as a contemporary resource to extract data from the past to manage biodiversity in the future (Hofreiter, 2008; Hoss et al., 1994; Nielsen & Bekkevold, 2012; Pääbo et al., 2004). More complete documentation of ancient samples and their process history (Figs. 2, 3 and 4) will be essential to confidently predict their suitability for successful molecular analyses, especially degraded samples and DNA extraction protocols which vary widely according to taxon, specimen age and preservation history (Dean & Ballard, 2001; Deagle et al., 2006; Habel et al., 2014; Mandrioli et al., 2006; Mitchell et al., 2005; Staats et al., 2011, 2013). The EU’s SYNTHESYS projects were created to improve access to collections and reduce the barriers to molecular research access to collections by optimising taxon-specific DNA extraction methods from difficult materials such as polyphenol-rich plants and invertebrates that contain high levels of polysaccharides.

For living collections, the in situ, ex situ and in vitro conservation of biodiversity is represented by different functional levels: (a) niches, habitats, ecosystems, biomes; (b) species and species assemblages; (c) totipotent germplasm; (d) culturable and non-culturable organisms and cells; (d) viable and non-viable organisms and cells; (e) DNA, replicable and non-replicable genetic resources; (f) non-viable biospecimens derived from cells, tissues and organs; (g) cellular and sub-cellular analytes. Conservation research linked to systematics informs population genetics studies in situ and supports evidence based decisions as to what should be conserved ex situ in genebanks and culture collections (Harding et al., 2013).

Clinical and non-clinical biorepositories have cooperated to generate BPs and guidelines to improve the quality of their collections and there is a strong, united consensus across biobanking communities for standardization (Benson et al., 2011a, 2011b, 2013; Harding et al., 2013; ISBER, 2012; Nussbeck et al., 2013, 2016; Mackenzie-Dodds et al., 2013). The need for interdisciplinary cooperation about data standards and data management...
was debated at an interactive session of the European, African and Middle Eastern Society for Biopreservation and Biobanking (ESBB) conference. The participants proposed the following action steps: raise awareness about data management across different biobank sectors, develop and deliver training workshops, improve data standards that use common vocabularies (Nussbeck et al., 2016).

As Biospecimen Science focuses on identifying, annotating and where feasible, controlling pre-analytical variability it has relevance for biodiversity preserved and conserved in all types of collections, especially: (a) sensitive and labile samples, (b) storage recalcitrant viable germplasm, (c) one-off/limited samples from high risk/rare species, (d) opportunistic sampling when sub-optimal practices may be unavoidable, (e) acquisitions compromised by difficult, complicated and remote sampling logistics, (f) large-scale collaborative infrastructures projects/networks, (g) harmonizing BPs across federated institutions for which reporting pre-analytical variables is desirable and (h) future-proofing collections. The relevance of Biospecimen Science tools for biodiversity collections quality management can be exemplified by the application of Next Generation Sequencing (NGS). This is now used to study historical samples (Fig. 2) as pre-analytical variables can interfere with other types of analyses where museum collections have been preserved using traditional methods. There is an increased awareness about the importance of developing minimum information checklists and data standards in the life sciences sector (Vogt, 2013). This motivates the application of BRISQ and SPREC as quality management tools as improved systemization helps to assure biodiversity samples are fit-for-purpose and remain so in the long-term. However, collection managers will have to consider the additional resources needed to implement BRISQ and SPREC which may be viewed as unnecessary burdens that lack sufficient relevance and flexibility to meet the needs of diverse taxa and sample types. On the other hand, the risks of inadequate data reporting may be revealed
downstream should sample quality become compromised by a lack of information and insufficient detail about the critical pre-analytical variables to which they have been exposed. These fears can be allayed in part by proof-of-concept evidence from the other biobanking sectors that use BRISQ and SPREC routinely (Moore et al., 2011; Nussbeck et al., 2013). Their experiences affirm that different data may be accommodated to ensure tangible benefits arise from investing in their implementation. For these reasons it is timely to explore the use of BRISQ and SPREC in biodiversity collections quality management, particularly as their clients have increasing expectations as new sophisticated downstream analyses come on line that are affected by pre-analytical variability.

**Biospecimen reporting for improved study quality (BRISQ)**

The importance of minimum information checklists in biodiversity and systematics research has been discussed by Vogt (2013), the goal being to create robust data standards for eScience that use general reporting structures to convey details about a specific type of data. Similarly, the requirement for comprehensive process chain annotation was first acknowledged by clinical biobanks because a significant number of historical biospecimens were of inadequate quality and analytical results derived from sub-optimally processed samples put at risk data interpretation (Betsou et al., 2010; Nussbeck et al., 2013; Ransohoff and Gourlay, 2010). The purpose of BRISQ is to improve the quality of research generated through the use of biospecimens by presenting in publications and documentation a systematic, standard way of reporting critical information about samples and how they are handled and stored (Moore et al., 2011). The scope of the clinical BRISQ informs about the biospecimen and the variables to which it is exposed. Other associated information related to regulations, permissions, administrative identifiers is not generally considered in BRISQ as this is recorded elsewhere in Laboratory Information Management Systems (LIMS) and
CMS (Collection Management Systems). To avoid the input of inappropriate items a biodiversity BRISQ would need to qualify up front the range and type of data incorporated. For example, the clinical BRISQ is confined to primary samples and does not include cell lines, derivatives, DNA or sub-cellular analytes. The journal Biopreservation and Biobanking recommends BRISQ summary reports to be incorporated in clinical manuscripts and the Nature Publishing Group announced the need to reduce irreproducibility in papers by reporting technical details more fully (Nature Editorial, 2013; Nature Publishing Group, 2013) and mentions BRISQ in their guidelines (Moore et al., 2011). The incorporation of a BRISQ in research outputs is becoming routine in clinical biobanking on the basis that the interpretation, comparison and reproduction of results needs to be improved (Simeon-Dubach & Moore, 2014; Simeon-Dubach & Perren, 2011). The National Cancer Institute recommend the inclusion of BRISQ in their evidence-based BPs (Engel et al., 2014).

**Constructing a prototype BRISQ for biodiversity collections**

As far as the authors are aware BRISQ has not to date been routinely used in biodiversity collections although provision for adopting BRISQ has been made in the GGBN data standard specification (G. Droege personal communication) and the need for comprehensive checklists has been proposed for eSciences data standards in the life sciences (Vogt, 2013). A biodiversity BRISQ will need to: (a) be sufficiently broad to encompass the applications of multiple types of stored biological materials; (b) contain generic elements to allow flexible reporting of a variety and often integrated conservation strategies, and (c) accommodate diverse taxa. It is envisaged that a more robust approach to reporting will enhance the quality, value and utility of biodiversity collections, now and in the future. As described by Moore et al. (2011) BRISQ construction involves several steps:
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1. Mapping a systematic biospecimen process chain to calibrate BRISQ reporting elements (also termed ‘items’) in a logical, step-wise sequence.

2. Compiling sample information (e.g. developmental, physiological and disease status).

3. Creating a quick reference (check list) of critical elements to produce a basic BRISQ report.

4. Creating a comprehensive list that describes reporting items in detail and prioritizes them in a three-tiered (check list) report according to their relative importance:

- **Tier 1** critical items recommended for reporting;
- **Tier 2** items beneficial to report, but are less crucial than those in Tier 1;
- **Tier 3** additional items that provide information about parameters and conditions that may be useful, but it is not known if they influence results outcomes, including parameter information that is not always available.

Tier 1 items are recommended for reporting in research publications, Standard Operating Procedures (SOPs) and BPs; they include critical information about: (a) biospecimen or sample pre-acquisition status (see Tables 1 and 2) and (b) processing details which can be generated from validated SOPs. Tier 2 and Tier 3 data provide more detail in appendices or as supplementary online information. The clinical BRISQ developed by Moore et al. (2011) has been adapted to investigate its application for the preservation and *ex situ* conservation of biospecimens and biological resources as demonstrated in the following steps which explain how to construct a prototype ‘biodiversity BRISQ’ using natural history and culture collections as exemplars.

*Step 1 Process chain systemization*
Constructing comprehensive process chains (Figs. 3 and 4) is the first step towards identifying the critical information used to populate BRISQ templates. As described by Moore et al. (2011) BRISQ comprises five reporting elements each corresponding to a stage of the clinical biospecimen process chain: (I) pre-acquisition, (II) acquisition, (III) stabilization and preservation, (IV) storage and transport and (V) QA that are relevant to extracted products before analyte extraction and evaluation. The authors have modified the clinical BRISQ for biodiversity collections and included additional elements to accommodate: (a) the complexity of biodiversity collection acquisitions and difficult sampling logistics; (b) the flexibility required to report multiple stabilization treatments, intermediate transfers and storage regimes and (c) the diverse types of samples, collections and processing combinations (Figs. 3 and 4).

Step 2 Creating a quick reference biodiversity BRISQ checklist

The involves creating a quick reference check list of Tier 1 BRISQ elements. The biodiversity example (Table 1) adapted from the clinical format (Moore et al., 2011) is not comprehensive, rather it illustrates a range of Tier 1 elements using different types of collections, samples and applications as examples; this basic template can be adapted to meet the needs of individual collections or research consortia. Using Table 1 to demonstrate this, sample acquisition data is often missing or incomplete, in which case BRISQ records all available Tier 1 information regarding stabilization and shipping parameters pertinent for labile samples collected from remote field sites. Another example, concerns axenicity which would be designated as Tier 1 for biospecimens used in genomic and taxonomic research as non-axenic samples compromise sensitive molecular analyses. Similarly, rewarming protocol would be Tier 1 for cryopreserved cultures because it is a vital step for recovering viable cells after cryostorage, particularly when this takes place many years after the
bioresource was originally cryopreserved. These selected examples show how BRISQ
records and retains critical process information that may otherwise be overlooked or lost
over time or by changes in biorepository personnel.

Step 3 Creating a comprehensive BRISQ template

Each check list element (Table 1) is expanded to produce a comprehensive BRISQ template
which collates all relevant sample data and as appropriate the related information associated
with each element at each stage of the sample process chain (Table 2). Thus, elements I-VII
correspond to processing steps (Figs. 3 and 4) that are further delineated into
alphanumerically coded ‘items’ as described by Moore et al. (2011). These items provide
more details about samples and the processing options (e.g. preservation or conservation)
for different types of collections (e.g. dead or living) and sample types (e.g. cells, tissues,
organs, organisms). For instance (see Table 2) the BRISQ code for I.p (toxicology status)
reports information about sample toxicology which would become a Tier 1 element for
biospecimens used in pollution monitoring or as biopsies for wildlife crime analyses
investigating cases of poisoning. The standardization of reporting is consistent with good
quality management and creating comprehensive BRISQ templates (Table 2) allows
curators to select those reporting options that reveal the most critical information that could
affect sample quality and usability. Once recorded this data can be used at a later time to
help decide on the suitability of a sample for a particular analysis, application or project.

The level of detail reported for BRISQ elements depends upon the scope and criticality
of information and care should be taken to avoid unnecessary duplication across other data
fields and the production of overly complex reports. Associated data related to accession
details, collectors names, permits and regulations (Table 2 provenance section I.f.1; history
of ownership) would usually be cross-referenced with administrative history recorded in the
curatorial fields of existing CMS or LIMS. In contrast, the annotation of provenance data
that describes the habitat from which a sample was taken (Table 2, provenance section I.f.2, place of origin) might be included in a BRISQ because it provides a record of the conditions and variables to which a sample has been exposed that could impact on its quality and usability. These various examples demonstrate how the level of detail reported in a template can be refined and where extra information may be needed as indicated by Moore et al. (2011). It is not possible in this review to include all possible sub-level options for all BRISQ elements, therefore living collections elements IV-VII (Table 2) will need to be expanded to provide critical information about in vitro culture, storage and cold chain management. Similarly, QA/QC measures (element VII, Table 2) will encompass sub-level reporting to assure the fitness-for-purpose of specific samples and collections.

**Step 4 Compiling a prioritized BRISQ report**

Finally, BRISQ information is ranked according to reporting priority (Tiers 1-3) for each type of collection, biospecimen or bioresource; ranking is based on the criticality of sample and process chain information on the quality of the sample, its usability and future use. Only the most relevant information is recorded and to avoid creating over-complicated reports decisions are rationalized as to which elements to exclude and include. This varies according to sample type, process chain complexity and the relevance of each Tier for satisfying performance indicators (Figs. 1 and 2). Although not all BRISQ items will need to be included it is recommended to future-proof each tier for biodiversity samples that are expected to be used for molecular analyses at a future time, especially those involving collaborative projects.

How to generate a BRISQ prioritization report is demonstrated as follows using the Vietnamese endangered land snail (*Camaena* sp.) derived from the molecular collection of the Natural History Museum, London as a case study for ranking elements as Tiers 1, 2 or
1. Items selected from Table 2 can be further sub-divided in prioritized reports; using *Camaena* sp samples as an example this can include provenance (Table 2, I.f.2) which is further itemized as place of origin I.f.2.i (Tier 1); substrate/habitat I.f.2.ii (Tier 2) and altitude/depth I.f.2.iii (Tier 3).

**PRIORITIZED BIODIVERSITY BRISQ EXAMPLE**

**Vietnamese Endangered Land Snail (*Camaena* Sp.)**

**TIER 1 ELEMENTS RECOMMENDED TO REPORT**

**I. PRE-ACQUISITION**

- ✓ I.c Selection criteria (quality): high quality specimens: authentic, stable and pure.
- ✓ I.d Collection modality: preservation: frozen tissue sub-samples.
- ✓ I.e Collection category: animal tissue: foot muscle subsamples and whole specimens
- ✓ I.f.2.i Provenance: CucPhong National Park, N Thailand, (+ GPS data, withheld for species protection purposes) September 2013, 11:00 AM
- ✓ I.h Taxonomy: Camaena sp.
- ✓ I.i Biological donor: fresh specimen

**II ACQUISITION, STABILISATION, TRANSPORT**

- ✓ II.b Time from collection to stabilisation: specimens viable for up to 24 hours during transfer to labs >100km from field collection site.
- ✓ II.c Stabilisation: foot muscle tissue excised from fresh (humanely euthanized) specimens and immediately placed in -80°C freezer.
II.d Shipping parameters stage 1: frozen samples shipped from Hanoi to London on dry ice (~28 hours).

II.e Interim storage: -80°C freezer over the weekend before accessioning to cryo-facility.

III PRESERVATION, FIXATION, STORAGE

III.a Preparation: as above for II.c (stabilization) and II.d (shipping).

III.f Long-term storage: LN vapour phase (-196°C).

VI DISPATCH, TRANSPORT, COLD CHAIN

VI.a Frozen tissues in cryovials, shipped in dry shipper (<-150°C) to end user.

VII QUALITY ASSURANCE AND QUALITY CONTROL MEASURES

VII.a Frozen sample thermal history, freeze/thaw records; DNA/RNA quality/quantity audits.

TIER 2 ELEMENTS BENEFICIAL TO REPORT

I. PRE-ACQUISITION

I.f.2.ii Substrate/habitat: limestone rocks in tropical forest.

I.i Biological donor is an individual specimen.

I.k Vital state: fresh subsample from live, healthy specimen.

I.m Gender: hermaphrodite.

I.q Axenicity: sample free from other organisms.

I.r Disease and pathology status: no known parasites and/or disease present.

➢ Risk note: this species is known to carry parasitic nematode Angiostrongylus cantonensis which can cause eosinophilic meningitis in SE Asia.

II. ACQUISITION, STABILISATION, TRANSPORT
II.a Collection and sample container: hand collected in the field, live specimens placed in damp cloth bag, opening secured with drawstring. Specimens undamaged as they retreat inside shells during transportation in field.

VII QUALITY ASSURANCE AND QUALITY CONTROL MEASURES

VII.a Molecular/sequence data, Key Performance Indicators (KPIs), DQIs (Data Quality Indicators).

TIER 3: ELEMENTS ADDITIONAL TO REPORT

I. PRE-ACQUISITION

I.a Type of institution: new acquisition for UK University/NHM, London, project.

I.b Selection criteria (sample): ex-situ conservation, genetic resources, at risk species management with a partner conservation organization.

I.f.2.iii Altitude/depth (available from GPS data).

I.g Timescale: present day for an extant species.

I.n Life cycle/reproductive state: mature specimen.

I.o Health and nutritional status, healthy/well fed as observed by the collector.

I.p Toxicology status: no known exposure to xenobiotics.

VII QUALITY ASSURANCE AND QUALITY CONTROL MEASURES

VII.a Voucher specimen metadata: KPIs and DQIs.

Using the original clinical BRISQ format (Moore et al., 2011) the above report was generated for biospecimens of the Vietnamese endangered land snail with a view to testing and adapting the template for other types of biodiversity samples and collections.
**Defining significant BRISQ elements: preservation versus conservation**

The comprehensive BRISQ template (Table 2) reveals the potential significance of different processing treatments on sample quality as new molecular-genetic, epigenetic, omics and metagenomics technologies come on line. In contrast to the clinical BRISQ the authors decided to introduce more element options to accommodate the multiple processes used by different biodiversity collections. Preservation and conservation were designated as distinct BRISQ options, although they seem equivalent their ideological and technical differences have implications for future-proofing biospecimen quality and viable collection functionality (Fig.1; Tables 1-3).

In *sensu stricto*, preservation maintains the original state by protecting against change, decay or deterioration. Natural history collections use preservation treatments to ensure that a sample is ‘fixed’ in its original state at the point of collection (e.g. for Type, voucher, herbarium, reference specimens). Traditional natural history collections comprising non-viable biospecimens (e.g. universities, zoos, botanical gardens, genebanks, culture collections) preserve a variety of voucher specimens from taxa across the Tree of Life. These were initially preserved to maintain their original physical condition or morphological state which formed the basis of their analysis. A mixture of preservatives and treatments were used, many of them toxic to operators, or deleterious to DNA and other cellular contents (Staats et al., 2011). Preservatives used to eliminate and control pests (e.g. antimicrobial mercuric chloride in methylated spirits sprayed onto herbarium specimens; arsenic trioxide in soap solutions applied to furs, skins and feathers; insecticides, including naphthalene ‘moth balls’ in entomology for dry store collections) are now banned for health and safety reasons and hazardous or controlled substances have been replaced by safer physical treatments. Dehydration and chemical fixation alter the molecular structure of cells (e.g. formalin, glutaraldehyde cross links protein and DNA). They have been used by many
collections although museums now take measures to preserve cell contents and take sub-samples from fresh specimens in the field for molecular analyses. Preservation protocols vary for each taxon and sample type and are widely used across the process chain for collection, euthanizing, tissue sub-sampling, chemical fixation (treatments, preservatives), snap freezing, cold chain transfers and long-term storage (Corthals & Desalle, 2005; Dean & Ballard, 2001; Lorenz et al., 2005; Särkinen et al., 2012).

In contrast to preservation in natural history collections, conservation is usually associated with the sustainable use of ‘living’ biological and genetic resources (Figs. 1 and 2; Table 2). The ex situ conservation of biodiversity in culture collections and genebanks has three main purposes: (a) to underpin the sustainable management and utilization of totipotent biological and genetic resources to the benefit of humanity; (b) protecting biodiversity at risk in its native habitat and (c) supporting in situ conservation measures such as species introductions/re-introductions, captive breeding and restoration programmes.

Conserving biological resources in culture collections and genebanks necessitates their higher-order functionality (viability, replicability and totipotency) to be maintained and implicitly these cannot be ‘fixed’ as expressing developmental competencies involves a change from the original state at the point of acquisition. Possible exceptions are master cell lines of culturable organisms or germplasm cryopreserved for the long-term in base (equivalent to master cell lines) genebanks (Benson, 2008; Stacey, 2004). However viable cells and organisms are not fixed in their original physical condition as is assumed for reference, voucher and Type specimens as protective treatments such as acclimation and cryoprotection necessarily alter the morphogenetic, physiological and biomolecular state to enable successful post-storage recovery (Benson, 2008).

Exemplar: the potential use of BRISQ in future proofing natural history collections

URL: http://mc.manuscriptcentral.com/tsab
The potential application of BRISQ for biodiversity collection quality management is considered in this review using natural history museums as the primary exemplar, although natural history specimens can be held by institutions (e.g. universities, zoos, botanical gardens, libraries) other than museums. The perception of natural history museum collections as static exhibits is outdated, most are, or strive to become active repositories that comprise traditional collections with either new material being added or new information being generated from existing specimens. Curatorial identifications and storage locations are updated to increase the accuracy of collection data and new information is generated as new technologies (e.g. analytical biochemistry, DNA sequencing, 3D imaging) come online. It is essential to establish robust reporting procedures that connect the flow of data between the source material in the collection to downstream users. This can be facilitated by the generation of retrospective BRISQ priority reports and using them as QA tools to make evidence-based decisions about the suitability of traditionally preserved samples for modern analyses. Such an approach can be linked to other tools such as PrediCtoR™, (now thermal-age.eu) a web-hosted software tool (http://www.synthesys.info/joint-research-activities/synthesys-2-ijras/jra-1-predictor-software-tool/) that predicts the probability of successful ancient DNA being recovered from fossilised and non-fossilised bone in museum collections to avoid unnecessary destructive sampling (Smith et al., 2003). This decision-making software tool, developed within SYNTHESYS (European Union-funded Joint Research Activities) encourages dialogue between collection curators and researchers and helps quantify risks associated with the destructive analysis of specimens (Allentoft et al., 2012; Welker et al., 2015).

Museums, natural history collections and herbaria are continually developing new molecular technologies and bioinformatics tools to unlock the potential of ancient samples in new, diverse ways (see Besnard et al., 2014). Analysis of historical DNA in population
genetics and phylo-geographic research presents new opportunities to research evolutionary patterns over different temporal scales (Fig. 2); Navascués et al. (2000) refer to this as ‘heterochrony’. Although biodiversity samples are compromised by the degradation of DNA, RNA and metabolites (Colotte et al., 2009; Deagle et al., 2006; Särkinen et al., 2012) modern molecular biology tools such as Whole Genome Amplification (WGA) and NGS provide opportunities to generate deep coverage and whole genome quality data from previously unusable or degraded sample (Staats et al., 2013). Navascués et al. (2010) defines ancient DNA as that recovered from non-ideal biological materials derived from a host organism that is no longer alive, the samples (fossils, bones, teeth, museum/herbarium tissues) of which were preserved in a way that is suboptimal for DNA analysis (Fig. 2). According to Deagle et al. (2006) low quality biospecimens reduce the amount and quality of that which can be extracted and limit the scope of present and future research; in this context BRISQ has a potential use in the ‘future-proofing’ of historical collections.

The Standard Pre-analytical Code (SPREC)

Clinical biobanks report that biospecimen quality is modified by pre-analytical variables attributed to acquisition, handling and storage (Caboux et al., 2012; Lehman et al., 2012), extrapolating to biodiversity collections pre-analytical variables could potentially:

- reduce sample resilience to biopreservation treatments
- compromise the recovery of viable cells, tissues and organs after storage
- impair post-storage biomolecular analyses
- interfere with stored sample analyses and data interpretation
- compromise long-term, post-storage viability, totipotency, fitness-for-purpose.
Variability in handling compromises the post-storage recovery of viable biological resources and can affect sensitive downstream molecular analyses for which high quality samples are required (Benson, 2008, 2013; Colotte et al., 2009; Deagle et al., 2006; Harding et al., 2013; Särkinen et al., 2012). SPREC was devised by clinical biobankers to identify for stakeholders and end users the most critical pre-analytical variables associated with a biological sample (Fig. 5). To be included in a SPREC a variable is required to satisfy certain criteria: (1) it is known or suspected to impact the results of downstream analyses; (2) it is within the control of the biobank biorepository or collection and (3) it can be anticipated and standardized in SOPs. The most significant pre-analytical variables associated with a biospecimen or bioresource are reported using a standardized 7-element-long code (Benson et al., 2011a; Betsou et al., 2010; Lehman, et al., 2012). Each element is allocated a descriptor (e.g. terminal storage temperature) and its various options (e.g. -20°C, -80°C, -196°C) are identified as a short sequence of 2-3 letters which are the abbreviations for each possible pre-analytical variable for that element. If the option is unknown or inconsistent ‘X’ is used; if the option is known but does not correspond to a standard option ‘Z’ is used. Nussbeck et al. (2013) describe three methods for creating a SPREC:

1. Each element is selected from a list of coded items applied at the time of sample processing.
2. A default SPREC is created using existing SOPs, changes are only made where there are deviations from the SOP.
3. Codes are devised by automatic generation at time stamps (e.g. point in time at which data/biospecimen is added to the collection inventory/database) of a SPREC using a software tool that automatically calculates respective elements when data is entered.
SPRECalc is downloadable from the International Society for Biological and Environmental Repositories website (www.isber.org) and SPRECware are freely available (Lehmann et al., 2012; Nanni et al., 2012) for generating clinical SPREC data. Once created a SPREC can be updated in line with continuing improvements supported by evidence-based QC tools (Betsou et al., 2013). Biodiversity SPRECs can be produced using simple hand-written codes (Benson et al., 2011a; Harding et al., 2013) or alternatively software tools can be created that automatically generate computerized SPREC barcodes (Lehman et al., 2012; Nanni et al., 2011, 2012; Nussbeck et al., 2013). Once produced in either electronic or hand written formats the codes are permanently assigned to that specimen and all its derivatives and incorporated into quality management documents (Fig. 5). Dedicated SPREC software tools have the advantage of reducing errors generated by hand written records, they enhance the efficiency of data input and enable interoperability across institutions and consortia by facilitating the accurate, standardized transfer of information across (Nanni et al., 2012). It is possible to extract SPREC data and generate a code electronically by customizing existing LIMS and CMS and LIMS manufacturers now include SPRECs in their products (Fig. 5).

The SPREC has the potential to improve sample quality in several ways: (a) drawing attention to the impact of pre-analytical variables on sample fitness-for-purpose and informing practical measures, e.g. optimizing preparative centrifugation steps, to improve process manipulations, (b) assuring data, outcomes and results by providing documented evidence that a sample has been optimally processed and (c) providing a sample-specific record of deviations and departures from SOPs. An external QA survey tool developed by ISBER’s Biospecimen Science Working Group assesses biorepository pre-analytical traceability and procedures (BSWG, 2014) and Kristensen et al. (2013) describe how to self-
assess the pre-analytical phase and advise biobanks use surveys for managing pre-analytical variability.

(1) Exemplar: constructing a prototype SPREC for living collections

The information recorded in a SPREC is distinct from that usually assigned to biodiversity collections because it specifically concerns the translocation of technical processing details into systematic QA/QC records (Benson et al., 2011a; Betsou et al., 2010). A SPREC created for the collection, isolation, initiation and culture of viable algae is demonstrated below using the bold-highlighted codes (see Table 3; Fig. 5) to identify the appropriate option in sequence from each of 7 SPREC elements:

1. Sample type = plankton = B
2. Collecting method = plankton net = A
3. Sample container = polyethylene bottle = C
4. In transit stabilization = chilled = B
5. Transit time = 2 days = A
6. Isolation and initiation = droplet-liquid = DAL
7. Cultivation = non-axenic Erlenmeyer flask = NXB

The simple code is configured as a 7-element string of letters (e.g. B-A-C-B-A-DAL-NXB) which thereafter is attributed to the primary culture and all its derived samples, analytes, subcultures and DNA, throughout the lifetime of the sample and derivative(s). The example demonstrated is designated as SPREC A-01 (Fig. 5). The complete algal culture collection code is described by Benson et al. (2011a) which includes SPREC A-02 that annotates the procedures for algal cryopreservation in a 7-element code: (1) culture axenicity status, (2)
pregrowth/pretreatments, (3) cryoprotection, (4) cooling, (5) cryostorage, (6) rewarming and (7) post-cryostorage recovery. A prototype SPREC has been created for in vitro plant genebanks using tropical forest seeds and their derived in vitro cultures (Harding et al., 2013).

(2) **Significance of pre-analytical variability for biodiversity collections**

Once the SPREC of a biodiversity sample becomes ‘attached’ as either a text (hand written label) or digital barcode to its curatorial ID (e.g. strain, or accession number) it is possible to reveal details of the variables to which it has been exposed at a later stage by decoding the data (Fig. 5). One advantage of the simple 7-element long SPREC code is that it can be used by biorepositories and collections with limited physical, technical and IT infrastructures. Nanni et al. (2012) describe the advantages of SPREC for clinical biospecimens, these are also pertinent to biodiversity collections:

1. Properties attributed to the sample enable curators and users to determine if the bioresources, cell line or sample and its derivatives are suitable for requirements.

2. Critical information can be provided about historical collections:
   a. Viable cultures cryo-conserved stored for very long periods of time in cryobanks.
   b. Non-viable biospecimens preserved museum collections that may have been collected and processed using sub-optimal procedures for modern molecular analyses.
   c. Retrospective SPRECs can be generated based on available information.
   d. This information can be used to future-proof collections in anticipation of wider utilities.
Recipients of transferred cultures, samples, biospecimens can retrieve (de-code) information about a sample’s ‘life history’ from the SPREC and use this to assess the impacts of pre-analytical variables on its utility and fitness for purpose.

Regardless of taxa, the pre-analytical variability generated during processing can influence the present and future use of biospecimens and the resilience of viable bioresources to storage (Benson, 2008; Caboux et al., 2012). Although genetic analyses of ancient DNA, including those of archive collections are now common place, precautions and robust criteria are still needed to obtain data that represents authentic ancient DNA sequences (Pääbo et al., 2004). The relevance of pre-analytical variability for contemporary and historical biodiversity collection management is more apparent as: (a) increasingly stringent QA/QC measures are needed for ever more sensitive modern molecular biological techniques and (b) large-scale research networks undertake biodiversity research across diverse types of collections (Fig. 2). Lermen et al. (2014) recommend the standardization of sample acquisitions to control pre-analytical variability for multicenter studies involving environmental biospecimens and use mobile platforms which are customized collection vehicles equipped with environmental controls to achieve consistent collection and transport procedures. Sub-optimal and inconsistent handling have consequences for sample quality that is measured using performance indicators which define different levels of functionality (Figs. 1 and 2). Adoption by the wider conservation community of tools like BRISQ and SPREC provide potential solutions for managing variables, especially those that affect sensitive, complex molecular technologies (e.g. metagenomics, eDNA, epigenetics, omics) that extend beyond traditional biodiversity science.

**Scoping BRISQ and SPREC for biodiversity research and conservation**
The clinical reporting format developed by Moore et al. (2011) may be best considered as a starting point for scoping BRISQ for biodiversity collections and will need more options built in to accommodate different modus operandi, taxonomic range, complex acquisitions, difficult sampling logistics and intermediate transfers (Tables 1 and 2). Flexibility in reporting the types and combinations of elements will be essential, particularly for samples maintained in large, diverse collections comprising different types of samples. An advantage of BRISQ is that groups of elements are tiered according to priority so not all data will need to be included and decisions on which items to report can be rationalized according to sample type and processing. Establishing the criticality of tiers and their elements is a useful QA exercise with respect to satisfying specific performance indicators and biobank standards.

The Biospecimen Science Working Group of ISBER produced the first clinical SPREC to make explicit the pre-analytical variables attributed to the collection, processing and storage of clinical specimens (Betsou et al., 2010). A SPREC does not include pre-acquisition elements or detailed information about the physiological, developmental or pathological status of a sample (Tables 1, 2 and 3). The clinical SPREC is now internationally recognized by the human biobanking community as a quality management tool that supports: (a) the provision of high quality clinical samples for research, (b) improved interconnectivity and interoperability across federated clinical biobanks research infrastructures and (c) large-scale health care consortium projects (Lehman et al., 2012; Nussbeck et al., 2013). Prototype preanalytical codes have been devised for phytodiversity culture collections (Benson et al., 2011a; Harding et al., 2013) and provision for SPREC data has been made in the GGBN data standard (G. Droege personal communication). As far as the authors are aware the SPREC is not routinely implemented in biodiversity collections, extrapolating from the clinical SPREC several applications can be identified: (1) supporting
evidence-based conservation programmes involving geographically dispersed collaborative networks, (2) enabling biodiversity sampling, processing and biopreservation procedures, especially for labile, sensitive biospecimens and storage-recalcitrant germplasm and (3) improving the quality of stored biodiversity samples and the quality of molecular and systematics data generated by their use.

**Endangered species management: protected wildlife forensics and conservation**

Quality management extends beyond the routine curation of biodiversity collections as the interpretation of large amounts of biomolecular information generated from stored samples and their derivatives can be compromised by unaccounted for pre-analytical variability. Protected areas management, captive breeding and forensic measures against wildlife crime all depend upon reliable sample QA/QC which is essential for legal, ethical and regulatory aspects of wildlife and native flora protection. Conservation law enforcement requires the forensic analysis of biospecimens to be standardised wherever possible (Lorenz et al., 2005; Williams, 2007). The fight against wildlife crime involving Convention on International Trade in Endangered Species (CITES) infringements and the trafficking of endangered species is difficult to investigate without forensic identification. Stringent QA is essential for high quality samples used in forensic analysis particularly where DNA barcoding is applied to cases where species are used in products for human consumption, including bushmeat, or where investigation of health threats arise from virus transmission, mislabelling and the substitution of ingredients (Eaton et al., 2010; Smith et al., 2012).

There are limited standards for wildlife crime reference databases and older barcode data standards are inadequate for the new regulatory and forensic uses that demand sophisticated analyses. Chain of custody information and metadata for taxonomic reliability and quality
management (including pre-analytical variables) are requisites for forensic testing, especially where CITES legislation applies to the human food chain.

Managing sample quality is challenging for projects analysing traditional medicines which requires different expertise (biological, conservation, environment, traditional knowledge, ethnobotanical, cultural, socio-economic). Protected species management becomes even more complicated for associated species requiring forensic analysis of wildlife samples from different taxa. Some of the most difficult cases comprise mixes of unknown species or symbionts that are essential for medicinal efficacy but co-implicated in the illegal trade of an endangered species controlled by conservation law. As BRISQ and SPREC record accurate sample and process chain information they can inform endangered species conservation programmes and help reduce the risks of ambiguity about pre-acquisition and post-storage sample quality when dealing with CITES breaches, and poaching prosecutions.

Holt et al. (2014) review the latest developments in the conservation of endangered species, including the Frozen Ark, (http://www.frozenark.org/) and Frozen Zoo projects, (http://www.sandiegozooglobal.org/what_we_do_banking_genetic_resources/frozen_zoo/) which involve the cryopreservation of viable stem cells, gametes and embryos. Successful wildlife restoration programmes depend on accurate reporting and descriptions of all parts of the process, including assessing the impacts of taxon-specific controlled rate freezing and storage on downstream analyses, germplasm viability, stability and totipotency. Looking to the future, entire DNA sequences of the genomes of endangered species can now be preserved for future conservation initiatives, even if a species has become extinct in the wild. De-extinction, species resurrection and assisted breeding programmes (Ben-Nun et al., 2011) are controversial and where scientific collections are used it is prudent that
conservationists consider the risks of not implementing robust quality management measures, particularly when working with samples from multiple taxa.

**Sample and process chain annotation: from environmental DNA to citizen science**

Thorough documentation is essential for metagenomics and environmental DNA (eDNA) research which involves the study of genomes sampled from whole environments rather than individual species (Wooley et al., 2010). As defined by Thomsen and Willerslev (2015) eDNA is the genetic material obtained directly from environmental samples without obvious signs of the biological source material, it is used to study biodiversity in ancient and modern environments. Raising awareness as to the possible applications of BRISQ and SPREC is timely as it is anticipated that environmental biobanks, museum and culture collections will be increasingly required to store and distribute eDNA samples. Various studies suggest that sample acquisition and processing treatments (e.g. filtering, purification, transfers, extraction methods, storage) can influence eDNA analyses and they stress the importance of method validation and reporting provenance, abiotic and biotic factors (Dejean et al., 2011; Deiner et al., 2015; Foote et al., 2012; Strickler et al., 2015; Thomsen & Willerslev, 2015; Turner et al., 2014, 2015). Conveying critical information about the effects of pre-analytical variables on eDNA in formats such as BRISQ and SPREC (Table 2 and Fig. 5) is pertinent based on emerging evidence. Takahara et al. (2015) studied freeze/thaw treatments prior to analysis on the detection of eDNA in common carp finding it lower in frozen/thawed samples compared to unfrozen controls, concluding eDNA detection is affected by sample processing. McKee et al. (2015) found that dilution and post-extraction nucleic acid purification techniques influenced the accuracy, precision and inhibition of eDNA samples. Deiner et al. (2015) recommend that biases caused by choice of protocols used for eDNA
processing and analysis should be considered in order to produce more reliable and repeatable research outcomes.

Assessments of biodiversity using eDNA offer many advantages as they are non-invasive, cost effective and yield information about communities and species associations over time, consequently the monitoring of eDNA is increasing in environmental impact assessments and endangered species management (Foote et al., 2012; Thomsen & Willerslev, 2015). Citizen science involves volunteers from the general public, schools and local societies participating in research through observations, recording and collecting biospecimens. Increasingly citizen science is becoming a popular, scientific and educational tool, as the scale and coverage of conservation science is increased when significant numbers of people volunteer to gather information and samples from diverse and large areas. Consequently, eDNA technology is also being pioneered in citizen science biodiversity projects as a survey tool for rare, covert and difficult to monitor species. Biggs et al. (2015) tested the use of eDNA in a UK-wide project surveying the distribution of the great crested newt (Triturus cristatus) which involved comparing different methods and the ability of citizen science volunteers to collect DNA samples, concluding that eDNA-based surveying is highly effective. Similarly ‘Microverse’ is a collaborative citizen science research project between the Natural History Museum in London and schools and colleges across the UK (http://www.nhm.ac.uk/research-curation/life-sciences/genomicsmicrobial-diversity/research/index.html), the aim of which is to reveal and study the microbial diversity of urban ecosystems. In the future, sample acquisition for citizen science programmes may benefit from the use of a simplified, quick reference BRISQ check list (Table 1) to standardize collecting procedures and enhance survey value. Similarly, SPREC may have utility in eDNA surveys that are conducted by the general public as it records in a
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standardized format, critical information about pre-analytical variables that may otherwise be overlooked.

**Harmonizing quality management across different types of biodiversity collections**

Harmonization of standards and practices is complicated as individual institutions can hold diverse biospecimens and bioresources in large numbers and their quality management can range from *ex situ* conservation BPs and guidelines to DNA sequence standards (Benson, 2008; Benson et al., 2011b; Field et al., 2008; Ratnasingham & Hebert, 2007). Identifying appropriate performance indicators helps to refine QA measures and QC tests for collecting, sampling and storing biodiversity samples with contrasting conservation (viability/totipotency) and preservation (morphological fixing) priorities, this is pertinent where process chains (Fig. 3) conflict with a sample’s original or future utility. For example, chemical preservation in formalin fixes morphological structures but renders samples difficult or cost-prohibitive to use for DNA studies, in contrast, snap freezing is suitable for biomolecular studies but does not preserve the original structure. Next Generation Sequencing assembles damaged fragments and advances in DNA damage biochemistry and targeted repair mechanisms lead to the development of alternative protocols where NGS is unaffordable. As new technologies evolve it may become possible to extract DNA from specimens which presently only exist in a formalinised state. The conservation of viable, regenerable and totipotent organisms and cells also requires different *in vitro*, cryogenic and recovery treatments that can alter the morphogenetic and biomolecular state of the original sample, precluding their use as Type or voucher specimens (Figs. 1 and 2).

BRISQ and SPREC can help rationalize the dilemma of how best to harmonize the quality management of different types of biodiversity collections, especially where it is difficult to access and record critical information about pre-acquisition status and sampling.
involves rare or at risk species, difficult logistics and negotiating international borders. All of these issues require early consideration when writing grant proposals to budget for the appropriate storage technologies, such as controlled rate freezers, cryobanks and cold chain transport from field to biobank. Selection and recording of process chain elements, the identification of suitable performance indicators and robust QA/QC need to be in place from the start to achieve, at the least, good return-on-investment outcomes. It is prudent to build in flexible protocols and identify optional choices (e.g. preservation versus conservation) in BRISQ and SPREC to assure the future usability of biospecimens as new technologies become available and add value to original investments. These considerations are justified for samples and their associated data and apply to planned projects and unforeseen future research that will use historical collections and for which new QA/QC tools will be required to assess their suitability for analysis (Colotte et al., 2009).

**Future perspectives: new Quality Management Systems (QMS) for biodiversity collections**

A new framework for developing biodiversity collection Quality Management Systems (QMS) is advocated that includes Biospecimen Science tools such as BRISQ and SPREC to help standardize the annotation of historical, existing and future sample and process chain information. These tools are especially relevant for collections that are poorly annotated and consist of biospecimens or accessions with different levels of quality. Crucially, the proposed use of BRISQ and SPREC is not to replace existing curatorial practices, but rather to support and enhance them by guiding decisions about how best to rationalize archived, inherited and contemporary, collections, including those generated by citizen science projects. Vogt (2013) cautions that scientists might perceive new life sciences data standards as burdens rather than benefits and that in developing new standards it is important to
convey their practical value and application. Implementing standards is endorsed by the fact that sample quality issues are common across all types of biodiversity collections, presenting complex problems for both conservation science and systematics research. It can be difficult to put together cost efficient curation recommendations as to what samples and biospecimens to retain and what to discard. BRISQ and SPREC could provide a framework for making more obvious critical information about processes and procedures used in current field collecting and they can be retrospectively applied to curate legacy collections and the de-accession of lower value samples.

In the longer-term, implementing BRISQ and SPREC can be expected to have cost and efficiency benefits, although some investment will be required upfront to create and integrate the tools in existing collection QMS. Conversely, decisions informed by quality management tools must be scrutinized carefully as they will need to be countermanded by special cases, such as one off and precious genetic resources or samples from endangered or extinct species and ancient specimens or sample DNAs. Even though there is minimal data and the sample quality is poor their retention can be justified on the basis of rarity and one-time-only sampling. In these scenarios understanding the distinction between conservation and preservation becomes significant because they differentially impact on the fitness-for-purpose of bioresources and samples for contemporary and future molecular-based studies. Poorly annotated and sub-optimally preserved historical collections present quality management problems as do viable, storage-recalcitrant cultures, in which case treatments can be considered as pre-analytical variables that can be recorded in BRISQ and SPREC.

The clinical biobank community has created several information management tools (Betsou et al., 2010; Moore et al., 2011; Quinlan et al., 2014) and there may be a risk of over-producing too many with similar reporting formats without consolidating their common elements and delineating the unique use of each one. Significantly clinical SPREC
and BRISQ overlap in some elements, particularly pre-analytical variables (Tables 1-4); this poses special problems for biodiversity biobanks as in contrast to the clinical sector, collection curators have to deal with a huge range of taxa, species and sample types. Therefore, it will be necessary to: (a) scrutinize the validity of using the original clinical SPREC and BRISQ formats in biodiversity conservation and (b) investigate consolidating BRISQ and SPREC to produce ‘hybrid’ sample reporting tool(s) customized for specific types of biodiversity collections and applications. This poses the questions would it be better to use SPREC and BRISQ as individual tools, based on the original clinical format? Or, consolidate their formats to create one new tool which is sufficiently generic and versatile to be applied, with appropriate modification, across all types of biodiversity collection QMS? Which approach to take requires wider and further debate across the biodiversity community, a starting point may be to test and compare the utility of individual and hybrid BRISQ-SPRECs that are compatible with existing collection management software, but have sufficient built in flexibility to accommodate different types of biorepository, taxa, samples and applications.

*Intercalating BRISQ and SPREC with laboratory and collection management systems*

The SPREC and BRISQ share common elements that overlap with some metadata already recorded in existing collection information management systems (Tables 1, 2 and 3). Developing new sample reporting tools that are compatible with existing LIMS will ease retrospective updates and reviews when samples are re-analysed and edited data is re-submitted. This is exemplified by KE Software's Electronic Museum management system (KE EMu™) which together with other brands of commercially available software is purposely designed for museum collections and is used by the London Natural History Museum to record sample information, some of which is analogous to BRISQ and SPREC.
for part of the metadata it documents. For example, information about collection site (e.g. GPS, habitat, humidity, temperature), specimen/sample process chains, cold chain transportation to the museum, loan processing and other data (e.g. PCR and sequences) that are subsequently generated. Data sets in KE EMu™ are linked hierarchically to create a series of records pertaining to a ‘voucher’ specimen and data originating from this can be extended to describe the life cycle of the specimen and its derivatives. Metadata are also linked to other types of inventory systems such as molecular analysis and storage location FreezerPro™ and environmental (temperature, humidity) monitoring software.

BRISQ and SPREC are starting to be used by biodiversity consortia such as the GGBN (G. Droege, personal communications) as they have relevance for established data and information management systems, including Darwin Core (DwC)
(http://rs.tdwg.org/dwc/) and the Global Biodiversity Information Facility (GBIF
(http://www.gbif.org/). Links relevant to BRISQ and SPREC data fields can be mapped, integrated and exported, re-packaging existing information is justified as it highlights overlooked details critical for sample QA/QC. The impact of process chain variables on samples and their downstream analyses can be determined when existing metadata are represented in a way that reveals the precise relationship between sample quality and how it has been handled and stored. Reporting this information is desirable for sample utilization within the institution where it is stored, as well as for third party use, and it can help future-proof collections for end users and inform evidence-based decisions about choice of analyses. The extent and level of support and development required from institutional IT teams to create searches for existing data to populate BRISQ and SPREC will vary dependent upon staffing and IT resources. Reports generated by BRISQ and SPREC can be appended to quality management documents including SOPs and risk assessments (Harding & Benson, 2015).
Identification and standardization of robust performance indicators

For practical expediency BRISQ and SPREC could provide an evidence base for performance indicators because they record and standardize information about different types of samples and their storage. The level of sample, cell and organism functionality preserved or conserved (see section III 2) in different types of biodiversity collections can be calibrated on a potency scale (Fig.1) which defines: (a) collection purpose (e.g. voucher specimen preservation; genetic resources conservation) and (b) the sample quality required to meet biorepository operational standards and match the goals of a research project or end user needs. Functionality is best calibrated using performance indicators that scale sample ‘potencies’ ranging from full totipotency (i.e. germplasm from which a whole organism can be regenerated) at one end of the spectrum, to dead cells (e.g. for DNA extraction) at the other (Figs. 1 and 2). BRISQ and SPREC support the best possible performance outcomes by recording critical information about samples and the pre-analytical variables to which they are exposed during their preservation, conservation and processing (Figs. 3 and 4). Performance indicators can be used to validate storage methods and analytical protocols and they may be considered as quality standards for voucher, Type or genebank collections (including non-viable and viable samples) with respect to application (e.g. taxonomic classification, conservation, assisted breeding). Higher-order performance indicators assure that institutional standards are upheld and the requirements of collection stakeholders and end users are satisfied. Advances in analytical technologies have made it possible to perform genetic and cellular analyses on traditionally preserved specimens maintained in vintage and ancient biospecimen collections. Such developments benefit from an understanding of how processing treatments affect analyses performed on sub-optimally preserved samples. Where information is available, the retrospective coding of pre-analytical variables may be feasible.
for biospecimens from species that are extinct in the wild. The SYNTHESYS project PrediCtoR tool (Allentoft et al., 2012; Smith et al., 2003; Walker et al., 2015) may also be considered as a QA tool for performance indicators, although developed for ancient bones it could be expanded to other historic collections.

**Collections, conservation consortia and networks**

One of the greatest challenges for biodiversity scientists and conservationists is how best to adapt, refine and develop QMS for the huge range of taxa, species and sample types maintained and used by large-scale biodiversity consortia and research infrastructures. As more institutions, scientists, citizen scientists and practitioners participate in international networks a greater level of data standardization (Vogt, 2013) and harmonization of process reporting across physical and virtual infrastructures will be required. However, partnerships often involve diverse and geographically dispersed institutions that have varied technical operations and socio-economic constraints, these differences place extra demands on QMS development. Biospecimen Science focuses on improving the quality of collections and has pioneered BRISQ and SPREC to annotate, report and share critical information about samples, process chains and pre-analytical variability. The aim of this paper is to raise awareness about the significance of Biospecimen Science tools that communicate critical information about samples and the variability to which they are exposed an aspiration that is common across biodiversity conservation communities. Droege et al. (2014) report the need for GGBN, which links different types of biodiversity biobanks across the globe, to increase accessibility to compatible and harmonised data and samples by using standardised protocols to enhance sample value and improve research outcomes. This has relevance for all types of biological collections, especially those that have significant curation challenges,
ranging from future-proofing ancient and contemporary collections to building in sufficient flexibility to preserve and conserve diverse taxa and samples.

**Enriching sample and processing data enhances biodiversity sample value and usability**

The more information attributed to a sample the greater will be its intrinsic utility. This principle is based on the premise that rigorous quality management will enrich data, enable knowledge sharing and consequently enhance the value of collections used in biodiversity research and conservation. Implementing BRISQ and SPREC can make more accessible critical information about collections that can be used to improve sample metadata standardization and satisfy institutional KPIs. When used as QA/QC BRISQ and SPREC tools they can bridge the gap between sample and process quality management with the requirements of end users. Data that reveals critical information about the quality of sample and associated processing information, including pre-analytical variables, is relevant for natural history collections that are developing their digitisation programmes to support information sharing with others (Vos et al., 2014). Standardizing sample reporting is also useful for creating BPs, guidelines and generic SOPs across global conservation communities and genebanks.

Biorepositories involved in preserving and conserving biodiversity already have some BRISQ and SPREC elements in place but they are not always applied consistently and over time they may not be adhered to when there are changes in personnel, projects and funding. Whilst BRISQ and SPREC were first developed by the human biobank community which focuses on one species, two sample types (liquid or solid) and many diseases they have fulfilled their proof-of-concept as valuable sample reporting tools that support research and facilitate the management of millions of clinical samples used in ‘big-data’ studies. In contrast, BRISQ and SPREC will enable biodiversity curators and conservation practitioners
to quality-manage diverse collections comprising many taxa, sample types and timelines, including precious and one-off samples from endangered or extinct species. Clear descriptions of acquisitions and the accurate annotation of critical data attributed to provenance, process chains and pre-analytical variability will especially contribute to the scientific advancement of biodiversity science and conservation involving molecular technologies. Relative to the clinical application of BRISQ and SPREC, biodiversity collections are presented with additional and often extreme sample quality issues, as is the case with difficult sampling logistics from remote regions. On this basis, it may be argued that the more complex the acquisition and processing of samples the more the biodiversity community needs to develop quality management tools like BRISQ and SPREC.

CONCLUSIONS

(1) Biodiversity collections support *in situ* and *ex situ* conservation, they are used in taxonomic and endangered species research and for the sustainable exploitation of bioresources; their utility is changing with advances in biomolecular technologies.

(2) Clinical biobanks recognized a significant number of historical collections were of inadequate quality, this motivated the emergence of Biospecimen Science which is dedicated to improving the quality management of collections.

(3) Biospecimen Reporting for Improved Study Quality (BRISQ) and the Standard PRE-analytical Code (SPREC) report critical information about samples, process chains and variability.

(4) Adopting a Biospecimen Science framework benefits biodiversity collections because sample quality equates to the quality of systematics research and conservation outcomes.
International conservation networks need to harmonize collections quality management to improve sample, data and knowledge exchanges within and between institutions.

Stakeholders and users of biodiversity collections have rising expectations as new, sophisticated downstream analyses come on line that are affected by pre-analytical variability.

It is timely to explore BRISQ and SPREC as quality management tools that help assure collections are fit-for-purpose and remain so in the long-term.

Retrospective BRISQ and SPRECs extend the utility of historical collections and inform decisions about the suitability of traditionally-preserved samples for modern molecular analyses, including those used in systematics research.

Consideration is required regarding the resources needed to implement biodiversity BRISQ and SPREC; reservations regarding this investment can be allayed by proof-of-concept from the clinical biobanking community.

To summarize, SPREC and BRISQ are relevant for biodiversity collections used for systematics research and conservation science. In support of their implementation it will be necessary to: (a) harmonize reporting with existing data collection and laboratory management systems, (b) consider the customization of ‘hybrid’ BRISQ-SPREC reporting tools and (c) stimulate wider debate across the biodiversity community as how best to rationalize their formats.
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45 (Systematics & Biodiversity)


Caboux, E., Lallemand, C., Ferro, G., Hémon, B., Mendy, M., Biessy, C., ... Hainaut, P. (2012). Sources of pre-analytical variations in yield of DNA extracted from blood samples: Analysis of 50,000 DNA samples in EPIC. *Public Library Of Science ONE, 7*, e39821. doi:10.1371/journal.pone.0039821


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Table Titles and Legends

Table 1. Prototype Biospecimen Reporting for Improved Study Quality (BRISQ) quick-reference checklist with examples of Tier 1 elements applicable in biodiversity collections. Adapted from Moore et al. (2011).

Table 2. BRISQ reporting elements for biodiversity preservation and conservation in natural history, museum and culture collections. Adapted from Moore et al., (2011) with italicized explanatory notes. Tier 1 = recommended to report, Tier 2 = beneficial to report, Tier 3 = additional to report. BRISQ information is recognized by attaching the report to a sample’s unique accession number, strain identifier or curatorial ID. Element options can be delineated into a third level code. BRISQ elements in common with existing information management systems are indicated as: the Standard PRE-analytical Code (SPREC) e.g. for environmental biobanks (Benson et al., 2011); Collection Management Systems (CMS) e.g. the London Natural History Museum software for museum management (KE EMu™) and general Laboratory Information Management Systems (LIMS). BRISQ elements IV - VII will need to be expanded dependent upon sample and collection type.

Abbreviations: ABS = Access and benefit sharing, BRC = Biological Resource Centre; CBD = Convention on Biological Diversity, CITES = Convention on International Trade in Endangered Species of Flora and Fauna, CPD = critical point drying, DMSO = dimethyl sulphoxide, GPS = Global Positioning System, FFPE = Formalin Fixed Paraffin Embedded, GTS = geological timescales, LN = liquid nitrogen, LTS = long-term storage, MC = moisture content, MTA = material transfer agreement, MTS = medium-term storage, N/A = not applicable, QA = quality assurance, QC = quality control, QM = quality management,

Table 3. A 7-element Standard PRE analytical Code (SPREC) for sample collection, processing and culture initiation using the algal culture collection SPREC a-01 as an example. Full details for SPREC A-01 are provided in the complete code compiled for algal culture collections (Benson et al., 2011). Code options highlighted in bold (B, A, C, B, A, DAL, NXM) indicate how sample and technical details can be recorded in the 7-element code.
Figures Titles and Legends

**Fig. 1.** The typography and dimensions of biodiversity collections. BRC = Biological Research Centre(s).

**Fig. 2.** Impacts of biopreservation, temperature and pre-analytical variables on the functionality and fitness-for-purpose of different natural history, museum and culture collection holdings. IMS = industrial methylated spirit; LN = liquid nitrogen; NGS = Next Generation Sequencing.

**Fig. 3.** Examples of museum and natural history collection process chains constructed for different types of biospecimen acquired as either: (a) fresh, field-collected samples (i-iii) or (b) biospecimens sampled from existing, catalogued collections within the museum (iv-vi). FTA = Fast Transfer Application; LN = liquid nitrogen (cryobank at -196°C); QA = quality assurance; QC = quality control.

**Fig. 4.** Examples of viable culture collection process chains constructed for different types of organisms represented by: (a-c) of fresh, field-collected whole organisms (a = microalga), sexual reproductive germplasm (b = tree seed) and asexual clonal germplasm (c = vegetative propagules). LN = liquid nitrogen (cryobank at -196°C); QA = quality assurance; QC = quality control.

**Fig. 5.** Schematic demonstrating how to annotate, code and track a SPREC. Example is based on the algal SPREC A-01 (Benson et al., 2011) created for the sampling, initiation
and *in vitro* culture of microalgae (see Table 3). The schematic is adapted from Lehmann *et al.* (2012); Nussbeck *et al.* (2013); Nanni *et al.* (2012). CMS = collection management system; ID = identifier; LIMS = laboratory information management system; SPREC = Standard PRE-analytical Code.
Biodiversity Conservation *In Situ*: Biosphere - Biomes - Ecosystem - Habitat

Conservation *Ex Situ* in Reserves, Field Collections, Arboreta, Botanical Gardens, Zoos

---

**Preservation & Conservation *Ex Situ* in Biorepositories**

- **Biorepository Typography & Function**

  - **Museum Collections**
    - **Dry Collections**
      - skins, bones, dry/pinned arthropods
    - **Feathers, herbarium sheets**
  - **Spirit Collections**
  - **Frozen Collections**
    - **Tissues, cells, organs, subcellular extracts, DNA RNA, proteins**
  - **Scientific Collections**
    - **Research, Taxonomy Type, Voucher Specimens**

  - **Living Collections**
    - **Biobanks**
      - Environmental Biobanks
    - **Genebanks**
      - *In Vitro* Genebanks
      - *In Vitro* Active Genebank
      - *In Vitro* Base Genebank
    - **Sperm, Pollen, Seed Banks**

  - **Biological Research Centres (BRCs)**
  - **Culture Collections**
    - Working, Master
  - **Cryobanks**
    - Vaults

  - **Sustainable Use & Conservation**
    - Biological & Genetic Resources Type, Reference Strains

---

**Dimension 1 - Diversity**

**End User Fitness for Purpose**

**Dimension 2 - Functionality**

**End User Fitness for Purpose**

**Dimension 3 - Time**

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### Collection Holdings

**Museum Collections**
Non-viable / non-culturable biospecimens - traditional wet / dry collections: ancient, ‘vintage’ & archival specimens; labile non-viable samples, dry & pinned arthropods, herbarium sheets, shells, mammal & bird skins, bones, hair, horn, plant material with silica gel, FTA™ cards, spirit collections (in ethanol, IMS, formalin); RNALater™, frozen tissue collections: biomolecular extracts, eDNA; genetic resources

**Culture Collections**
Cultures initiated from viable, replicable cells, tissues, organs, totipotent germplasm (clonal propagules, spores, gametes, embryos, pollen, seed, meristems, stem cells), organisms, assemblages of organisms (parasitic, symbiotic, mycorrhizal). Maintained as active cultures, under growth retarding conditions; cryopreserved in base & master collections

### Preservation

**Type & Voucher Specimens**
Preservatives: fixatives, chemicals, desiccants, silica gel, low oxygen

**Conservation**

### Temperature

**Ambient**
environ-control
10 - 25°C

**Refrigerated**
chilling
4° - 10°C
mechanical freezer
-20°C
-70°C / -80°C
-150°C

### Pre-Analytical Variables

**Low impact**
Physical structure, morphology
NGS

**High impact**
Cell function
viability
tissue extractions
sensitive molecular analyses

### Functionality

**Dead**
Cell & bio-structure intact
transient metabolism

**Viable**
Replicable
totipotent growth

**Performance Indicators**
Viability, totipotency
molecular analyses

**Conservation**

**Low impact**
Viability, totipotency
molecular analyses

**High impact**
Structural & morphological characters
(stress) biomarkers

---

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# Museum and Natural History Collection Biospecimen Process Chains

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Collection</th>
<th>Transit Stabilization</th>
<th>Processing</th>
<th>Storage</th>
<th>Recovery</th>
<th>Dispatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Fresh field</td>
<td>ii. Beetle</td>
<td>Fresh field/ museum</td>
<td>Fresh field / museum (a-b; i-vi) - 80˚C on dry ice or -150˚C in dry shipper (LN)</td>
<td>Fresh field / museum (a-b; i-vi) -196˚C LN - vapour cryobank FTA slides</td>
<td>Fresh field / museum (a-b; i-vi) fast thaw in class II hood; aliquots to 2 D barcoded storage tube</td>
<td>Fresh field / museum (a-b; i-vi) - 80˚C on dry ice or -150˚C in dry shipper cryogenic (LN)</td>
</tr>
<tr>
<td>i. Land snail</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ii. Beetle</td>
<td>iii. Plant</td>
<td>Fresh field/ museum</td>
<td>Fresh field / museum (a-b; i-vi) - 80˚C on dry ice or -150˚C in dry shipper (LN)</td>
<td>Fresh field / museum (a-b; i-vi) -196˚C LN - vapour cryobank FTA slides</td>
<td>Fresh field / museum (a-b; i-vi) fast thaw in class II hood; aliquots to 2 D barcoded storage tube</td>
<td>Fresh field / museum (a-b; i-vi) - 80˚C on dry ice or -150˚C in dry shipper cryogenic (LN)</td>
</tr>
<tr>
<td>b. Museum</td>
<td>iv. Pinned butterfly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv. dry store</td>
<td>v. Formalin-preserved fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v. spirit collection</td>
<td>vi. Dried plant, fungi, algae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fresh field/ museum</td>
<td>Fresh field / museum (a-b; i-vi) - 80˚C on dry ice or -150˚C in dry shipper (LN)</td>
<td>Fresh field / museum (a-b; i-vi) -196˚C LN - vapour cryobank FTA slides</td>
<td>Fresh field / museum (a-b; i-vi) fast thaw in class II hood; aliquots to 2 D barcoded storage tube</td>
<td>Fresh field / museum (a-b; i-vi) - 80˚C on dry ice or -150˚C in dry shipper cryogenic (LN)</td>
</tr>
</tbody>
</table>
### Culture Collection Process Chains

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Collection</th>
<th>Transit Stabilization</th>
<th>Processing</th>
<th>Culture</th>
<th>Storage</th>
<th>Recovery</th>
<th>Dispatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>b. Post-dehiscent mature tree fruit / seed</td>
<td>b. Hand-collect from tropical rainforest floor</td>
<td>b. RH / T°C stabilized + antifungal treatments</td>
<td>b. Disinfect, remove from fruit, excise embryo from surface-sterilized seed</td>
<td>b. Germinate seedling from excised zygotic embryo</td>
<td>b. Cryobank meristems excised from in vitro seedlings</td>
<td>b. Rewarm, meristems, regrow in vitro, transfer plants to glasshouse</td>
<td></td>
</tr>
<tr>
<td>c. Vegetative plant clonal propagule (shoot, bud, tuber, bulb, rhizome)</td>
<td>c. Excise from donor plant or propagule</td>
<td>c. Aseptic in vitro field stabilization</td>
<td>c. Surface sterilize explants + phytosanitary treatments a - b QA / QC documents</td>
<td>c. Initiate culture from explant, clonal propagation / serial culture</td>
<td>c. In vitro genebank Active or (slow growth) Base (cryobank)</td>
<td>c. Rewarm, and / or transfer to standard culture, acclimatize regenerated plants</td>
<td></td>
</tr>
</tbody>
</table>

**QA / QC documents**

- a.
- b.
Option 1
SPREC Tools

Option 2
Extracting SPREC data from existing LIMS/CMS

Option 3
Simple handwritten records

SPRECs attached to a sample’s ID + sub-samples, derivatives, analytes

Dedicated software

SPREC encoding options

Customized software

SPREC Decoder

Sample or accession ID

B-A-C-B-A-DAL-NXB

Evidence-based evaluation of preanalytical variable impacts on sample quality, usability, stability and analyses

Improved sample quality standards

Pre-analytical variables revealed

Systematics and Biodiversity

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### Process Stages

<table>
<thead>
<tr>
<th>Check</th>
<th>Data Elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of institution</td>
<td>Research infrastructures, museum on loan biospecimens, new acquisitions</td>
</tr>
<tr>
<td>Selection criteria (sample)</td>
<td>Museum type specimens, culture collection reference strains, indicator organisms</td>
</tr>
<tr>
<td>Selection criteria (quality)</td>
<td>Biological Resource Centre (BRC) quality criteria (authenticity, purity, stability)</td>
</tr>
<tr>
<td>Collection modality</td>
<td>Dead, living, replicable, totipotent, dictates biospecimen or bioresource usability</td>
</tr>
<tr>
<td>Collection category</td>
<td>Museum herbarium, spirit, molecular, type, reference, voucher specimen; active / base genebank, working / master</td>
</tr>
<tr>
<td>Provenance</td>
<td>Biodiversity access and benefit sharing</td>
</tr>
<tr>
<td>Time scale</td>
<td>Geological timescales for climate change research; long-term conservation at cryogenic temperatures ad infinitum</td>
</tr>
<tr>
<td>Taxonomy</td>
<td>Species verification, new species identification</td>
</tr>
<tr>
<td>Biological donor</td>
<td>Population genetics, taxonomic research, metagenomics</td>
</tr>
<tr>
<td>Anatomical site</td>
<td>Tissues used for storage and extraction; explants used to initiate cultures</td>
</tr>
<tr>
<td>Vital state</td>
<td>Post mortem evidence for wildlife forensics, viability of living collections</td>
</tr>
<tr>
<td>Physiological &amp; developmental state</td>
<td>Viable culture initiation</td>
</tr>
<tr>
<td>Gender</td>
<td>Species identification, genetic resources conservation, assisted breeding</td>
</tr>
<tr>
<td>Life cycle &amp; reproductive state</td>
<td>Genebanks, assisted breeding, species re-introductions, wildlife management</td>
</tr>
<tr>
<td>Health &amp; nutritional status</td>
<td>Endangered, at risk species management, exploitation of cultures for natural products biotechnology</td>
</tr>
<tr>
<td>Toxicological status</td>
<td>Xenobiotic, radiation, environmental impact studies, poisons</td>
</tr>
<tr>
<td>Axenicity</td>
<td>Purity critical for molecular genetics, omics research; non-axenicity for parasitology, symbioses, assemblages, eDNA</td>
</tr>
<tr>
<td>Disease and pathology status</td>
<td>Epidemiological studies, safe transfer of biospecimens and bioresources</td>
</tr>
</tbody>
</table>

### I. Pre-acquisition & associated sample date

- **Collection & sample container**: Stabilization of all types of biospecimen, sample, eDNA, biological/genetic resource, organism; especially when biodiversity is sampled and dispatched from remote, extreme or difficult locations (e.g. polar regions, conflict zones) and when cold chain and the chain of custody, security, monitoring and logistics are compromised by lack of infrastructure

### II. Acquisition, stabilization & transport

- **Preparation**: Prevention of deterioration, particularly fragile, labile biospecimens (e.g. Lepidoptera, molluscs, insects) before preservation
- **Chemical fixation**: Preservation of the original state e.g. herbarium samples, tissues, horn, feathers, organisms in spirit collections, eDNA
- **Preservation by desiccation & drying**: Stabilization of permanent museum collections under environmentally controlled conditions
- **Flash / snap freezing**: Sample quality impacts on thermo-labile biospecimens; repeated sub-sampling from the same specimen

### III. Preservation, fixation & storage

- **Freeze/thaw parameters / cycles**: Sample quality impacts on thermo-labile biospecimens; repeated sub-sampling from the same specimen

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<table>
<thead>
<tr>
<th>IV. In vitro culture</th>
<th>□</th>
<th>Culture</th>
<th>Multiple steps involved in the successful initiation and serial sub-culture of viable cells, tissues, organs, organisms <em>in vitro</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>□</td>
<td></td>
<td><em>in vitro conservation</em></td>
<td>Conservation of active and base collections in culture collections, genebanks, BRCs</td>
</tr>
<tr>
<td>V. Conservation storage &amp; recovery</td>
<td>□</td>
<td>Cryopreservation</td>
<td>Conservation of viable cells, tissues, organs, organisms in cryobanks <em>ad infinitum</em></td>
</tr>
<tr>
<td>□</td>
<td></td>
<td>Rewarming &amp; recovery</td>
<td>Recovering fit-for-purpose viable, functional, totipotent cells, tissues, organs, organisms after cryostorage</td>
</tr>
<tr>
<td>VI. Dispatch transport &amp; cold chain security</td>
<td>□</td>
<td>Shipping temperature &amp; conditions</td>
<td>Cold chain, chain of custody, security, monitoring, logistics for shipping viable biological/genetic resources, organisms</td>
</tr>
<tr>
<td>VII. Quality assurance &amp; quality control measures</td>
<td>□</td>
<td>Quality management</td>
<td>All types of biospecimen, sample, biological/genetic resource especially for endangered and at risk species</td>
</tr>
</tbody>
</table>
**COMMON ELEMENTS IN OTHER DATA SYSTEMS**
e.g. CMS, SPREC, LIMS

<table>
<thead>
<tr>
<th>ELEMENT CODES</th>
<th>REPORTING DATA FOR CREATING A GENERIC BIODIVERSITY BRISQ</th>
<th>EXAMPLES OF BRISQ ELEMENTS FOR BIODIVERSITY SAMPLES MAINTAINED IN REPRESENTATIVE TYPES OF NON-VIABLE (MUSEUM) AND VIABLE (CULTURE) COLLECTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. PRE-ACQUISITION - ASSOCIATED SAMPLE DATA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>I.a</strong></td>
<td><strong>Type of institution</strong></td>
<td>Institution/organization type and primary context in which the biospecimens, samples or organisms are acquired, exchanged, donated, on loan or accrued.</td>
</tr>
<tr>
<td><strong>I.b</strong></td>
<td><strong>Selection criteria (sample)</strong></td>
<td>Scientific, research and usability criteria used to inform, choice of biospecimen, organism for preservation and/or conservation.</td>
</tr>
<tr>
<td><strong>I.c</strong></td>
<td><strong>Selection criteria (quality)</strong></td>
<td>Sample quality standards for acceptance and rejection criteria.</td>
</tr>
<tr>
<td><strong>I.d</strong></td>
<td><strong>Collection modality</strong></td>
<td>Preservation of non-viable biospecimens in frozen, desiccated, fixed, pinnied, spirit (LIMS, absolute ethanol, formaldehyde, formalin, molecular (RNLater) collections.</td>
</tr>
<tr>
<td><strong>I.e</strong></td>
<td><strong>Collection category</strong></td>
<td>Biospecimen, sample, biological resource, genetic resource, Type, reference, voucher.</td>
</tr>
<tr>
<td><strong>CMSSPREC</strong></td>
<td><strong>MUSEUM COLLECTIONS</strong></td>
<td><strong>CULTURE COLLECTIONS</strong></td>
</tr>
<tr>
<td><strong>(a)</strong></td>
<td>Accession-strain identifier or unique curatorial ID</td>
<td></td>
</tr>
</tbody>
</table>

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| CMS, LIMS | l.f | **Provenance**  
Documented authentication.  

**2.1.f.1 History of Ownership.** Formal documented evidence providing historical context of ownership. Legal provenance records used for specimen authentication in archival inventories demonstrating chronological, traceable chains of custody as sequences of formal ownership (e.g. transfer of title), including location/storage attached to primary samples, all derivatives and downstream processing requests. Compliant with International conventions (CBD, Nagoya Protocol ABS, CITES) and permits for collecting the specified species in the specified area, and MTA agreements to share, utilise/analyse the specimens collected with the country of origin.  

**2.1.f.2 Place of Origin.** Place of origin and/or sample site (including GPS) of strains, isolates, propagules, plants, explants, seeds, organisms that are used to generate culture collections that comprise expertly preserved, authenticated viable cell lines, replicable cultures, microbial strains or whole organisms of known provenance (origin). GPS location of the collection site, descriptive details of habitat, ecological zone e.g. marine, freshwater aquatic, water chemistry; terrestrial e.g. soil type, vegetation type; substrate e.g. lithosphere, cryosphere. Habitat attributes: geological geographical features, topography, slope, depth, altitude, sedimentary, eDNA, soil. |
| CMS, LIMS | l.g | **Time scale**  
Collection timelines  

Geological timescales (GTS). Deep time denoted as epochs with distinctive features (stratigraphy) used by geologists, palaeontologists, to record the Earth’s history as timelines depicting specific events. Epoch (10s millions of years); ancient (millennia), archival (centuries), vintage (decades). Present time to biopreservation timescales (days - multiple decades) representing storage regimes. Medium term storage (MTS - months 1 - 2 years) in expansion, distribution, working, and active collections; long-term storage; (LTS - multiple decades) in base or master collections (cryobanks – ad infinitum). |
| CMS, LIMS | l.h | **Taxonomy**  
Taxa - species conserved.  

All kingdoms represented, identified to species, sub-species; taxonomic identifier authenticated by taxonomic ID guarantee, see VII.b, metagenomics, eDNA. Individual or groups of taxa representing thematic (microbial, protist, plant, animal) or functional, socioeconomic (crops, pathogens, yeasts, forestry, environmental) collections, identified to species, sub-species; strain, cultivar, genotype level as appropriate. |
| CMS, LIMS | l.i | **Biological donor**  
Donor of original sample, specimen.  

Individual organism, historical or ancient specimens, present time, fresh. Multiple or individual donors from specific or multiple taxa, associated species/organisms, parasites, symbionts, assemblages. Sub samples collected from in situ populations. |
| CMS, LIMS | l.j | **Anatomical site**  
Organ or tissue of origin of sample.  

Any anatomical part (bone, muscle, hair, horn) of an organism from where biospecimen, or sample is collected, usually non-viable and non-replicable. Cells, tissues, organs, spores, clonal propagule, totipotent germplasm (gametes, ova, pollen, sperm, oocytes, eggs), explant, seed, zygotic embryo, embryonic axis, shoot meristem, hyphae, mucilaginous matt, symbiotic or parasitic assemblage. |
| CMS, LIMS | l.k | **Vital state**  
Viable or non-viable  

Usually non-viable, mainly collected post mortem. Details of agonal state (physical condition immediately preceding death) and cause of death important for certain biospecimens (e.g. wildlife forensics, toxicology, epidemiology studies). Viable. |
| CMS, LIMS | l.l | **Physiological & developmental state**  
Functional status - morphogenetic, growth, developmental competence, totipotency.  

Non-viable, replicable (DNA) from dead cells. Viable/non-culturable or viable/culturable; autotrophic, heterotrophic, mixotrophic, log, lag, stationary culture, sporulating, meristematic, totipotent i.e. capacity to regenerate whole new cells, organs, organism. Morphogenetically and biosynthetically (1˚ - 2˚ metabolism) competent. |
| CMS, LIMS | l.m | **Gender**  
Male, female, hermaphrodite. |
<table>
<thead>
<tr>
<th><strong>l.n</strong></th>
<th><strong>Life cycle &amp; reproductive state</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage in life cycle when sample was taken from organism or functional part (seed, fruit, pollen, embryo) of organism.</strong></td>
<td></td>
</tr>
<tr>
<td>Juvenile, mature, quiescent, ageing, senescent; sexual or asexual; life cycle stage: gametophyte (haploid), sporophyte (diploid); clonal propagule (e.g. bulb, corm, tuber); sexual hybrid (e.g. mature/immature seed, ripe/unripe fruit).</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>l.o</strong></th>
<th><strong>Health and nutritional status</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Healthy or sub-optimal condition.</strong></td>
<td></td>
</tr>
<tr>
<td>Healthy or damaged, injured, traumatized, stressed (biotic, abiotic), nutrition optimum or nutritionally compromised.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>l.p</strong></th>
<th><strong>Toxicology status</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Xenobiotic exposure.</strong></td>
<td></td>
</tr>
<tr>
<td>No exposure, or exposed to pollutants, toxins, poisons, xenobiotics, radiation.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>CMS</strong></th>
<th><strong>l.q</strong></th>
<th><strong>Axenicity</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Axenic - free from other organisms.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axenic or non-axenic e.g. systemic, covert, endogenous co-contaminants, symbiotic partnerships, mycorrhizae, obligate /non-obligate parasitic associations (see Disease and Pathology Status <strong>l.r</strong>).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>l.r</strong></th>
<th><strong>Disease &amp; pathology status</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnostic test outcomes required for epidemiology and risk management.</strong></td>
<td></td>
</tr>
<tr>
<td>Disease free state confirmed; presence of parasites, pests, poxes test-confirmed.</td>
<td></td>
</tr>
<tr>
<td>Disease-free or infected with pathogens, mycoplasmas, phytoplasmas, viruses, retroviruses, bacteria, yeast, fungi, pests; test-confirmed as a pathological positive test or certified healthy, pathogen, virus, pest-free.</td>
<td></td>
</tr>
</tbody>
</table>

### II. ACQUISITION, STABILIZATION & TRANSPORT

<table>
<thead>
<tr>
<th><strong>CMS, LIMS, SPREC</strong></th>
<th><strong>l.a</strong></th>
<th><strong>Collection &amp; sample container</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>How samples are obtained from field site, conditions to which exposed.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyethylene bottle/bag/tube, glass tube, jar, sterile bottle, Petri dish, cryovial. Sterile scalpel, small muscle section, multiple others.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyethylene bottle/bag/tube, glass tube, jar, sterile bottle, Petri dish, cryovial. In vitro field collection, climbing tree, ground, soil, water, snow, litho sampling.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>LIMS, SPREC</strong></th>
<th><strong>l.b</strong></th>
<th><strong>Time</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>From collection to stabilization.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minutes, hours, days, months.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minutes, hours, days, months.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>CMS, LIMS, SPREC</strong></th>
<th><strong>l.c</strong></th>
<th><strong>Stabilization</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>How samples are stabilized during and immediately after collection from the field.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF, buffer on wet ice, ± multiple others. Humane euthanasia using chemicals and drugs under licensed procedures e.g. Insects: killing jar with alcohol or ethyl acetate; fish/amphibians: tricaine methanesulphonate, benzocaine hydrochloride, 2-phenoxyethanol. Snap freezing in LN2/dry shipper.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature ambient, refrigerated, on ice, in LN (Dry Shipper); ± low RH dried, desiccated (air, chemical desiccant, silica gel) to a specific MC (e.g. 5 - 15% fresh weight); disinfection, antifungal, antimicrobial treatments; dark, light, low light, photoperiod.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SPREC</strong></th>
<th><strong>l.d</strong></th>
<th><strong>Shipping parameters stage 1</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transport container and conditions, time in transit before interim storage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient or controlled temperatures, Dry LN shipper; dry ice, desiccant, silica gel; minutes, hours, days, months. Log of environmental conditions (T°C, RH, light).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient, controlled environment facility or container; minutes, hours, days, months. Log of environmental conditions (T°C, RH, light).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SPREC</strong></th>
<th><strong>l.e</strong></th>
<th><strong>Interim storage</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Storage container, duration, RH; temperature, light</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass tube, jar, bag, cryovials; minutes, hours, days, months; ambient, low temperatures 4°C, -20°C, -80°C; 80%; RH &lt;40% with silica gel; light, dark, diffuse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyethylene bottle/bag, glass tube, jar, sterile bottle, Petri dish cryovials; minutes, hours, days, months, ambient, low temperatures 4°C, -20°C, -80°C, RH: 80%, 15%; light, dark, diffuse, photoperiod.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SPREC</strong></th>
<th><strong>l.f</strong></th>
<th><strong>Shipping parameters stage 2</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transport container and conditions, time in transit before interim storage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient or controlled temperatures, Dry LN shipper; dry ice, desiccant, silica gel; minutes, hours, days, months. Log of environmental conditions (T°C, RH, light).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient, controlled environment facility or container; minutes, hours, days, months. Log of environmental conditions (T°C, RH, light).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| SPREC | II.g | Short-term storage  
Storage container, duration, RH temperature, minimal growth. Storage of sample at main biorepository. | Glass tube, jar, bag, cryovials, mins, hours, days, weeks, glass tube, jar, bag. ambient, controlled, 25°C, 4°C, -20°C, -80°C. 80%, <40% with silica gel. limiting growth conditions N/A for non-viable collections. | Glass tube, jar, bag, cryovials; mins days, weeks, months; ambient, 25°C, 4°C, -20°C, -80°C, LN. 80%, 15%; minimal nutrients, light, growth factors, T°C |
| CMS, LIMS | III.a | Preparation  
Procedures used to prepare and prevent sample deterioration before storage; duration of exposure to treatments Chemical preservatives prevent degradation by pests, microorganisms, limit chemical reactions oxidation, hydrolysis, physical treatments arrest biological activity and eliminate pests. | Multiple preparations. Organisms or biospecimens prepared in IMS, 80% (v/v) ethanol; molecular collections prepared in absolute ethanol, RNALater, DMSO/NaCl buffers. Low temperature pre-treatments (-20°C) dehydration, low RH evaporative desiccation (silica gel), freeze drying, inert atmospheres, low O₂. Treatment exposure times, minutes, hours, days, weeks. | N/A for viable, replicable collections. see section IV, V. |
| CMS | III.b | Chemical fixation  
Process by which biospecimens are ‘fixed’ to preserve them as close as possible to original state. Altering biochemical state to preserve the physical form. | Multiple procedures. Zoological spirit collections: formalin/formaldehyde fixation, transferred to spirit; tissue slices: FFPE slide preparations; chemical dehydration using glutaraldehyde fixation; CPD for SEM samples and pinned insects; fresh cells lysed and DNA fixed on a paper matrix by chemicals in FTA cards. | N/A for viable, replicable collections. see section IV, V. |
| LIMS | III.c | Preservation by desiccation and drying  
Reducing sample MC to preserve original structures, morphological attributes before storage | Chemical dehydration CPD for SEM (pinned insects); herbarium plant mounting freezing at -30 °C for 1 week, dehydration in drying room (days), mounted on acid free paper in plant presses. | N/A for viable, replicable collections. see section IV, V. |
| CMS, LIMS | III.d | Flash / snap freezing  
Ultra rapid cooling to fix the biomolecular state of the original sample before transferring to terminal storage temperatures. | Plunge whole specimen or sub-sampled tissue from non-viable specimen into liquid LN or vapour phase LN in dry shipper. | Some viable orthodox seeds, pollen, spores, cysts, dormant buds, extremophile microorganisms can be preserved using ultra rapid cooling without the need for cryoprotection and recovered in the viable state after storage see section V. |
| III.e | Preservation at low temperatures  
Methods used to preserve non-viable samples at low and ultra low (cryogenic temperatures). | Multiple methods. Preserved using water or buffers, with or without protective additives applied at various temperatures, exposure times, cooling rates. | N/A for viable collections see section Va, Vb |
| SPREC | III.f | Long-term storage  
Conditions in which (usually) non-viable specimens are permanently stored in ambient, or environmentally controlled conditions or at low and ultra low temperatures. | Multiple types of storage. Storage vessel, sample container (e.g. cryovials, cryotank). Long-term storage of biospecimens, organs, organisms in IMS spirit collections at 15°C < flash point of ethanol; plant material stored at RT with desiccant, traditional dry stores: ambient, 40% RH (optimal), molecular collections stored at ambient temperatures, various RH (+ with silica gel); FTA card, freeze dried material stored in RH and O₂ controlled cabinets; frozen collections stored at 4°C, -20°C, -80°C, -196°C, LN vapour. | Some (e.g. orthodox seeds, pollen, spores, cysts, dormant buds, extremophile microorganisms) viable cells, tissues, organs, organisms can be stored at low MC at ambient or low temperatures without the need for cryopreservation and recovered in the viable state after storage. see section IV, V. |
### III. Freeze/thaw Parameters/cycles

**Conditions to which specimens are exposed when thawed; number of freeze/thaw cycles.**

Multiple types of freeze/thaw conditions slow, rapid, stepwise rewarming, temperatures, times at which specimens are held between thawing an analysis and re-freezing. Number of freeze/thaw cycles.

*N/A for viable collections see section Va, Vb*

### IV. IN VITRO CULTURE

**LIMS SPREC**

#### IV.a Culture

- Isolation, disinfection, anti-microbial treatments, culture vessel, culture initiation, propagation, cultivation
- Subculture, serial culture, serial passage, regrowth, regeneration
- Controlled environment parameters. Procedures involved in the initiation, maintenance, (sub-culture, serial culture) regrowth, regeneration, transfer of replicable cultures.

*N/A for non-viable museum collections.*

Aseptic, *in vitro*, microbiological techniques for the sampling and isolation of microorganisms, protists, explants, animal cells; antimicrobials (disinfectants, bleach, hypochlorite solutions, surfactants, antibiotics, fungicides, miticides).

Culture vessel (size, type, sealant, ventilation). Media composition (macronutrients, micronutrients, gelling agents, hormones, vitamins, growth regulators, carbon source, antioxidants, special additives, pH) for culture initiation, proliferation, subculture and transfer regimes (to stimulate morphogenesis, regeneration or return to *ex vivo* growth).

RH, T °C, light intensity, irradiance, quality, photoperiod (dark, light, diurnal) environmentally-controlled (light, T °C, O2, CO2) regimes applied to simulate *in vitro* and natural life cycles (growth, clonal propagation, morphogenesis, embryogenesis, dormancy, rejuvenation, acclimation).

### V. CONSERVATION, STORAGE & RECOVERY

**LIMS SPREC**

#### V.a In vitro conservation

- Culture vessel, duration, temperature, RH, growth limiting conditions, sub-culture, serial culture regimes (transfer intervals)
- Procedures used to maintain viable active culture collections in serial culture or slow (arrested, limited) growth (medium-term storage).

*N/A for non-viable museum collections.*

Petri dish, glass jar, culture vessel (size, type, sealant, ventilation) months, years, ambient, 25 °C, 4 °C, -20 °C, -80 °C, RH, 80%, 20%, 15%, 5%; arrested metabolism, minimal nutrients, T °C light, growth factors, growth inhibitors; subculture cycle, weeks, months, years.

#### V.b Cryopreservation

- Pre-growth, pre-treatment cryoprotection regime, cooling regime, cryogenic state, temperature, storage duration
- Procedures used to establish and maintain base collections of viable cells, tissues, organs in long-term storage. Also termed cryo-conservation.

*N/A for non-viable collections.*

Osmotics and special additives, colligative, non-colligative cryoprotectants, alginate, controlled rate, rapid, ultra rapid cooling programmable freezer, Mr Frosty®, frozen, partially vitrified, vitrified, -196 °C (LN vapour phase > -130 °C), mechanical freezers, years, decades, multiple decades.
| SPREC | VI.a | Shipping temperature & conditions
Shipping parameters, freeze/thaw cooling/rewarming,
duration of thaw/rewarming, time from thaw/rewarming to end use;
temperature between thaw/use
Stabilizing conditions applied to samples transferred, relocated, dispatched to end user. | ambient, chilled (wet ice), -20°C (dry ice), -80°C (dry ice), -196°C (dry shipper), log of sample temp and shipment time. | In vitro cultures: ambient/chilled; cryopreserved: -196°C (or LN, vapour phase ca. >130°C), dry ice, Dry Shipper, log of critical cold chain parameters freeze/thaw cool / rewarming. |
| LIMS | VII.a | Quality management
Measures that assure sample quality outcomes, down-stream analyses.
QA/QC comprise validated measures [including acceptance / rejection thresholds, quality standards] used to test procedures and sample quality before and after storage, and dispatch, to assure fitness-for-purpose for end users. | Authentication and taxonomic ID guarantee (for museum collections) QA/QC fitness-for-purpose testing: multiple end point analyses and functional biomarkers, e.g. (viability, apoptosis, regrowth, totipotency, morphogenetic), metabolic, biosynthetic, epigenetic, genetic stability. Fulfilment of BRC principles: authenticity, purity, stability. Self assessments for QA/QC: pre-analytical variables / SPREC; BRISQ check lists. Incorporation of SPREC, BRISQ tools into QMS and CMS data bases. External quality assurance measures. |
<table>
<thead>
<tr>
<th>Sample type</th>
<th>Collecting method</th>
<th>Collecting container</th>
<th>In transit &amp; stabilization</th>
<th>Transit time</th>
<th>Isolation &amp; culture initiation</th>
<th>Cultivation procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerophytic</td>
<td>A</td>
<td>Glass bottle A</td>
<td>Ambient</td>
<td>1 - 2 days</td>
<td>A</td>
<td>Wire loop inoculation to agar slopes</td>
</tr>
<tr>
<td>Plankton</td>
<td>B</td>
<td>Polyethylene bottle B</td>
<td>Chilled (ice, refrigerated 0°C - 4°C)</td>
<td>3 - 5 days</td>
<td>B</td>
<td>Vortex algal drops in liquid medium, transfer to fresh liquid culture</td>
</tr>
<tr>
<td>Periphyton</td>
<td>C</td>
<td>Polyethylene bag C</td>
<td>Frozen (-18°C to -20°C)</td>
<td>1 week</td>
<td>C</td>
<td>DAS</td>
</tr>
<tr>
<td>Benthic</td>
<td>D</td>
<td>Unknown X</td>
<td>Hydrated, ambient</td>
<td>2 - 3 weeks</td>
<td>D</td>
<td>DAL</td>
</tr>
<tr>
<td>Epilithic</td>
<td>E</td>
<td>Unknown X</td>
<td>Hydrated, chilled (ice, refrigerated 0°C - 4°C)</td>
<td>1 month</td>
<td>E</td>
<td>CAS</td>
</tr>
<tr>
<td>Endolithic</td>
<td>F</td>
<td>Other Z</td>
<td>Hydrated frozen (-18°C to 20°C)</td>
<td>1 - 2 months</td>
<td>F</td>
<td>CAL</td>
</tr>
<tr>
<td>Epiphytic</td>
<td>G</td>
<td>Other Z</td>
<td>Ambient</td>
<td>2 - 3 months</td>
<td>G</td>
<td>DCS</td>
</tr>
<tr>
<td>Phycobiont</td>
<td>H</td>
<td>Other Z</td>
<td>Chilled + antimicrobial</td>
<td>3 - 4 months</td>
<td>H</td>
<td>Non-axenic: grown in Erlenmeyer flasks</td>
</tr>
<tr>
<td>Assemblage</td>
<td>I</td>
<td>Other Z</td>
<td>Frozen + antimicrobial</td>
<td>4 - 5 months</td>
<td>I</td>
<td>NXC</td>
</tr>
<tr>
<td>Snow</td>
<td>J</td>
<td>Other Z</td>
<td>Hydrated ambient + antimicrobial</td>
<td>≥ 6 months</td>
<td>J</td>
<td>Unknown</td>
</tr>
<tr>
<td>Hot springs</td>
<td>K</td>
<td>Other Z</td>
<td>Hydrated, chilled + antimicrobial</td>
<td>Unknown</td>
<td>X</td>
<td>Other</td>
</tr>
<tr>
<td>Intertidal</td>
<td>L</td>
<td>Other Z</td>
<td>Hydrated frozen + antimicrobial</td>
<td>Other</td>
<td>Z</td>
<td>Other</td>
</tr>
<tr>
<td>Urban building</td>
<td>M</td>
<td>Other Z</td>
<td>Unknown</td>
<td>Other</td>
<td>Z</td>
<td>Other</td>
</tr>
<tr>
<td>Unknown</td>
<td>X</td>
<td>Other Z</td>
<td>Other</td>
<td>Other</td>
<td>Z</td>
<td>Other</td>
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<tr>
<td>Other</td>
<td>Z</td>
<td>Other Z</td>
<td>Other</td>
<td>Other</td>
<td>Z</td>
<td>Other</td>
</tr>
</tbody>
</table>

Example codes for growth type, habit, ecological zone from which organisms are sampled:
- Aerophytic
- Plankton
- Periphyton
- Benthic
- Epilithic
- Endolithic
- Epiphytic
- Phycobiont
- Assemblage
- Snow
- Hot springs
- Intertidal
- Urban building
- Unknown

Example codes for type of collecting method (e.g. by hand, net, use of tools to remove from surfaces):
- Plankton net
- Squeezing
- Scraping
- Grasping
- Unknown

Example codes for type of containers used to collect samples:
- Glass bottle
- Polyethylene bag
- Polyethylene bottle
- Unknown

Example codes for how samples are stabilized in base camps, field sites; in transit to biobanks:
- Ambient
- Chilled (ice, refrigerated 0°C - 4°C)
- Frozen (-18°C to -20°C)
- Hydrated, ambient
- Hydrated, chilled (ice, refrigerated 0°C - 4°C)
- Hydrated frozen (-18°C to 20°C)
- Ambient + antimicrobial
- Chilled + antimicrobial
- Frozen + antimicrobial
- Hydrated ambient + antimicrobial
- Hydrated, chilled + antimicrobial
- Hydrated frozen + antimicrobial
- Unknown

Example codes for holding and transit times in and from base camps, field sites to biobanks:
- 1 - 2 days
- 3 - 5 days
- 1 week
- 2 - 3 weeks
- 1 month
- 2 - 3 months
- 4 - 5 months
- ≥ 6 months

Example codes for isolation & culture initiation:
- Wire loop inoculation to agar slopes
- Vortex algal drops in liquid medium, transfer to fresh liquid culture
- Comb-streak algae in drops on agar plates
- Wire loop transfer of algae to agar slopes
- Vortex algae in liquid medium, transfer to fresh liquid medium
- Combined comb + drop inoculation method

Example codes for cultivation procedure:
- Axenic: + antibiotics, grown in glass tubes
- Axenic: Dakin solution disinfection, grown in glass tubes
- Axenic: + antibiotics, grown in Erlenmeyer flasks
- Non-axenic: grown in glass tubes
- Combined comb + drop inoculation method

Example of simple codes for axenicity, antimicrobial treatments, cultivation

SPREC A-01 provides full technical details for initiation of cultures from free swimming, soil, substrate growth types

SPREC A-01 provides full technical details for culture regime, medium, vessel, environmental parameters (e.g. 18 - 20°C, 12 : 12 h light : dark photoperiod ; irradiance 30 - 40 µmol m^2 s^-1)