A. Biospecimen Deposit Guidelines

1. Introduction

The Biospecimens Deposit Guidelines describe the regulatory requirements, instructions, and required documentation for submitting H3Africa biospecimen collections to host H3Africa biorepositories for sharing with authorized researchers. The principles governing access to collections stored at host biorepositories are in the **H3Africa Data and Biospecimen Access Guidelines.**

2. Initiating a Deposit

H3Africa genomics researchers should contact their closest host H3Africa biorepository in their country or geographical region when a collection is ready for deposit. Contact information and status of the host biorepositories are as follows:

Biorepository	Principal Investigator (PI)	Status
Clinical Laboratory Services, Johannesburg South Africa	Dr. Elizabeth Mayne elizabeth.mayne@nhls.ac.za	Receiving biological samples for outside distribution after embargo period ends.
Makarere University, Kampala, Uganda	Dr. Moses Joloba mlj10@case.edu	Planning for receipt of biological samples.
Institute of Human Virology Nigeria, Abuja, Nigeria	Dr. Alash'le Abimiku aabimiku@ihvnigeria.org	Planning for receipt of biological samples.

3. Regulatory and Policy Compliance

The following requirements should be met prior to deposit:

a. **Ethics:** The Ethics Committee of the country of origin oversees the consent process and other ethical aspects related to the acquisition and subsequent use of biospecimens, including protection of study participant (donor) privacy and confidentiality of associated data. In cases where the local Ethics Committee must approve each new use of shared biospecimens, responsibility for obtaining approval lies with authorized users of the collection rather than the original study investigators.

b. Biospecimen Deposit Material Transfer Agreement (BDMTA): The PI and signing official of the submitting institution should sign the **BDMTA**, which establishes the terms and conditions of biospecimen transfer and use. The BDMTA is co-signed at the host biorepository and a signed copy is returned to the submitting institution. If required, submitting institutions may use their own MTA with similar terms and conditions,

addressing the purpose of transfer, and the requirements and restrictions for biospecimen use.

c. **Permits:** The submitting institution and host biorepository should ensure that they have applied for, received and exchanged the relevant import and export permits for biospecimen shipment and receipt. Copies of permits must be available at both sites.

4. Preparation of Shipments

a. **Local storage:** Submission sites must have appropriate local storage conditions; risk management processes and QA procedures relating to storage of DNA. Except where ambient temperature storage is used, DNA should be stored in a -80° C freezer monitored continuously or at least twice daily for temperature to ensure maintenance of the cold chain prior to shipping and during shipping. Freezers and boxes should be free of frost and mold. Adequately pre-cooled backup storage space should be available for emergency transfers in case of freezer failure.Liquid nitrogen should be considered as an alternative source for providing a pre-cooled temporary backup storage space in regions with frequent and unreliable power supply. Cryovialsshould be labeled in a way that protects donor privacy and confidentiality, in accordance with local laws and policies.

b. **Preparing biospecimens to ship to host biorepository:** Biorepository staff will work with each submitting site to assist submitting staff and address any site-specific issues. Ideally, one sample per biospecimen typeshould be submitted in a tightly sealed (preferably screw top)2D-etched cryovial or plain tube with barcoded label. As a general rule for all types of biospecimens, volume of the biospecimen should occupy up to 60% of the tube size chosen to avoid dehydration. Regardless of the tube type chosen, submission sites should ensure that individual cryovialshave a scannablebarcode and a matching human-readable number that uniquely identifies each biospecimen and enables its linkage to donor-specific phenotype information in associated de-identified data sets. Exceptions will be considered on a case-by-case basis. The barcode should directly contain or link to an accompanying dataset that directly contains8 variables in the following sequence:

1	2	3	4	5	6	7	8
Unique specimen ID	**De-identified participant ID**	**Study name**	**Prefix of country where collected**	**Specimen typeand aliquotID**	**Date of collection**	**Gender**	**Age at collection**

For DNA submissions, higher concentrations are more stable in the long term and therefore preferable. An ideal concentration would be 100 ng/µl, with a recommended range of 50 – 150 ng/µl and an acceptable range of 20 - 200 ng/µl.Furthermore, an ideal amount of DNA would be 5 micrograms (i.e., 5 µg, or

5000 ng), with a recommended range of 2 - 20 µg, and a minimum amount of 500 or 1000 µg. At the recommended concentration of 100 ng/µl and amount of 5 µg DNA, acryovialwould have a volume of 50µl of liquid.

c. Shipping costs and payment: Couriers base their quotes on kilograms, volume, distance and temperature requirements. The host biorepository will determine the most cost-effective courier and frequency of batch shipments, and will pre-pay the cost of DNA shipments from submission sites. While one aliquot with sufficient DNAis currently recommended,pediatric biospecimens or biospecimens collected from donors with a compromised health status may not yield the recommended amounts of DNA in a single aliquot. In such cases, the host biorepository will cover the cost of shipping multiple aliquots to achieve the recommended amount of DNA, regardless of the concentration or volume per aliquot. The submission sites should pre-pay shipping costs for other types of biological materials with adequate arrangement with the host biorepository, but may choose not to ship such samples if funds are not available.

d. **Shipping requirements/standards:** Biospecimens such as infectious substances, diagnostic specimens, genetically modified organisms and microorganisms are considered dangerous goods. Biospecimens should be classified according to all governing transport regulations to ensure safe packaging and handling. Air shipments should conform to International Air Transport Association (IATA) standards. Ground shipments should conform to applicable national standards. The courier should be consulted for assistance in figuring out the right requirements and to ensure compliance. All personnel involved in shipping should be trained for both air and ground shipments.

e. Shipping Conditions and Packaging: DNA shipments can be sent under the following conditions:

Ambient (15-25° C): use insulated packing to protect from extreme heat or cold conditions.

Refrigerated (2-8° C): use gel packs conditioned at -10° C designed for refrigerated conditions or reusable shipping boxes.

Frozen (-20^{o}C or -45^{o}C): Use frozen gel packs conditioned to maintain a frozen state of the biospecimens or reusable shipping boxes.

Frozen (-80° C): use dry ice pellets at the bottom, middle and top of shipper. Shipment within African countries might require replacement of dry ice or gel packs by shipper due to unanticipated delays with customs. Make sure that the shipper provides this option or that you can arrange for this to be done.

Shipments that have critical temperature requirements should include a device that can verify temperatures throughout the transport cycle. The quantity of biospecimens

shipped will affect the type of packing and amount of refrigerant needed to maintain the cold chain. Container size should be appropriate for the amount of refrigerant needed and number of biospecimens shipped, as described in the Sample Shipping and Transportation Standardized Operating Procedure (SOP).

Batch shipments should be sent quarterly, or else the frequency can be based on time needed to fill one container or other considerations, but should be discussed with the host biorepository first. The submission site should consult the courier and host biorepository with questions, and refer to the relevant SOP and shipping guidelines for further information.During biospecimen transfer, the submission site and host biorepository will share responsibility for tracking shipments.

The impact of freeze/thaws on biospecimen integrity must be carefully considered when determining shipping conditions. Freeze/thaw cycles compromise integrity and downstream analysis. As much as possible, maintain the temperature at which the samples were stored for shipment by using frozen ice packs and dry ice.

5. Submission of Collections

Regulatory documentation: The study informed consent and ethics approval, BDMTA and import/export permits should be exchanged. The BDMTA only needs to be signed once and exchanged with consent and ethics documents when the first batch of biospecimens are submitted to the host repository, unless local requirements specify otherwise.

a. **Forms submitted by biospecimen submission sites:** To initiate and document shipments, thesubmission site should notify the host biorepository of a pending shipment and prepare the following required forms. The Shipment Checklist is for internal use at the submission site to prepare the shipment. The Shipment Manifest and the Shipment Receipt Confirmation and Query Form should be sent by email to the host biorepository at time of shipment.

H3Africa Submission Forms	**Purpose**
Shipment Checklist	Documents that all deposit requirements have been met by the submission site
Shipment Manifest	Details the shipment contents
Shipment Receipt Confirmation and Query Form	Documents hand over of a shipment to the courier

The submission site may also send the H3Africa PI Input Questions on Future Use of Biospecimens Collections. This is an optional form for providing recommendations to the Data and Biospecimen Access Committee and potential users of the biospecimencollection. The submission site should also send the courier's waybill

number, and include printed copies of the commercial invoice and permits with the shipment.

c. **Submitting associated data:** Biospecimens deposited in the host biorepository must be accompanied by a minimum set of associated data to annotate the collection. The minimum set includes the variables in the barcode described in section 4b and the fields in the Shipment Manifest Form. The submission site may use a web-based laboratory information management system (LIMS) to transfer the minimum data or generate a HL7 file. If interconnectivity with the biorepository LIMS is not possible, an Excel or Access workbook may be sent by email. The workbook must be password protected to prevent mismanagement of data. The data checklist inside the Shipment Checklist Form may be used to facilitate dataset preparations.

   In addition to receiving the minimum associated data in the 8-variable barcode andthe other information on the Shipping Manifest Form, the host biorepository will work with the submission site to transfer available biospecimen-level quality control (QC) data such as DNA QC test results and SPREC codes for various biospecimen types. Aggregate information about the collection, such as study description and informed consent or local restrictions applicable to biospecimen sharing, will also be obtainedto describe the collection in theH3Africa Biospecimen Catalogue.

d. **Biospecimen mapping across data systems:**The host biorepository will work with submission sites and H3ABioNetto post descriptive study-level information, as well as de-identified individual-level and biospecimen-level information to the H3Africa Biospecimen Catalogue, so that potential users can request access to biospecimens of interest and link them to corresponding phenotype-genotype data housed at the European Genotype Archive (EGA).

   Once a biospecimen is submitted to the host biorepository or data from a biospecimen are submitted to H3ABioNet, an H3Africa Biospecimen Catalogue number will be assigned to that biospecimen. This catalogue number may be generated from a combination of the Participant ID and study name 2 digit code, the unique specimen ID, or a newly generated unique identifier in cases where such a number is required for de-identification according to ethical or regulatory requirements. It is important that the required identifiers are provided at both biospecimen and data submission points in order for H3ABioNet to maintain the appropriate records associated with each catalogue number and ensure accurate mapping between biospecimens, data, and EGA accession numbers. In addition, when new identifiers are generated, the submission site must document and audit their internal processes, and ensure that at least two internal people are capable of performing mapping, since only the site will know key linking information.

6. QC at Submission Sites and Host Biorepositories

a. Submission sites: While quality assurance measures such as SOPs, training, and validation of equipment and assays help to optimize biospecimen integrity, QC testing helps assess the quality of biospecimens.QC testing should be performed on all DNA biospecimens intended for deposit in H3Africa biorepositories. Biospecimen-level QC results for all samples should be transferred to the host biorepository along with the minimum associated data in the Shipment Manifest Form. As QC procedures may differ by site, if QC results other than concentration and purity are sent, the content of that QC data file will be determined through discussions with the host biorepository. All DNA concentration and purity results must be documented in the Shipment Manifest. Submission sites must communicate in advance with the host biorepository regarding the format in which other types DNA QC results will be submitted (such as gels).

DNA purity and concentration of the QC biospecimens should be determined using H3Africa SOPs.If available, gel electrophoresis is also recommended to test for degradation.QC on serum and plasma biospecimens entails visual grading for turbidity (lipemia) and hemolysis according to the Plasma and Serum Visual Grading SOP. At this time, there is no recommended QC test for urine biospecimens and submission sites should discuss this directly with the host biorepository.

b. Host biorepository: During the first year of biospecimen deposit, 10% of received DNA biospecimens in a shipment will be randomly subjected to QC testing. After one year, 1% should be tested. However, the host biorepository may expedite this timeline if the first two shipped batches meet high quality standards.

Specifically, for DNA, 90% of the tested biospecimens should have an A260/280 of 1.7-2.0, and be of high MW by gel electrophoresis. An acceptable QC outcome indicates that the submission site prepared high quality DNA and the shipment was maintained as per IATA requirements. After QC evaluation and acceptance, DNA biospecimens will be divided into two or more aliquots for storage in separate independent freezers. Any QC deviations or indicators of inferior quality, including trends, will be documented in the Shipment Receipt Confirmation and Query Form, and sent to the submission site for investigation and resolution. The host biorepository will work with the submission site to implement a corrective action plan if a shipment does not meet the above standard. Collections with persistent serious QC deviations may be declined and eliminated from the H3Africa Catalogue.

For serum and plasma, hemolysis of the samples will be graded 0 (no hemolysis) to 3 (significant hemolysis) at the biorepository and specific issues will be discussed with the PI. As with DNA, QC deviations for serum, plasma and other types of biospecimens will be addressed through a corrective action plan before a final decision is made to accept or reject the collection.