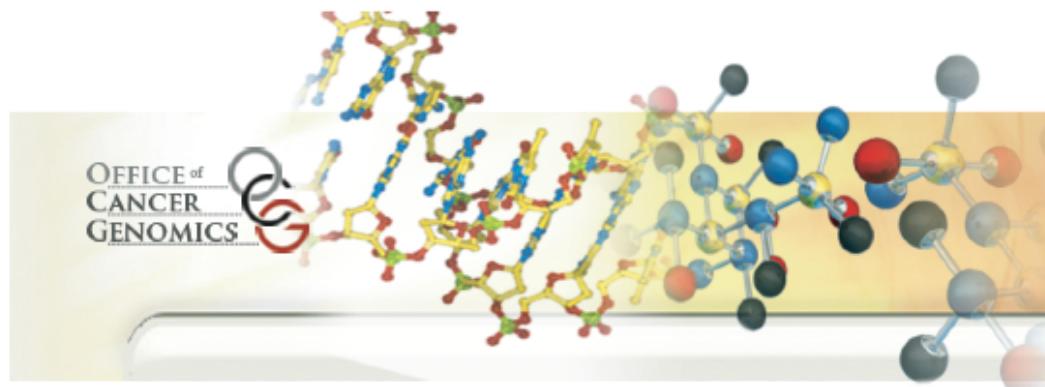


National Cancer Institute



# **OCG TUMOR MOLECULAR CHARACTERIZATION PROJECTS**

## **STANDARD OPERATING PROCEDURES MANUAL (version 3.0 11/14/2012)**

U.S. DEPARTMENT  
OF HEALTH AND  
HUMAN SERVICES  
National Institutes  
of Health



# OCG Tumor Molecular Characterization Projects

## Standard Operating Procedures Manual

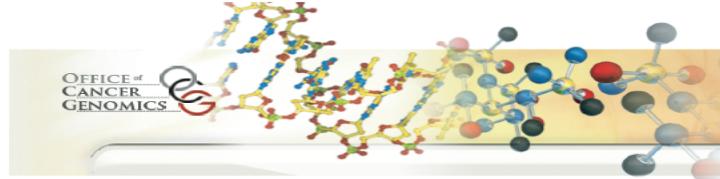
NOTE: The pages listed below can be accessed directly using the bookmark menu in the left side bar of your PDF reading application.

<b>Table of Contents</b>	<b>iii</b>
<b>Preface</b>	<b>vii</b>
<b><u>Templates</u></b>	<b>1</b>
<b>Template #101:</b> HTMCP Biology Protocol Template	<b>3</b>
<b>Template #102:</b> Suggested language for <u>prospective</u> tissue collections in genomic-scale projects	<b>13</b>
<b>Template #103:</b> Suggested language for <u>retrospective</u> tissue collections in genomic-scale projects	<b>19</b>
<b>Template #104:</b> Material Transfer Agreement (MTA) between Tissue Source Site and Processing Facilities	<b>27</b>
<b>Template #105:</b> Institutional Certification Letter for Adult Cancers	<b>35</b>
<b>Template #106:</b> Institutional Certification Letter for Pediatric Cancers	<b>37</b>
 <b><u>HTMCP General Protocols</u></b>	 <b>39</b>
<b>SOP#100:</b> Document requirements for sample submission to the HTMCP project	<b>41</b>
<b>SOP#102:</b> Processing tissue for molecular characterization of HIV+ Tumors	<b>43</b>
<b>SOP#103:</b> Processing blood samples for the HIV+ tumor characterization project.	<b>47</b>
<b>SOP#104:</b> Shipping cryoports containing frozen biosamples for processing and extraction of nucleic acids	<b>51</b>

<b>SOP#105:</b> Sample shipping guidelines for the HIV+ tumor characterization project	<b>57</b>
<b>SOP#106:</b> Sample identifier standards for the HIV+ tumor characterization project	<b>59</b>
<b>SOP#108:</b> Disposition form for remaining macromolecules/tissues contributed to the HIV+ tumor characterization project	<b>61</b>
<b>SOP#109:</b> Very useful Instructions on how to complete a study protocol request to the institutional Review Board (IRB) for the HIV+ Tumor Molecular Characterization Project	<b>63</b>
Data release policy for the HIV+ tumor molecular characterization project	<b>65</b>
HTMCP DLBCL Contact Sheet	<b>69</b>
HTMCP Lung Contact Sheet	<b>71</b>
HTMCP Cervical/Anal Contact Sheet	<b>73</b>
<b><u>HTMCP Tumor Specific Protocols</u></b>	
<b>A) Diffuse Large B-Cell Lymphoma</b>	<b>75</b>
<b>SOP#101A:</b> Prospective sample submission procedure for the HTMCP Project	<b>77</b>
Tissue Requirements	<b>81</b>
Clinical Data Elements	<b>83</b>
<b>SOP#107A:</b> Centralized pathology review process for HIV+ tumor molecular characterization project.	<b>91</b>
<b>SOP#201A:</b> Sectioning tissue for molecular characterization of HIV+ Tumors	<b>95</b>
<b>B) Lung Cancer</b>	<b>99</b>
<b>SOP#101B:</b> Prospective sample submission procedure for the HTMCP Project	<b>101</b>
Tissue Requirements	<b>105</b>
Clinical Data Elements	<b>107</b>
<b>SOP#107B:</b> Centralized pathology review process for HIV+ tumor molecular characterization project.	<b>113</b>

<b>C) Cervical Cancer</b>	<b>117</b>
<b>SOP#101C:</b> Prospective sample submission procedure for the HTMCP Project	<b>119</b>
Tissue Requirements	123
Clinical Data Elements	125
<b>SOP#107C:</b> Centralized pathology review process for HIV+ tumor molecular characterization project.	<b>133</b>
<b>D) Anal Cancer</b>	<b>137</b>
<b>SOP#101D:</b> Prospective sample submission procedure for the HTMCP Project	<b>139</b>
Tissue Requirements	143
Clinical Data Elements	145
<b>SOP#107D:</b> Centralized pathology review process for HIV+ tumor molecular characterization project.	<b>153</b>
<b><u>BLGSP Protocols</u></b>	<b>157</b>
<b>SOP 001</b> BLGSP Document Requirements for Sample Submission	<b>159</b>
<b>SOP 002</b> BLGSP Tissue Processing Protocol	<b>163</b>
<b>SOP 003</b> BLGSP Sample Submission Process	<b>167</b>
Clinical Data Elements	174
<b>SOP 004</b> BLGSP Blood Processing	<b>179</b>
<b>SOP 005</b> BLGSP Sample Shipping Guidelines	<b>183</b>
<b>SOP 006</b> BLGSP Shipping Protocol	<b>185</b>
<b>SOP 007</b> BLGSP Naming Standard	<b>189</b>
<b>SOP 008</b> BLGSP Instructions to complete IRB protocol	<b>191</b>
<b>SOP 009</b> BLGSP Pathology Review	<b>193</b>
<b>SOP 010</b> BLGSP Hematoxylin & Eosin Staining Protocol	<b>197</b>





Dear Colleague,

You are about to review the latest version of the National Cancer Institute Office of Cancer Genomics book of Standard Operating Protocols (SOPs) that should be followed when you contribute samples and data to our large-scale genomic characterization project(s).

The sample and data acquisition process is explained in comprehensive detail to ensure that all materials contributed will be of sufficient quality to be utilized in the projects. However, the actual process is simple and requires only six basic steps:

1. Creation of an IRB approved protocol and informed consent forms.
2. Institutional Certification of patient consent.
3. Acquisition and freezing of tumor samples.
4. Acquisition and freezing of patient-matched normal samples (e.g., blood).
5. Acquisition of unstained formalin-fixed paraffin-embedded sections for pathology review.
6. Shipment of tissues and data.

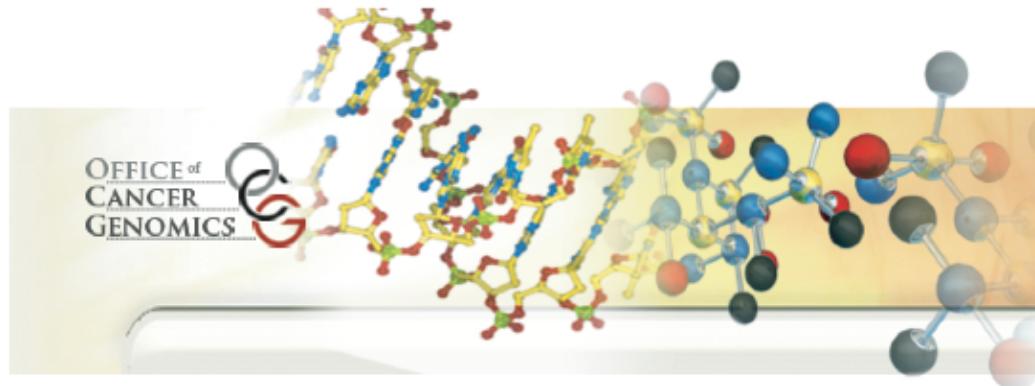
The book is divided into general protocols and templates that apply to all projects, as well as tissue/disease specific ones. Although many protocols are included in this book, only a handful of them may apply to yourself, depending on your role in the acquisition process :

- Clinical Practitioners:
  - IRB approved protocol and informed consent templates.
  - General guidelines on the process and clinical data requirements (SOP#101).
- Institutions:
  - Institutional certification letter.
  - Material Transfer Agreement (MTA) template and instructions on how to fill it (SOP#109).
- Laboratory or research personnel:
  - General guidelines on the process and clinical data requirements (SOP#101).
  - Processing tissue for molecular characterization (SOP#102).
  - Processing blood samples (SOP#103).
  - Shipping frozen biosamples in cryoports (SOP#104) and FFPE slides for pathology review (SOP#107)

Should you require any clarification on the protocols and/or process, please do not hesitate to contact the appropriate OCG personnel listed in your SOPs



National Cancer Institute



## GENERAL CLINICAL TEMPLATES

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## HIV+ Tumor Molecular Characterization Project (HTMCP)

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**Statistician**

## Table of Contents

Section	Page
1.0 Schema	3
2.0 Background and Rationale	4
2.1 HIV-Associated Malignancies	4
2.2 Rationale	5
3.0 Objectives	5
4.0 Eligibility Criteria	5
5.0 Sample and Data Acquisition and Processing	6
5.1 Tumor Sample Acquisition	6
5.2 Matched Normal Tissue Acquisition	6
5.3 Sample and Data Storage	7
5.3.1 Sample Identification and Assurance of Anonymity	7
5.3.2 Storage and Release of Samples and Medical Information	7
5.4 Sample Shipment	7
5.5 Research Plan Outline	7
5.6 Clinical Data Collection	8
5.7 Data Dissemination	8
6.0 Financial Compensation/Costs	8
7.0 Patient Risks / Benefits	8
7.1. Potential Benefits of Participating in the Project	8
7.2. Potential Risks of Participating in the Project	9
7.2.1 Physical Risks	9
7.2.2. Psychological or Social Risks Associated with Loss of Privacy	9
8.0. Project Results	9
9.0. Alternatives to Participating in the Project	9
9.1 Voluntary Participation	9
9.2 Withdrawal from the Project	9

## 1.0 Schema

### Tumors to be accrued:

- HIV-Associated Diffuse Large B Cell Lymphoma
- HIV-Associated Non-Small Cell Lung Cancer
- HIV-Associated Cervical Cancer
- HIV-Associated Anal Cancer

### Procedures:

- Samples (tissues) to be obtained prior to oncologic treatment (e.g. neo-adjuvant therapy):
  - Tumor tissue biopsy, tissues from surgical resection and/or tumor bone marrow aspirate (for lymphomas)
  - Case matched normal peripheral blood mononuclear cells; buccal cells or adjacent normal tissues. Blood mononuclear cells are purified and frozen  
All tissues must be snap frozen
- Tissue block or unstained slides from formalin fixed, paraffin-embedded (FFPE) tissue (tumor and/or adjacent normal) and/or bone marrow biopsy must be available

### Sample Distribution:

- Frozen tissues, bone marrow, and/or peripheral blood mononuclear cells will be shipped to British Columbia Genome Science Center, Vancouver, Canada.
- Unstained slides of formalin fixed tissue and/or bone marrow biopsy will be shipped to the appropriate designated central pathology lab.

### Data Submission:

- Clinical report forms are submitted to the NCI Data Coordinating Center.

## 2.0 Background and Rationale

### 2.1 HIV-Associated Malignancies

HIV infection is associated with a variety of malignancies, including “AIDS-defining cancers” and “non-AIDS-defining cancers” [1]. The AIDS-defining cancers are non-Hodgkin’s lymphomas, Kaposi’s sarcomas, and cervical cancer. AIDS-defining non Hodgkin’s lymphomas are predominantly diffuse large B-cell lymphomas, Burkitt’s lymphomas, and less commonly primary effusion lymphomas and plasmoblastic lymphomas. Non-AIDS defining cancers that are increased in prevalence among HIV-1 infected individuals include anal carcinomas, Hodgkin’s lymphomas, non-small cell lung cancers, and hepatocellular carcinomas.

The cause for increased prevalence of malignancies in HIV-1 infected individuals is poorly understood, and no systematic molecular characterization of these neoplasias has been reported to date. Many HIV-associated malignancies are also associated with other oncogenic virus infections. These include members of the human papilloma viruses and gamma herpes viruses, including Epstein-Barr virus and Kaposi’s sarcoma herpes virus (KSHV), however not all AIDS associated malignancies have been linked to such co-infections. Viruses are associated with a variety of malignant and pre-malignant conditions [2]. Human papilloma viruses are the cause of almost all anogenital carcinomas, and approximately 50% of oral malignancies [3, 4]. Epstein-Barr virus is associated with Burkitt’s lymphoma, nasopharyngeal and gastric carcinomas, NK/T cell lymphomas, AIDS lymphomas, Hodgkin’s lymphomas, post-transplant lymphoma, and pediatric AIDS-associated leiomyosarcomas [5]. KSHV (human herpes virus 8, HHV8) is associated with Kaposi’s sarcoma, primary effusion lymphomas, and multicentric Castleman’s disease [6]. Human T-cell leukemia virus (HTLV) type 1 causes adult T-cell leukemia and HTLV-associated myelopathy, as well as pneumopathy, uveitis, and immunosuppressive conditions [7]. A recently discovered polyoma virus, Merkel’s carcinoma virus, is associated with the majority of cases of Merkel’s neuroendocrine skin malignancies. Hepatitis viruses type B (HBV) and C (HCV) are associated with hepatocellular carcinoma, and HCV is also associated with splenic marginal zone lymphomas. Another recently identified virus, xenotropic murine leukemia-related virus (XMRV) may be associated with human prostate malignancy and chronic fatigue syndrome, although this remains controversial [8]. Other viruses have been implicated in collagen vascular, hepatobiliary, and other malignancies, but definitive information is currently lacking [9, 10]. These infections may be pathogenic in immunosuppressed individuals as a result of an impaired cell-mediated immune response resulting in chronic and incompletely suppressed infection. Malignancies may also arise from cytokine release from activated T cells induced by HIV infection or other opportunistic infectious agents complicating HIV infection.

HIV-1 and -2 are associated with immunodeficiency, which predisposes individuals to infections by opportunistic infectious agents, including oncogenic viruses. HIV-associated immunodeficiency also inhibits anti-tumor mechanisms that result in an increased frequency of a variety of tumors [11, 12]. Thus, HIV-1 infection is associated with markedly increased prevalence in AIDS-defining malignancies, such as Kaposi’s sarcoma, non-Hodgkin-s lymphoma, and cervical malignancies, as well as increased prevalence of non-AIDS defining malignancies, including Hodgkin’s lymphoma, anal carcinomas, as well as plasma cell neoplasms, hepatocellular malignancies, lung and testicular malignancies. The effects of HIV and other viruses on mechanisms of tumorigenesis remain to be defined, and this information may provide a solid foundation for new therapeutic approaches.

Comprehensive sequencing of genomes and transcriptomes in cancers that arise in HIV-infected individuals through the HIV+ Tumor Molecular Characterization Project (HTMCP, [http://cgap.nci.nih.gov/Cancer\\_Types](http://cgap.nci.nih.gov/Cancer_Types)) may provide a starting point for a systems biology approach towards understanding differences in pathway activation among identical histological subtypes of cancers in immunocompetent and immunodeficient patients. The results obtained should provide important clues to the pathways that either allow tumors to counteract immune surveillance mechanisms or are redundant in the presence of an extrinsic oncogenic influence such as oncogenic viruses.

## 2.2 Rationale

Rapidly evolving sequencing and informatics tools are substantially diminishing costs of comprehensive characterization of tumor transcriptomes and tumor genomes. These advances have resulted in detailed information on the repertoire of alterations in cancers. Novel approaches of genomic sequencing analyses have provided new tools of pathogens discovery and new information on cellular genetic alterations associated with viral pathogenesis.

The availability of high quality, clinically annotated patient samples is crucial for the study of biologic factors that influence the progression and treatment response of HIV-1 malignancies. Comprehensive genomic sequence of HIV-associated cancers may identify diagnostic or prognostic disease signatures, and recurrent “driver” alterations that may be targets for new therapies. It is also possible that the comparison of transcriptomes and genomes between lymphomas from HIV<sup>+</sup> and HIV<sup>-</sup> individuals might identify novel non-human sequences that could potentially suggest the presence of transcripts from hitherto undiscovered oncogenic viral agents.

## 3.0 Objectives

The primary objective of this HTMCP biological protocol is to support investigation of the hypothesis above by accrual of high quality, clinically annotated tissue from patients with HIV-1 malignancies. This material will be used to study clinical, genetic, and immunologic parameters that might have prognostic significance and/or are involved in the initiation and progression of HIV-1 malignancies in the context of the HTMCP initiative. The project include complete genomic and transcriptomic sequencing of HIV-associated diffuse large B cell lymphomas, lung, cervical and anal cancer and matched normal tissue from the same individuals.

## 4.0 Eligibility Criteria

1. **Diagnosis.** Patients must have a diagnosis of one of the HIV-associated malignancies aforementioned or clinical findings suggestive of a possible HIV-associated malignancy. Patients that had undergone neo-adjuvant therapy are not eligible for the HTMCP.
2. **Age.** Patients must be ≥ 18 years old.
3. **Informed Consent.** Patients must have signed an IRB-approved informed consent document that permits the use of the samples for genomic-based molecular characterization project(s).

## 5.0 Sample and Data Acquisition and Processing

Samples will be obtained and processed using protocols developed for HTMCP ([HTMCP Standard Operating Procedure Manual](#)).

### 5.1 Tumor Sample Acquisition

Samples will be obtained from HIV positive patients who had diagnosis of any of the cancers listed in page 3 and will undergo either surgery or biopsy from which sufficient quantity of tissue will available along with case matched blood, buccal cells and/or normal adjacent tissue. Not all samples accrued yield RNA and DNA in sufficient quantities or meet the technical quality criteria (DNA: 80% of molecular weight 10,000 or higher; RNA: RNA Integrity Number (RIN) of seven or higher).

Specifically, this protocol requests:

- Permission to obtain solid tumor tissues donated by the patient at the time of the surgery; OR
- Biopsy tissue from a lymph node or other organ involved with malignancy that remains after the necessary samples are used for optimal medical care of the patient. The sample may be obtained by either surgical biopsy(ies) or needle core biopsies (concurrent additional biopsies taken at the same time as biopsy for pathological diagnosis are acceptable).
- The minimum requirement of tumor tissue amount varies with the cancer type, however, as a general rule, 100 mg of tissue is necessary for the HTMCP. All tissues must be snap frozen in liquid nitrogen within 20 minutes of removal following the established protocol provided in HTMCP SOP #102
- About 4 tablespoons of blood drawn from a vein. If the patient objects to having blood drawn, an alternative is to collect normal tissue by swabbing cells from the inside of their cheeks.
- Tissue block (or in its absence, unstained slides) from FFPE tumor must be submitted for centralized pathology (for lymphoma and lung malignancies, HTMCP SOP #107A and B respectively)
- Permission to collect information from the patient medical records, including age, ethnic background, diagnosis, disease history, medical treatments, surgical pathology, and response to treatments.

### 5.2 Case-matched Normal Tissue Acquisition

All participants in this study will have a 10 mL sample of peripheral blood drawn by venipuncture or cannulation of an indwelling venous access device. Samples will be placed in sterile EDTA, or sodium citrate or heparin anticoagulant vacutainer tubes, and cryopreserved following the established protocol (HTMCP SOP #103). This blood draw may occur at the same time as a blood draw for routine medical care.

In cases when blood draw is not possible, buccal cells will be collected. Adjacent normal tissue from surgery samples could be collected as well.

## **5.3 Sample and Data Storage**

### **5.3.1 Sample Identification and Assurance of Anonymity**

All biological materials and medical information will be coded in HTMCP. Only the designated gatekeeper at each Institution will keep the code key that matches the project identifying number to the personally identifiable information

(<http://datacenter.cit.nih.gov/interface/interface241/PIIguide.html>; Note: this is applicable in the US, other countries may have different regulatory frames that must be complied with) using procedures in place and approved by the local institution. Researchers, including those who will be working with the patient samples and medical information, will not have access to any of the traditionally used identifying information about the patient. All materials submitted to the HTMCP will be labeled with a project-assigned ID (as described in HTMCP SOP #106).

### **5.3.2 Storage and Release of Samples and Medical Information**

The coded tissue samples will be sent to the Genome Science Center of the British Columbia Cancer Agency (BC-GSC), which is the characterization center for the HTMCP. The samples will be processed there and the molecular analytes extracted from samples will be used for sequencing. Any remaining samples will be stored at the BC-GSC until the end of the project. At the end of the project, any remaining samples will be handled in accordance with the protocol of contributing institution as designated in the disposition form (HTMCP SOP #108).

Data stripped of identifiers, in compliance with the definition specified in the HIPAA Limited Data Set definition (<http://hipaa.wisc.edu/ResearchGuide/limiteddatasets.html>), will be submitted by the contributing institution to the Data Coordinating Center (DCC). The DCC serves as a central HTMCP project database. The DCC also stores the molecular profiling data generated with the DNA and RNA.

## **5.4. Sample Shipment**

The complete sample sets (tumor and case-matched normal DNA source) will be shipped to the BC-GSC following the procedures explained in HTMCP SOP #104 and 105.

## **5.5. Research Plan Outline**

Samples will be processed and analyzed at the GSC by high-coverage genomic and transcriptomic sequencing. The results will be analyzed will be made between tumor and normal DNA to identify the somatic changes present in the cancer tissues. These alterations include detection of chromosomal changes, such as, but not limited to, amplification (and levels), deletions, loss of heterozygosity, translocations, etc., expression profiling as well as detection of transcripts resulting from translocations and mutations, including single nucleotide variants, insertions, deletions etc.. The results from the tumors of one type will be examined for patterns of common changes, including mutations as a first step to identify the molecular changes that drive the cancer etiology. The alterations will also be analyzed within the context of biological pathways and systems biology.

## 5.6. Clinical Data Collection

For patients whose samples will become part of HTMCP, clinical information will be collected as described in the clinical report form (for lymphoma lung and cervical malignancies, HTMCP SOP #101A, B and C respectively). These patients will be followed prospectively in order to record the types of treatment given and treatment outcome and toxicity. Follow-up information will include the results of subsequent laboratory and imaging tests, pathology, cytogenetic and molecular diagnostic reports, and records describing the patient's course in the inpatient and outpatient setting. (Note: this enumeration of datapoints is specific for HTMCP project but might not be necessary in the protocol depending on your IRB practices).

## 5.7. Data Dissemination

- Information (data) from analyses of the coded samples and the coded medical information will be deposited into publicly available databases. These databases will be accessible by the Internet. Medical information and molecular characterization results on the coded samples will be stored in a controlled-access database. The information in this database will be available only to researchers who have received approval from the NCI Data Access Committee after their institutions have certified their adherence to patient data protection policies for the project (<http://epi.grants.cancer.gov/dac/charter.html>).
- Anonymous information from the analyses will be put in a public database, available to anyone on the Internet.

## 6.0. Financial Compensation/Costs

Patients will not be paid to participate in this project. Tissue samples and the medical information will be used only for research purposes and will not be sold. It is possible that some of the research conducted using the samples or information eventually will lead to the development of new diagnostic tests, new drugs or other commercial products. Should this occur, the patient will not receive any part of the profits generated from such products.

The patient will not incur any expenses from participating in this project.

The chance that the patient will be physically injured as a result of participating in this project is very small. However, if the patient is physically injured as a result of participating in this project, emergency medical treatment for the patient's research-related injury will be provided to the patient at no cost. (Note: this paragraph might not be applicable to your institution, if so, please remove).

## 7.0. Potential Patient Risks/Benefits

### 7.1. Potential Benefits of Participating in the Project

The patient should not expect to personally benefit from this research. The main reason the patient may want to participate is to help researchers and health professionals around the world to better understand the causes of cancer and so that they can find better ways to prevent, detect, treat, and cure the disease in the future.

## 7.2. Potential Risks of Participating in the Project

This project is considered a *minimal risk* protocol

### 7.2.1 Physical Risks

- If a blood sample is NOT taken, there are no physical risks associated with this project.
- If a blood sample is taken, the physical risks are minimal. Possible risk from blood draw include mild pain, bleeding, bruising, and infection at the site of the needle insertion. Short faint or light-headedness can sometimes occur..

### 7.2.2. Psychological or Social Risks Associated with Loss of Privacy

Breach of confidentiality is likely the greatest risk of participating in this study. Every effort will be exerted to minimize this risk. There also may be other privacy risks that we have not foreseen. While we believe that the risks to the patient and his/her family are very low, we are unable to tell exactly what all of the risks are.

Despite the extensive security measures employed to protect the identities of patients and their donated tissue specimens, there is a possibility that the identities of patients enrolled in this study could be discovered or linked to genetic sequence data obtained from their tissue specimens. Consequently, it is possible to use this information to link them to the identities of their children, parents, siblings, and other relatives. It may be possible to identify patients as carriers of genetic mutations. It is also possible that there could be violations of the security used to store the codes linking patient's genetic information. In the case of such breach, there could be risks of denial of employment, insurance, etc.

## 8.0. Project Results

Individual results from this research project will not be given back to the patient or put into the patient's medical records. If research from this project is published in professional journals, there will be no traditionally-used identifying information, such as the patient's name, address, telephone number, or social security number, included in the publications. Some publications from this project will be found at the HTMCP website [[http://cgap.nci.nih.gov/Cancer\\_Types](http://cgap.nci.nih.gov/Cancer_Types)].

## 9.0. Alternatives to Participating in the Project

The alternative option is not to participate.

### 9.1 Voluntary Participation

The choice to participate in this research by consenting the use the patient's donated tissues and medical information for the HTMCP project is completely up to the patient. No matter what the patient decides to do, his/her decision will not affect their medical care.

### 9.2 Withdrawal from the Project

Once the molecular analysis and patient information have been transferred to the DCC, it will not be possible to destroy those data. At the end of the project, unused tissue samples will be destroyed or

returned to the contributing institution as is specified in protocol (HTMCP SOP #108).

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12. Silverberg MJ, Abrams DI: **AIDS-defining and non-AIDS defining malignancies: cancer occurrence in the antiretroviral therapy era.** *Current Opinion in Oncology* 2007, **19**:446-451.

**NOTE:** Highlighted text of this document has to be used as provided in your Institution's informed consent forms for the samples to be acceptable to the project

## **OFFICE OF CANCER GENOMICS SUGGESTED LANGUAGE FOR PROSPECTIVE TISSUE COLLECTIONS IN GENOMIC-SCALE PROJECTS**

### **Purpose of the Project**

We would like to invite you to participate in a research project called **[Project Name]**. The purpose of the **[Project Name]** project is to discover genetic changes associated with cancer, thus potentially leading to better prevention, detection and treatment of cancer, and perhaps other diseases as well.

This project is being sponsored by the National Cancer Institute (NCI), part of the government agency known as the National Institutes of Health (NIH).

Bodily tissues are made up of cells containing DNA, which is part of the unique genetic material carrying the instructions for your body's development and function. Cancer can result from changes in this genetic material, thereby causing cells to divide in an uncontrolled way and possibly to travel to other organs. Some of the genetic changes leading to cancer are currently known, however many remain to be discovered.

The **[Project Name]** project is designed to identify genetic changes that can cause cancer in humans. As such, we would like to study the genetic material obtained from your tumor tissue as part of the **[Project Name]**. We will compare the genetic material from your cancerous tissue with the genetic material from your normal tissue to find any differences that may exist. By combining information about genetic differences between normal and disease tissues along with information contained in your medical records, it may be possible to identify the genetic changes that are associated with your particular type of cancer. This same process will be performed with normal and cancerous tissues obtained from a number of other people who have agreed to participate in this research project. In this way, we expect to identify most of the genetic changes associated with many different kinds of cancer. By comparing treatment responses of patients with various cancers (through recorded medical information), this project could also lead to more knowledge about why certain cancers respond differently to treatments. With such knowledge, future treatment options could potentially become customized to a patient's unique genetic make-up.

### **Description of the Research**

#### **Collection of Samples and Medical Information**

- Your scheduled surgery is part of the medical treatment that you agreed upon with your doctor. During surgery, cancerous tissue will be removed. Usually, when cancerous tissue is removed, very small amounts of nearby normal tissue are removed as well. Your surgery is not part of the **[Project Name]** research project. We will receive some of these cancerous and normal tissues following your

## OCG Template #102

surgery.

- We will collect a sample of blood (approximately 4 tablespoons), drawn from a vein in your arm, as a second type of normal tissue.
- Should you object to having blood drawn, we will instead swab cells from inside of your mouth through gentle sweeping of the inner cheeks to obtain a secondary source of normal tissue.
- We will also collect information from your medical records, including your age, ethnic background, diagnosis, disease history, medical treatments, and response to treatments.

#### Coding of Tissue Samples and Medical Information

- Your tissues, blood or buccal (cheek swab) sample, and medical information will be labeled with a confidential project-assigned ID.
- Only Dr. [Physician Name] at [Institution Name] will have the information that matches the code to traditionally-used identifying information, such as your name, address, phone number, or social security number. Dr. [Physician Name] will keep the information that matches the confidential code to this identifying information in a safeguarded database. Only authorized personnel, who have specifically agreed to protect your identity, will have access to this database. All materials conveyed to the [Project Name] will be labeled with a project-assigned ID, removing traditionally-used identifying information, such as your name, address, phone number, or social security number. All other researchers and personnel, including those who will be working with your samples and medical information, will not have access to any of the traditionally-used identifying information about you.

#### Storage and Release of Samples and Medical Information

- Your coded tissue samples will be sent to an NCI-sponsored storage facility. The facility will process the samples and then send portions of your samples to different types of laboratories for analysis as part of this project. One type of laboratory will analyze your DNA by a method called sequencing. Other laboratories will study your samples by different methods. The remaining tissue from your samples might be stored for an unlimited period of time for use in future research related to cancer, or perhaps in other research projects.
- Information obtained from analyses performed on your coded samples and medical information will be entered into Internet-accessible databases along with information acquired from the other research participants in this project.
  - Anonymous information from the analyses, which cannot be traced to any individual patient, will be available to anyone in a completely public Internet database.
  - Information obtained from more detailed analyses, along with your confidential coded medical information, will be put into a controlled-access database. The information in this database will be available only to researchers who have received approval from an NIH Data Access Committee. In gaining access to such information, researchers have to agree to use the data only for research projects and not to ever try to use it

## OCG Template #102

in order to identify the donor of the material. However, despite all of the safety measures that we will use, we cannot guarantee that your identity will never become known.

*Please note that traditionally-used identifying information about you, such as your name, address, telephone number, or social security number, will NOT be put into either the public or controlled-access databases for this project.*

**Recontact**

- In the future, we may want to obtain additional samples or follow-up information about your health or medical care. Should this be needed, a person from [Institution Name] will contact you with an explanation of the reasons for any follow-up and to ask whether you would be interested in participating in this additional research.

**Financial Compensation/Costs**

You will not be paid to participate in this project. Your tissue samples and your medical information will be used for research purposes only and will not be sold. It is possible that some of the research conducted using your tissue samples or medical information will eventually lead to the development of new diagnostic tests, drugs or other commercial products. Should this occur, you will not receive any part of the profits generated from such products.

You will not incur any expenses from participating in this project. The chance that you will be physically injured as a result of participating in this project is highly unlikely. However, if you are physically injured as a result of participating in this project, emergency medical treatment for your research-related injury will be provided to you at no cost.

**Potential Benefits of Participating in the Project**

You should not expect to personally benefit from this research, aside from the knowledge that your participation will help researchers and health professionals around the world to better understand the causes of cancer and other diseases. Research projects such as this lead to better ways to prevent, detect, treat, and cure such illnesses.

**Potential Risks of Participating in the Project****Physical Risks**

- There are very few physical risks associated with this project. Possible side effects from drawing the blood sample include mild pain, bleeding, bruising, and infection at the site of the needle insertion. Fainting or light-headedness can sometimes occur, but usually lasts only a few minutes. Every precaution will be taken to minimize these effects.

**Psychological or Social Risks Associated with Loss of Privacy**

- Your privacy is very important to us, and we use many safety measures to protect your privacy. However, despite all of the safety measures that we will use, we cannot guarantee that your identity will never become known. Although your genetic information is unique to you, you do share some genetic information with your children, parents, brothers, sisters, and other relatives. Consequently, it may be possible that genetic information from them could be used to help identify you. Similarly, it may be possible that genetic information from you could be used to help identify them.
- While neither the public nor the controlled-access databases developed for this project will contain information that is traditionally used to identify you (your name, address, telephone number, or social security number), technology may be developed in the future that would allow someone to link your genetic or medical information in our databases back to you. For example, someone could compare information in our databases with information from you (or a relative) in another database and be able to identify you (or your relative). It is also possible that there could be violations to the security of the computer systems used to store the codes linking your genetic and medical information back to you. Because some genetic variations can help to predict the current or future health problems of you and your relatives, this information may be of interest to employers, health providers, insurance companies, and others. Patterns of genetic variation also can be used by law enforcement agencies to identify a person or his/her relatives. Therefore, your genetic information could potentially be used in ways that could cause you or your family distress, such as by revealing that you (or a relative) carry a genetic disease or by leading to the denial of employment or insurance for you (or a relative).
- There also may be other privacy risks that we have not foreseen.

While we believe that the risks to you and your family are very low, we are unable to tell you exactly what all of the risks are. There are some state laws that protect against genetic discrimination by employers or insurance companies, but there is currently no federal law that prohibits such discrimination. We believe that the benefits of learning more about cancer and other diseases outweigh these potential risks.

**Confidentiality**

We will make every attempt to protect your confidentiality and to ensure that your personal identity remains anonymous. This signed consent form will be stored in a locked file that will be accessible only to authorized people involved with this project. We will carefully follow the coding, storage, and release plan explained in the *Description of the Research* section on pages 1 and 2 of this document.

**Project Results**

Your individual results from this research project will not be given back to you or put

## OCG Template #102

into your medical records. If research from this project is published in professional journals, there will be no traditionally-used identifying information, such as your name, address, telephone number, or social security number, included in the publications. Some publications from this project will be found at the [Project Name] website.

**Alternatives to Participating in the Project**

The alternative option is not to participate in this project.

**Voluntary Participation**

The choice to participate in this research by donating your tissues and medical information is completely up to you. **No matter what you decide, your decision will not affect your medical care.**

**Withdrawal from the Project**

Once your coded samples have been distributed to the participating research laboratories and centers, and your information transferred to the appropriate databases, you will **not** be able to withdraw your information from this research project. However, you may be able to request the return or destruction of the tissue samples if you so desire.



## Contact Information

If you have any questions about the project or your participation, [please use specific institutional language here, but do not automatically promise ability to withdraw].

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### Agreeing to Participate in the Project

To participate in this research, you must agree to ALL of the following statements:

- I voluntarily agree to donate cancerous tissue and normal tissue to be used for this and for other research projects.
- I agree to release information from my medical records for this and for other research projects.
- I agree to have my coded genetic information and coded medical information placed into Internet-accessible databases as described in the *Storage and Release of Samples and Medical Information* section on page 2 of this document.
- I understand that my coded genetic information and coded medical information contained in the Internet-accessible databases will be used in this and in other research projects.
- I understand that there is a risk that someone in the future may be able to use information in these databases to identify me or possibly my relative(s).
- I agree to be contacted in the future about my willingness to provide additional samples or follow-up information about my health or medical care if it is required.

Please sign your name here if you agree to the six statements listed above.

Your signature: \_\_\_\_\_

Date: \_\_\_\_\_

Signature of Doctor/Nurse/Other Witness \_\_\_\_\_

**NOTE:** Highlighted text of this document has to be used as provided in your Institution's informed consent forms for the samples to be acceptable to the project

## **OFFICE OF CANCER GENOMICS SUGGESTED LANGUAGE FOR RETROSPECTIVE TISSUE COLLECTIONS IN GENOMIC-SCALE PROJECTS**

### **Purpose of the Project**

We would like to invite you to participate in a research project called **[Project Name]**. The purpose of the **[Project Name]** project is to discover genetic changes associated with cancer. This should lead to better ways to prevent, detect, and treat cancer and, perhaps, other diseases as well.

This project is being sponsored by the National Cancer Institute (NCI), part of the government agency known as the National Institutes of Health (NIH).

Body tissues are made up of cells. Cells contain DNA, which is part of your unique genetic material that carries the instructions for your body's development and function. Cancer can result from changes in a person's genetic material, that cause cells to divide in an uncontrolled way and, sometimes, to travel to other organs. Currently, researchers and doctors know some of the genetic changes that can cause cancer, but they do not know all of the genetic changes that can cause cancer.

The **[Project Name]** project is designed to identify most of the genetic changes that can cause cancer in people. Therefore, we would like to study the genetic material from your cancer tissue as part of the **[Project Name]**. We will compare the genetic material from your cancer tissue to the genetic material from your normal tissue to find the differences that exist. By combining this information with information from your medical records, it may be possible to identify the genetic changes that are associated with your particular type of cancer. We will perform this same process with many (hundreds of) other people who have agreed to participate in this research project. By studying many different kinds of cancer in this way, we expect to identify most of the genetic changes associated with different kinds of cancer. Since we also will combine genetic information with information from medical records, such as the responses of different kinds cancers to different treatments, this project could lead to more knowledge about why certain cancers respond differently to treatments. With such knowledge, future treatments potentially could become customized to a patient's unique genetic make-up.

### **Description of the Research**

#### **Collection of Samples and Medical Information**

- You already have had surgery as a part of the medical treatment that you agreed upon with your doctor. During your surgery, cancerous/tumor tissue was removed. As usually happens, when your cancerous tissue was removed, very small amounts of nearby normal tissue were removed along with it. Your surgery

was not part of the [Project Name] research project. For this research project, we seek permission to receive some of these cancerous and normal tissues.

- If a second type of normal tissue (e.g., blood) was collected from you before or after your surgery, we request permission to obtain some of this tissue and genetic material that already may have already been extracted from this tissue.
- If an adequate blood sample is not available for this project, we will collect a sample from you by drawing approximately 4 tablespoons of blood from a vein in your arm. If you object to having blood drawn, we will collect normal tissue from you by swabbing cells from the inside of your cheeks.
- We will also collect information from your medical records, including your age, ethnic background, diagnosis, disease history, medical treatments, and response to treatments.

#### Coding of Tissue Samples and Medical Information

- Your tissues, blood or buccal (cheek swab) sample, and medical information will be labeled with a project-assigned ID.
- Only Dr. [Physician Name] at [Institution Name] will have the information that matches the code to traditionally-used identifying information, such as your name, address, phone number, or social security number. Dr. [Physician Name] will keep the information that matches the code to this traditionally-used identifying information in a safeguarded database. Only authorized people, who have specifically agreed to protect your identity, will have access to this database. All materials conveyed to the [Project Name] will be labeled with a project-assigned ID, removing traditionally-used identifying information, such as your name, address, phone number, or social security number. All other researchers and personnel, including those who will be working with your samples and medical information, will not have access to any of the traditionally-used identifying information about you.

#### Storage and Release of Samples and Medical Information

- Your coded tissue samples will be sent to an NCI-sponsored storage facility that will process the samples and then send portions of your samples to different types of laboratories as part of this project. One type of laboratory will analyze your DNA by a method called sequencing. Other laboratories will study your samples by different methods. The remaining portions of your samples will be stored for an unlimited period of time for future use in research related to cancer, or perhaps in other research projects.
- Information obtained from analyses performed on your coded samples and your coded medical information will be put into databases along with information from the other research participants. These databases will be accessible by the Internet.
  - Anonymous information from the analyses will be put into a completely public database, available to anyone on the Internet.
  - Your coded medical information and information from more detailed analyses of your coded samples will be put into a controlled-access database. The information in this database will be available only to researchers who have received approval from an NIH Data Access

Committee. In gaining access to such information, researchers have to agree to use the data only for research projects and not to ever try to use it in order to identify the donor of the material. However, despite all of the safety measures that we will use, we cannot guarantee that your identity will never become known.

*Please note that traditionally-used identifying information about you, such as your name, address, telephone number, or social security number, will NOT be put into either the public or controlled-access databases for this project.*

### **Recontact**

- In the future, we may want to obtain additional samples or follow-up information about your health or medical care. Should this be needed, a person from [Institution Name] will contact you to ask whether you would be interested in participating in this additional research.

### **Financial Compensation/Costs**

You will not be paid to participate in this project. Your tissue samples and your medical information will be used only for research purposes and will not be sold. It is possible that some of the research conducted using your samples or information will eventually lead to the development of new diagnostic tests, new drugs or other commercial products. Should this occur, you will not receive any part of the profits generated from such products.

You will not incur any expenses from participating in this project. It is unlikely that you will be physically injured as a result of participating in this project. However, if you are physically injured as a result of participating in this project, emergency medical treatment for your research-related injury will be provided to you at no cost.

### **Potential Benefits of Participating in the Project**

You should not expect to personally benefit from this research. The main reason you may want to participate is to help researchers and health professionals around the world to better understand the causes of cancer, and other diseases, and potentially to find better ways to prevent, detect, treat, and cure such illnesses. We hope that you will feel good knowing that you may be helping future cancer patients, as well as patients with other diseases.

### **Potential Risks of Participating in the Project**

#### **Physical Risks**

- If no blood sample is taken from you, there are no physical risks associated with this project.
- There are very few physical risks if a blood sample is taken from you. Possible side effects from drawing the blood sample include mild pain, bleeding, bruising,

and infection at the site of needle insertion. Fainting or light-headedness can sometimes occur, but usually last only a few minutes.

### **Psychological or Social Risks Associated with Loss of Privacy**

- Your privacy is very important to us, and we use many safety measures to protect your privacy. However, despite all of the safety measures that we will use, we cannot guarantee that your identity will never become known. Although your genetic information is unique to you, you do share some genetic information with your children, parents, brothers, sisters, and other relatives. Consequently, it may be possible that genetic information from them could be used to help identify you. Similarly, it may be possible that genetic information from you could be used to help identify them.
- While neither the public nor the controlled-access databases developed for this project will contain information that is traditionally used to identify you (your name, address, telephone number, or social security number), technology may be developed in the future that would allow someone to link your genetic or medical information in our databases back to you. For example, someone could compare information in our databases with information from you (or a relative) in another database and be able to identify you (or your relative). It is also possible that there could be violations to the security of the computer systems used to store the codes linking your genetic and medical information back to you. Because some genetic variations can help to predict the current or future health problems of you and your relatives, this information may be of interest to employers, health providers, insurance companies, and others. Patterns of genetic variation also can be used by law enforcement agencies to identify a person or his/her relatives. Therefore, your genetic information could potentially be used in ways that could cause you or your family distress, such as by revealing that you (or a relative) carry a genetic disease or by leading to the denial of employment or insurance for you (or a relative).
- There also may be other privacy risks that we have not foreseen.

While we believe that the risks to you and your family are very low, we are unable to tell you exactly what all of the risks are. There are some state laws that protect against genetic discrimination by employers or insurance companies, but there is currently no federal law that prohibits such discrimination. We believe that the benefits of learning more about cancer and other diseases outweigh these potential risks.

### **Confidentiality**

We will make every attempt to protect your confidentiality and to make sure that your personal identity remains anonymous. This signed consent form will be stored in a locked file that will be accessible only to a very small number of authorized personnel involved in this project. We will carefully follow the coding, storage, and release plan explained in the *Description of the Research* section on pages 1-3 of this document.

## Project Results

Your individual results from this research project will not be given back to you or put into your medical records. If research from this project is published in professional journals, there will be no traditionally-used identifying information, such as your name, address, telephone number, or social security number, included in the publications. Some publications from this project will be found at the [Project Name] website.

## Alternatives to Participating in the Project

The alternative option is not to participate in this project.

## Voluntary Participation

The choice to participate in this research by donating your tissues and medical information is completely up to you. **No matter what you decide to do, your decision will not affect your medical care.**

## Withdrawal from the Project

Once your coded samples have been distributed to the participating research laboratories and centers, and your information transferred to the appropriate databases, you will **not** be able to withdraw your information from this research project. However, you may be able to request the return or destruction of the tissue samples if you so desire.

## Contact Information

If you have any questions about the project or your participation, [please use specific institutional language here, but do not automatically promise ability to withdraw].

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## Agreeing to Participate in the Project

**To participate in this research, you must agree to ALL of the following statements:**

- I voluntarily agree to donate cancerous tissue and normal tissue to be used for this and for other research projects.
- I agree to release information from my medical records for this and for other research projects.
- I agree to have my coded genetic information and coded medical information placed into databases accessible by the Internet, as described in the *Storage and Release of Samples and Medical Information* section on page 2 of this document.
- I understand that my coded genetic information and coded medical information in the Internet-accessible databases will be used in this and in other research

- projects.
- I understand that there is a risk that someone in the future might be able to use information in these databases to identify me or possibly my relative(s).
  - I agree to be contacted in the future to see if I am willing to provide additional samples or follow-up information about my health or medical care if they are needed.

Please sign your name here if you agree to the six statements listed above.

Your signature: \_\_\_\_\_

Date: \_\_\_\_\_

Signature of Doctor/Nurse/Other Witness \_\_\_\_\_

Template



## Material Transfer and Data Use Agreement

This Material Transfer and Data Use Agreement (the “Agreement”) is entered into by and between \_\_\_\_\_ (“Provider”) and \_\_\_\_\_ (“Recipient”), regarding the transfer of human specimens and associated data to the Recipient as part of tumor characterization projects and associated research coordinated by the National Cancer Institute’s Office of Cancer Genomics (“the Projects”), including [Project Name]. Throughout this Agreement, Provider and Recipient are collectively referred to as the “Parties” and individually as “Party.” This Agreement will become effective upon the date of the last signature affixed below (the “Effective Date”).

WHEREAS, in order to improve the ability to diagnose, treat, and prevent cancer, the National Cancer Institute (“NCI”), a member institute of the National Institutes of Health, an agency of the federal government, has undertaken the Projects as a comprehensive and coordinated research effort to accelerate the understanding of the molecular basis of cancer through the application of genome analysis technologies, including large-scale genome sequencing;

WHEREAS, the Projects are managed by the NCI Office of Cancer Genomics;

WHEREAS, under the Projects, clinically annotated tissue samples will originate from several clinical Tissue Source Sites, and the tissue samples and associated data will be processed by centralized core facility(ies);

WHEREAS, Recipient has been selected to act as a centralized core facility, pursuant to a subcontract with NCI’s Operations and Technical Support (“OTS”) contractor, SAIC-Frederick, Inc. or directly with the NCI (either, the “OTS Contractor”), and the tasks with which it is charged include receiving and processing human biospecimens, derivative materials and associated data and distributing all of the foregoing to NCI approved characterization centers (“the Centers”) and distributing only the associated data to a data coordinating center that is operated by NCI (“DCC”);

WHEREAS, Recipient, as a subcontractor of NCI’s OTS Contractor, desires to receive and, in conjunction with subcontractors of Recipient and the NCI and/or SAIC-Frederick, Inc. (collectively, “the Project Subcontractors”), process biospecimens, derivative materials and associated data from the Provider and distribute the same to the Centers and a DCC, as appropriate;

WHEREAS, Provider, acting as a Tissue Source Site under the Projects, desires to transfer certain human biospecimens, derivative materials, and associated data to Recipient for further distribution to the Centers and a DCC, as appropriate;

WHEREAS, the Centers and the DCC, pursuant to policies and practices established as part of the Projects, may not make a claim for intellectual property rights in the MATERIAL (as defined below), nor may they make a claim for intellectual property rights in DATA (as defined below) prior to its public availability;

WHEREAS, Provider and Recipient desire to protect the privacy and provide for the security of certain information disclosed to Recipient in compliance with applicable laws and regulations; and

WHEREAS, Provider, if an entity of the United States of America (“U.S.”), may be a covered entity subject to the Health Insurance Portability and Accountability Act of 1996, as

amended (“HIPAA”), and, if not a U.S. entity, desires to protect the privacy of certain information disclosed to the Recipient in a manner consistent with HIPAA and the applicable laws of its jurisdiction that are similar in nature.

NOW, THEREFORE, in consideration of the mutual promises in this Agreement and for other good and valuable consideration, the sufficiency of which is hereby acknowledged, the Parties hereby agree as follows:

**1. DEFINITIONS.** Within this Agreement, the following terms will have the same meaning and effect as those used in the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 CFR Parts 160 and 164 (“HIPAA Privacy Rule”). These terms are repeated here for convenience.

- (a) Under 45 CFR 160.103 (“Definitions”), a “covered entity” is an organization, individual, institution, or other entity that is subject to the standards, requirements, and implementation specifications of the HIPAA Privacy Rule with respect to protected health information.
- (b) Under 45 CFR 164.514 (“Other requirements relating to uses and disclosures of protected health information”), “De-identified” information is information that formerly contained individually identifiable health information but which has had all unique identifying information, numbers, characteristics, and codes removed such that the information a record contains cannot be used alone or in combination with other information to identify the individual who is the subject of the information. Identifying information includes, but is not limited to, the 18 categories of identifiers described in 45 CFR 164.514(b)(2).
- (c) Under 45 CFR 164.103 (“Definitions”), “Protected Health Information” or “PHI” means any information, whether oral or recorded in any form or medium: (i) that relates to the past, present, or future physical or mental condition of an individual; the provision of health care to an individual; or the past, present, or future payment for the provision of health care to an individual, and (ii) that identifies the individual or with respect to which there is a reasonable basis to believe the information can be used to identify the individual.
- (d) Under 45 CFR 164.514(e)(2) (“Implementation Specification: Limited data set”), a “limited data set” (herein “LDS”) is protected health information that excludes the 16 direct identifiers listed in that section. Any such information that identifies the individual who is the subject of the PHI, his or her relatives, employers, or household members must be removed for the PHI to constitute an LDS.

## **2. DESCRIPTION OF MATERIAL AND DATA.**

- (a) The material to be transferred (“ORIGINAL MATERIAL”) is a set of human biospecimens described specifically as: Human Tumors, Matching Normal Specimens or Blood, and Formalin Fixed Paraffin Embedded Tissues.
- (b) The data to be transferred to Recipient are clinical, biological, technical and/or other information describing the ORIGINAL MATERIAL (“DATA”). Some of the DATA may be Protected Health Information and will be transferred in the form of an LDS.

**3. COLLECTION OF MATERIAL AND DATA.** The Provider represents and warrants to Recipient that: (a) as necessary, all ORIGINAL MATERIAL and DATA provided to Recipient by

Provider were collected pursuant to and in accordance with a protocol approved by an Institutional Review Board (“IRB”); (b) the IRB’s oversight of the collection of any ORIGINAL MATERIAL and DATA included a review of all necessary informed consents and authorizations, which consents do not prohibit redistribution of the ORIGINAL MATERIAL or materials derived from the ORIGINAL MATERIAL, e.g., DNA and RNA products (“DERIVATIVE MATERIAL,” together with the ORIGINAL MATERIAL, the “MATERIAL”) or DATA in the manner described in Section 4 of this Agreement; (c) the transfer, processing and analysis of the ORIGINAL MATERIAL and DATA, as part of the Projects and for the Purpose (as defined below), is authorized by or consistent with the general principles of the informed consent of the patient supplying such ORIGINAL MATERIAL and DATA, as determined by an IRB; and (d) the collection of the ORIGINAL MATERIAL and DATA was conducted in compliance with all applicable laws, regulations and policies for the protection of human subjects, including, in the case where Provider is a covered entity, 45 CFR Part 46, “Protection of Human Subjects” (the “Common Rule”) and the HIPAA Privacy Rule, and any necessary approvals, authorizations, human subjects assurances, informed consent documents, and IRB approvals were obtained.

**4. TRANSFER OF ORIGINAL MATERIAL AND DATA; PURPOSE.** (a) Provider agrees to provide to Recipient the ORIGINAL MATERIAL and DATA, in the form of an LDS pursuant to Case Report Forms provided by the Recipient to the Provider, in accordance with applicable laws, regulations and policies, including but not limited to the Common Rule, the HIPAA Privacy Rule, and any necessary authorizations, human subjects assurances, informed consent documents, and IRB approvals. The sole and limited purpose of the Provider’s transfer to Recipient of the ORIGINAL MATERIAL and the DATA is to enable Recipient to receive, process and distribute the MATERIAL and the DATA, in the appropriate form as indicated below, to the Centers, a DCC, and the Project subcontractors in fulfillment of its contractual obligations to NCI’s OTS Contractor (the “Purpose”). If Provider is a HIPAA Covered Entity, the Parties expressly intend for this Agreement to constitute a Data Use Agreement, authorizing use and disclosure only in furtherance of the Purpose, in accordance with 45 CFR 164.514(e)(4). Provider is responsible for removing all of the prohibited direct identifiers from the DATA, such that the DATA will be in the form of an LDS, before transfer to Recipient.

(b) Provider has the authority and hereby grants Recipient explicit permission to further distribute the MATERIAL and De-identified DATA to the Centers and the Project Subcontractors.

(c) Provider has the authority and hereby also grants Recipient explicit permission to further distribute the DATA, in the form of an LDS, to a DCC upon execution by both Recipient and NCI of a Data Use Agreement that is consistent with the requirements of the HIPAA Privacy Rule. Furthermore, Provider acknowledges and agrees that Recipient may allow the DCC to provide all or part of the LDS to third parties pursuant to separate Data Use Agreements that are no less restrictive than this Agreement and that prohibit such third parties from further distributing the LDS.

(d) The Agreement does not restrict the Provider’s right to distribute the MATERIAL and DATA to third parties.

## **5. RESPONSIBILITIES AND AUTHORIZATIONS OF RECIPIENT**

(a) Recipient’s IRB has approved the Recipient’s participation in the Projects (IRB approval number: IRB 12-00222). Recipient agrees to handle and distribute the MATERIAL in accordance with all

applicable laws, regulations and policies, including, as applicable, the Common Rule, the HIPAA Privacy Rule, and any necessary human subject's assurances, informed consents and IRB approvals.

(b) Recipient further agrees that it will only use and/or disclose the DATA for the Purpose described herein and shall not use or disclose the DATA in a manner inconsistent with the HIPAA Privacy Rule.

(c) Recipient is not authorized and shall not further disclose the DATA other than as permitted by this Agreement or as otherwise required by law. Recipient shall not distribute the DATA to other third parties without written consent from Provider and the NCI Program Director or designee for the particular Project in question.

(d) Recipient shall use appropriate administrative, technical, and physical safeguards to prevent use or disclosure of the DATA other than as provided for in this Agreement.

(e) Recipient shall notify Provider in writing within five (5) working days of its discovery of any use or disclosure of the DATA not permitted by this Agreement of which Recipient, its officers, employees, or agents become aware. Recipient shall take (i) prompt corrective action to cure any deficiencies or (ii) any action pertaining to such unauthorized disclosure required by applicable federal law.

(f) Recipient shall ensure that any of its agents or subcontractors agree with Recipient in writing that such agent or subcontractor will hold any DATA transmitted from the Recipient to such agent or subcontractor confidential and will use or disclose the information only for the purpose for which it was used or disclosed to the agent or subcontractor, or as required by law. Additionally, the agent or subcontractor shall notify Recipient of any instances, of which it is aware, in which the DATA has been used or disclosed inconsistent with this Agreement.

(g) Recipient agrees to not identify or contact any donor, or living relative of a donor, who provided the MATERIAL or any DATA received by Recipient under this Agreement from Provider. Furthermore, Recipient will not attempt to obtain or otherwise acquire any PHI associated with the MATERIAL beyond that which is provided in the DATA by the Provider.

(h) Recipient will retain and abide by this Agreement for as long as it retains the DATA or other PHI received from the Provider, plus six (6) years after the date it returns or destroys all such information.

**6. BREACH OR VIOLATION.** Provider is not responsible for Recipient's violations of this Agreement, unless Provider knows of a pattern of activity or practice that constitutes a material breach or violation of this Agreement, in which case it must take reasonable steps to cure the breach, end the violation or withhold the LDS or other PHI delivered to Recipient. If this is not possible, the breach will be reported to the Secretary of the Department of Health and Human Services ("DHHS").

## **7. THE MATERIAL AND DATA ARE NOT FOR USE IN HUMAN SUBJECTS OR FOR THE TREATMENT OR DIAGNOSIS OF HUMAN SUBJECTS.**

**8. DISCLAIMER.** Any MATERIAL delivered pursuant to this Agreement is understood to be experimental in nature and may have hazardous properties. SUBJECT TO THE REPRESENTATIONS IN SECTION 3 ABOVE WITH RESPECT TO THE MATERIAL OR DATA, PROVIDER MAKES NO REPRESENTATIONS AND EXTENDS NO WARRANTIES OF

ANY KIND, EITHER EXPRESSED OR IMPLIED. THERE ARE NO EXPRESS OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR THAT THE USE OF THE MATERIAL OR DATA WILL NOT INFRINGE ANY PATENT, COPYRIGHT, TRADEMARK, OR OTHER PROPRIETARY RIGHTS.

**9. DISPOSAL OF MATERIAL AND DATA.** At the end of its subcontract with the NCI's OTS Contractor or upon the termination of this Agreement by either Party, Recipient will dispose of the MATERIAL and DATA in its possession in the manner decided at the sole discretion of the NCI Office of Cancer Genomics or designee for the particular Project in question and consistent with law and the informed consent of the individual providing the ORIGINAL MATERIAL. Such disposition may include, but is not limited to, continued storage on behalf of Provider for future research, transfer to the Provider, use in an expansion of the Projects, transfer to another organization acting on NCI's behalf, or destruction. NCI shall be responsible for ensuring that any directive given to the Recipient regarding the disposition of the MATERIAL and DATA is consistent with the informed consent of the patient who provided the ORIGINAL MATERIAL. Provider acknowledges that any ORIGINAL MATERIAL transferred by Recipient to the Centers may be destroyed as a consequence of the analyses conducted in accordance with the Projects.

**10. INTELLECTUAL PROPERTY.** Provider explicitly retains ownership of ORIGINAL MATERIAL and DATA. Provider acknowledges and agrees that it does not by virtue of this Agreement acquire any intellectual property rights in the future inventions or discoveries made by third parties using the MATERIAL or DATA distributed by Recipient. Recipient acknowledges that it serves only as the custodian of the MATERIAL and DATA, and therefore agrees that it does not by virtue of this Agreement acquire any intellectual property rights in the MATERIAL or DATA, nor any future intellectual property rights in any research conducted by third-parties using the MATERIAL or DATA.

**11. ASSIGNMENT; SUCCESSORS AND ASSIGNS; NO THIRD-PARTY RIGHTS.** Recipient may not assign its rights or cause to be assumed its obligations hereunder without the prior written consent of Provider, which consent shall not be unreasonably withheld or delayed. Subject to the foregoing, this Agreement shall apply to, be binding in all respects upon and inure to the benefit of the Parties hereto and their respective successors and assigns. Nothing expressed or referred to in this Agreement shall be construed to give any person or entity other than the Parties hereto any legal or equitable right, remedy or claim under or with respect to this Agreement or any provision of this Agreement.

**12. COST.** The MATERIAL and DATA are provided at no cost to Recipient.

**13. SHIPPING.** Provider will notify Recipient when the ORIGINAL MATERIAL and DATA are ready for shipment. Recipient will be responsible for the pick-up and shipment, including shipping costs, of the ORIGINAL MATERIAL and DATA.

**14. ENTIRE AGREEMENT.** This Agreement constitutes the entire agreement between the Parties with respect to the subject matter hereof, and supersedes and replaces all prior agreements, understandings, commitments, communications and representations made between the Parties, whether written or oral, with respect to the subject matter hereof. This Agreement may not be amended, supplemented, or otherwise modified except by a written agreement executed by each of the Parties.

**15. TERMINATION.** Either Party has the right to terminate this Agreement at any time upon sixty (60) days prior written notice to the other Party.

**16. INDEMNIFICATION.** Each party shall indemnify, defend and hold the other party and its parent and affiliates and their officers, directors, employees, and agents, harmless from and against any claims, charges, judgments, costs, liabilities, damages, losses, or expenses (including reasonable attorneys' fees and expenses of litigation) resulting from any third party claims, allegations, suits, actions, or demands (collectively "Claims") that arise out of or result from the indemnifying party's acts or omissions relating to this Agreement or the indemnifying party's failure to perform any obligation undertaken or covenant made in this Agreement. The indemnified party shall promptly notify and provide reasonable cooperation to the indemnifying party in the defense of any Claim for which indemnification is sought at the indemnifying party's expense. The indemnifying party shall have the right to settle Claims; provided, however, that the indemnifying party shall make no admission of fault or wrongdoing or other statement reflecting negatively on the indemnified party, without the indemnified party's prior express written consent.

**17. INSURANCE.** Each party shall maintain liability coverage of the types and at the levels that are usual and customary to insure its obligations and activities under this Agreement.

**18. NOTICE.** All notices, requests, demands, and other documentation required or permitted to be given under this Agreement shall be provided in writing and will be deemed to have been fully given and received (i) when delivered in writing personally; (ii) when sent by confirmed electronic message or facsimile; (iii) five (5) days after having been sent by registered or certified mail, return receipt requested, postage prepaid; or (iv) one (1) day after deposit with a commercial overnight carrier, with written verification of such receipt, to the addresses provided below.

**19. WAIVER.** No waiver by either Party of any term or condition of this Agreement, no matter how long continuing or how often repeated, shall be deemed a waiver of any subsequent act or omission, nor shall any delay or omission on the part of either Party to exercise any right, power, or privilege or to insist upon compliance with any term or condition of this Agreement be deemed a waiver of such right, power or privilege or excuse a similar subsequent failure to perform any such term or condition. All waivers must be in writing and signed by the Party granting such waiver.

**20. EXECUTION OF AGREEMENT.** This Agreement may be executed in two or more counterparts, each of which will be deemed to be an original copy and all of which, when taken together, will be deemed to constitute one and the same agreement. The exchange of copies of the Agreement and of signature pages by facsimile or electronic transmission will constitute effective execution and delivery of this Agreement as to the Parties hereto and may be used in lieu of the original Agreement for all purposes. Signatures of the Parties transmitted by facsimile or electronic transmission will be deemed to be their original signatures for all purposes.

[The rest of this page was left blank intentionally. Signature page follows.]

IN WITNESS WHEREOF, the Parties have executed this Agreement through their duly authorized representatives as of the Effective Date.

### **Signature for Provider**

Provider Scientist:

Provider Organization:

Address:

Name of Authorized Official:

Title of Authorized Official:

---

**Signature of Authorized Official      Date**

Certification of Provider Authorized Official: This Agreement \_\_has / \_\_has not been modified from the original template.

### **Signature for Recipient**

Recipient Scientist

Recipient Organization:

Address:

Name of Authorized Official:

Title of Authorized Official:

---

**Signature of Authorized Official      Date**



[This Institutional Certification should be submitted on the PI's institutional letterhead.]

Date: Month Day, Year

To: Elizabeth Gillanders, Ph.D.  
Branch Chief and NCI Data Access Committee Chair  
National Institutes of Health  
MSC 7324  
6130 Executive Blvd  
Rockville, MD 20892

Re: Certification of [name of PI's institution] to Accompany Submission of the Dataset for the [name of OCG initiative] to the NIH Database of Genotypes and Phenotypes (dbGaP).

Dear Dr. Gillanders:

[Name of PI's institution] adheres to the *Policy for Sharing of Data Obtained in NIH Supported or Conducted Genome-Wide Association Studies (GWAS)*, Notice Number: NOT-OD-07-088. [Name of PI's institution] provides the following Certification in regard to the Dataset for the [name of OCG initiative], which is being deposited into dbGaP.

- The data submission is consistent with all applicable laws and regulations, as well as institutional policies.
- All uses of these data that are deemed acceptable and approved per NIH policy and that follow the dbGaP procedures for access are allowable, with the following restriction:

#### **Data Use Limitation**

Use of the data is limited to scientific research relevant to the etiology, prevention, treatment, and late complications of treatment of cancer and for the development of applications proposing analytical methods, software, or other research tools.

- The identities of research participants will not be disclosed to dbGaP
- An Institutional Review Board has reviewed and verified that:
  - The submission of data to dbGaP and subsequent sharing for research purposes are consistent with the informed consent of the study participants from whom the data were obtained;
  - The plan for de-identifying datasets is consistent with the standards outlined above;

OCG Template #105

- [Name of institution] has considered the risks to the individuals, their families, and groups or populations associated with data submitted to dbGaP and determined risks to be reasonable in relation to anticipated benefits to society; and
- The phenotype data to be submitted were collected in a manner consistent with 45 CFR Part 46. .

The suggested Acknowledgement Statement to accompany the data set is:

**Acknowledgement Statement**

This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. [Funding mechanism].

If additional information is required, please do not hesitate to contact us.

Sincerely,

[Need signatures from both the signing official at the PI's institution and the PI for the Project.]

[This Institutional Certification should be submitted on the PI's institutional letterhead.]

Date: Month Day, Year

To: Elizabeth Gillanders, Ph.D.  
Branch Chief and NCI Data Access Committee Chair  
National Institutes of Health  
MSC 7324  
6130 Executive Blvd  
Rockville, MD 20892

Re: Certification of [name of PI's institution] to Accompany Submission of the Dataset for the [name of OCG initiative] to the NIH Database of Genotypes and Phenotypes (dbGaP).

Dear Dr. Gillanders:

[Name of PI's institution] adheres to the *Policy for Sharing of Data Obtained in NIH Supported or Conducted Genome-Wide Association Studies (GWAS)*, Notice Number: NOT-OD-07-088. [Name of PI's institution] provides the following Certification in regard to the Dataset for the [name of OCG initiative], which is being deposited into dbGaP.

- The data submission is consistent with all applicable laws and regulations, as well as institutional policies.
- All uses of these data that are deemed acceptable and approved per NIH policy and that follow the dbGaP procedures for access are allowable, with the following restriction:

#### **Data Use Limitation**

Use of protected datasets should be for research projects that can only be conducted using pediatric data (i.e., the research objectives cannot be accomplished using data from adults) and that have likely relevance to developing more effective treatments, diagnostic tests, or prognostic markers for childhood cancers.

- The identities of research participants will not be disclosed to dbGaP
- An Institutional Review Board has reviewed and verified that:
  - The submission of data to dbGaP and subsequent sharing for research purposes are consistent with the informed consent of the study participants from whom the data were obtained;

OCG Template #106

- The plan for de-identifying datasets is consistent with the standards outlined above;
- [Name of institution] has considered the risks to the individuals, their families, and groups or populations associated with data submitted to dbGaP and determined risks to be reasonable in relation to anticipated benefits to society; and
- The phenotype data to be submitted were collected in a manner consistent with 45 CFR Part 46. .

The suggested Acknowledgement Statement to accompany the data set is:

**Acknowledgement Statement**

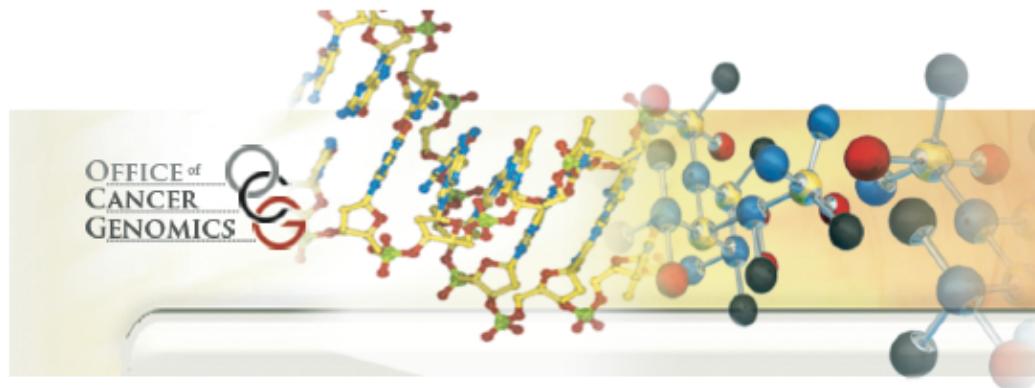
This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. [Funding mechanism].

If additional information is required, please do not hesitate to contact us.

Sincerely,

[Need signatures from both the signing official at the PI's institution and the PI for the Project.]

National Cancer Institute



## HTMCP GENERAL PROTOCOLS

U.S. DEPARTMENT  
OF HEALTH AND  
HUMAN SERVICES

National Institutes  
of Health



## HTMCP SOP #100

Adopted: \_\_\_\_\_ 4/26/2010  
2<sup>nd</sup> Version : \_\_\_\_\_ 09/01/2010  
3<sup>rd</sup> Version : \_\_\_\_\_  
Reviewed: \_\_\_\_\_  
4<sup>th</sup> Version : \_\_\_\_\_

## **DOCUMENT REQUIREMENTS FOR SAMPLE SUBMISSION TO THE HIV+ TUMOR MOLECULAR CHARACTERIZATION PROJECT**

### I. INTRODUCTION:

The National Cancer Institute's Office of Cancer Genomics (OCG) and the Office of HIV and AIDS Malignancy (OHAM) have developed an initiative to compare the cancer related alterations in HIV+ patients and HIV- patients. It is possible that the comparison of transcriptomes and genomes between tumors from HIV+ and HIV- individuals may or may not identify novel non-human sequences which could suggest the presence of transcripts from known or hitherto undiscovered oncogenic viral agents.

**It is imperative that all personnel involved in the project read all the protocols and adhere to them at all times. It is your responsibility as a contributor to the HTMCP to familiarize yourself with all aspects of the procedures and assure their compliance.**

### A. SCOPE AND PURPOSE:

1. To list all the documents needed in order to start collection of samples for the HIV+ Tumor Molecular Characterization Project (HTMCP).
2. This protocol applies to all Tissue Source Sites (TSSs) providing tissues prospectively.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times and which samples were affected. This information should be given within 48 hours of the occurrence to the project team by sending an e-mail to Dr. Jean C. Zenklusen ([jz44m@nih.gov](mailto:jz44m@nih.gov)) with the details.

### B. REQUIREMENTS:

1. Every TSS must have an IRB approved protocol in place that allows collection of tumor tissue, matched normal tissue (blood, whenever possible) and clinical data that can be used in a characterization project. The protocol must have explicit language

## HTMCP SOP #100

permitting the molecular characterization of the samples by genomic-scale methodologies, and subsequent deposition of the data into a public, but protected database.

2. Every patient accrued to the project must be enrolled in the protocol and agree to participate by signing an informed consent.
3. If you require assistance drafting such protocol or informed consent form, please contact the Project Team representative (PT, see address in contact sheet). OCG has templates that contain the appropriate language.
4. TSSs must have in place a materials transfer agreement (MTA) with both the Genome Science Center at British Columbia (GSC-BC, see address in contact sheet) and the Pathology Coordinator (see address in contact sheet) to allow transfer of tissues and pathology reports. A sample MTA can be provided by PT upon request.
5. OCG will store a copy of the IRB-approved protocol and a blank informed consent form. Additionally, certification that such protocol exists, and that patients have been consented, must be produced by the TSS Institution before the samples can be accepted and costs can be reimbursed. A template of such certification document can be found in Appendix A.
6. The completed Institutional certification must be sent to PT before any sample can be shipped.

## HTMCP SOP #102

Adopted: \_\_\_\_\_ 4/26/2010  
 2<sup>nd</sup> Version : \_\_\_\_\_ 9/01/2010  
 3<sup>rd</sup> Version : \_\_\_\_\_ 05/17/2012  
 Reviewed: \_\_\_\_\_  
 4<sup>th</sup> Version : \_\_\_\_\_

## **PROCESSING TISSUE FOR MOLECULAR CHARACTERIZATION OF HIV+ TUMORS**

### **I. INTRODUCTION**

#### **A. SCOPE AND PURPOSE:**

1. To establish a procedure for tissue processing and storage by Tissue Source Sites (TSS).
2. This protocol applies to all TSSs providing tissues prospectively.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times and which samples were affected. This information should be given within 48 hours of occurrence to the project team by sending an e-mail to Dr. Jean C. Zenklusen ([jz44m@nih.gov](mailto:jz44m@nih.gov)) with the details.

#### **B. SAFETY PRECAUTIONS:**

1. Wear personal protective equipment (PPE) such as lab coats and gloves.
2. Liquid nitrogen is extremely cold and can cause ‘burns’. Wear gloves that are specially made to withstand liquid nitrogen, eye protection (preferably Face Shield) and a lab coat to protect skin from splashes and spills. Liquid nitrogen is an asphyxiant; be sure to use in a well-ventilated area.
3. Acute overexposure to formaldehyde solutions and/or vapors causes severe eye, skin, and respiratory tract irritation.

#### **C. EQUIPMENT AND MATERIALS:**

1. Personal protective equipment (PPE) to include heavy duty gloves, eye protection (preferably Face Shield), lab coat and closed-toe shoes
2. Plastic cassette mold(s) for Formalin fixation.
3. Cryovials (2mL vials, e.g. ChartBiomed, Part Number 10778828)
4. Freezer resistant labels with project-assigned ID (obtained from Project Team, see HTMCP SOP #101)
5. Dewar thermo-flask
6. Isopentane
7. Liquid Nitrogen
8. Formalin (10% solution)

**LABEL ALL CONTAINERS WITH THE LABELS CARRYING PATIENT  
PROJECT-ASSIGNED ID OBTAINED PRIOR TO SURGERY.**

**II. PROCEDURE:**

- A. A lymph node or tissue diagnosed as tumor should be processed as follows:
    - 1. Wearing sterile gloves, cut the tissue into multiple 2 mm thin sections using a sterile scalpel.
    - 2. Place tissue into various containers as follows:
      - i. **24-HOUR FORMALIN FIXATION:** Submit two or three 2 mm representative tissue pieces for diagnosis, including lymph node capsule (1-2 blocks) to your Histology Lab. Tissue in formalin should be no more than 2 mm in thickness for proper fixation.
      - ii. **FREEZING TISSUE:** Select two or three representative pieces of tissue measuring about 10 x 10 x 2 mm in dimension (approximately 100mg). Do not freeze tissue pieces larger than aforementioned, if you have a larger tissue piece, fraction it into smaller pieces and freeze them separately. Freeze as many pieces as possible. Do not freeze the tissue with Freon.
- Freeze the tissues as described below:
- Note: Perform snap freezing of fresh tissue ASAP**
- It is generally accepted that for the best tissue preservation snap freezing should take place within 20 minutes after tissue is ablated from patient.
  - Do not perform snap freezing with bare hands. Wear gloves at all times.
- (i) **Set Up Freezing Station**
- a. Fill a small 100 ml metal beaker about 1/4 full with isopentane (2-methylbutane, certified grade).
  - b. Fill the Dewar thermo-flask about 1/3 full with liquid nitrogen.  
Use extreme caution when dispensing liquid nitrogen
- (ii) **Label Cryovials, as many as are needed for the tissue quantity obtained from the tumor**
- a. Use a cryovial for tissue snap freezing.
  - b. Label cryovial with freezer-resistant labels obtained prior to surgery (see H+TMCP SOP #101).
- (iii) **Freezing Tissue in Cryovial**
- a. Cut (razor blade or scissors) a 1cm by 3 cm strip of histowrap
  - b. Put a **single** tissue piece (no heavier than 100mg) on the histowrap strip. Weigh the piece to ensure weight limits are not exceeded.
  - c. Place the histowrap strip containing tissue into a labeled cryovial, using a pair of forceps. The forceps should be washed in 70% EtOH between handling individual tissue pieces.
  - d. Screw on the cap **tightly** or else isopentane will seep into the vial during freezing and become unwelcome liquid in the vial upon thawing.

## HTMCP SOP #102

- e. Prepare other cryovials with additional aliquots of tissue as described above. Store the tissue-containing cryovials awaiting freezing by placing them on dry ice.
  - f. Place all cryovials containing the aliquots of a single case into a 100 ml metal beaker containing 40 ml isopentane.
  - g. Lower the 100 ml metal beaker containing isopentane half-way into the liquid nitrogen for cooling. The liquid nitrogen will boil as the beaker is lowered, when the isopentane is reaching its freezing point the tone of the boiling will increase for 2-3 seconds.
  - h. Lift the beaker out of the liquid nitrogen once you see beads of solid isopentane at the bottom of the beaker (about 2 minutes).
  - i. Use long forceps to hold the cryovial down into the cooled isopentane. Hold for at least 1 minute.
  - j. Use the long forceps to take out the cryovial/ frozen tissue.
  - k. Store frozen tissue vial(s) in Liquid Nitrogen Storage Tanks.
- B. Make a gross report of the sample using the dictation template below.
- C. To evaluate the percent tumor nuclei (tumor cellularity) in your sample, a top and a bottom frozen section of each tissue piece must be produced. Creating frozen sections require the use of OCT (Optimal Cutting Temperature compound) to coat the surface to be cut to allow for stabilization. However, excessive use of OCT interferes with the DNA/RNA extraction protocol and must thus be avoided. Following these steps will enable you to obtain frozen sections without encasing the tissue in OCT:
- (a) Move a small tissue fragment(s) (~60 mg) from storage and transport it to the pathology lab on dry ice to prevent thawing.
  - (b) Cryostat chuck must be at least 20°C (the temperature of the cryostat cabinet) to ensure that the tissue does not thaw.
  - (c) Mount tissue to be cut by placing a small drop (~100 µl) of OCT on the chuck and putting the tissue block on the OCT.
  - (d) Slightly coat the top side (i.e the side which is NOT in contact with the chuck) of the tissue, up to the edge with OCT, by applying a small quantity with a wooden toothpick; discard the toothpick after single use. Allow the OCT to harden.
  - (e) Cut the frozen section so that adequate tissue is captured for evaluation (do not try to be too aggressive or will use precious sample) and place onto slide(s). Label slide(s) to indicate it is from the top of the tissue piece. Once finished, remove the remaining tissue from the chuck using a new razor blade, removing as much OCT as possible.
  - (f) To cut the bottom section slide, remount the tissue section on the chuck placing a small amount (~100µl) of OCT on the chuck and placing the tissue on it with the top side (the side that was microtomed first) facing down before the OCT hardens.
  - (g) Coat the bottom of the tissue piece (now facing up) up to the edges, with a thin coat of OCT with the help of a toothpick ; discard the toothpick after single use. Allow the OCT to harden. This step might not be necessary since

## HTMCP SOP #102

some of the OCT used to mount the piece in step 3 could be coating the whole of the face to cut. Use judgment, and don't overuse OCT if it is not necessary.

- (h) Cut the required frozen section(s) and place onto slide(s). Label one slide ahead of time to indicate that the sections are from bottom of tissue piece. Once finished, remove the remaining tissue from the chuck using a new razor blade, removing as much OCT as possible
- (i) Place the tissue piece back into the original cryovial, and store the vials in freezer or LN2 until shipment is arranged.

D. Any questions regarding this protocol should be directed to the appropriate Pathology Coordinators listed in the Contact List that can be found in the OCG Protocol manual.

**THE FROZEN SPECIMENS SHOULD BE KEPT FROZEN ON DRY ICE AT ALL TIMES DURING TRANSPORT TO AND FROM STORAGE TANKS.**

#### H+ TMCP STUDY GROSS DICTATION TEMPLATE

##### **History:**

The patient is a...

##### **Source/Gross:**

The specimen is received (**fresh vs. fixed**) in (# containers), each labeled with the project-assigned ID “#” and designated “#.” The specimen consists of (**gross to include number of fragments, size, appearance, etc.**)

Specimens submitted are:

Fixed in formalin for 24 hours – (**size, # of pieces in each block, and cassette designation**)

Snap Frozen – (**size and # of blocks**)

## HTMCP SOP#103

Adopted: \_\_\_\_\_ 4/26/2010  
 2<sup>nd</sup> Version : \_\_\_\_\_ 9/01/2010  
 3<sup>rd</sup> Version : \_\_\_\_\_  
 Reviewed: \_\_\_\_\_  
 4<sup>th</sup> Version : \_\_\_\_\_

## **PROCESSING NON-TUMOR SAMPLES FOR THE HIV+ TUMOR MOLECULAR CHARACTERIZATION PROJECT: BLOOD AND BUCCAL CELLS**

### **I. INTRODUCTION:**

#### **A. SCOPE AND PURPOSE:**

1. To establish a common procedure for processing non-tumor samples (either blood or buccal swabs) previous to shipment to the Genome Science Center at British Columbia (GSC-BC) by tissue source sites (TSS).
2. This protocol applies to all TSSs providing tissues prospectively.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times and which samples were affected. This information should be given within 48 hours of occurrence to the project team by sending an e-mail to Dr. Jean C. Zenklusen (**jz44m@nih.gov**) with the details.

#### **B. SAFETY PRECAUTIONS:**

1. Wear personal protective equipment (PPE) such as lab coats and gloves.

#### **C. EQUIPMENT AND MATERIALS:**

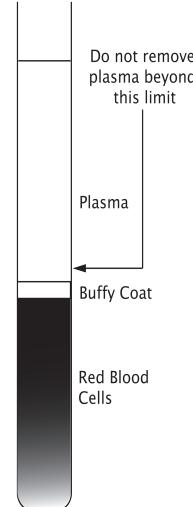
1. Personal protective equipment (PPE) to include heavy duty gloves, eye protection (preferably Face Shield), lab coat and closed-toe shoes
2. Clinical Centrifuge
3. Vortex
4. Cryovials (2mL vials, e.g., ChartBiomed, Part Number 10778828)
5. Cotton tipped swabs (e.g., Catch-All swabs, catalog # QEC091H; Epicentre Biotechnologies, Madison, WI, USA) OR Dacron swabs (e.g., MasterAmp™ Buccal Swab Brushes, catalog # MB100BR; Epicentre Biotechnologies, Madison, WI, USA)
6. Freezer resistant labels with project-assigned ID (obtained from Project Team, see HIV+TMCP SOP #101)
7. TE buffer (10 mM Tris·Cl; 1 mM EDTA, pH 8.0)
8. Dewar thermo-flask
9. Liquid nitrogen
10. Isopentane (2 methyl butane)

**MARK ALL CONTAINERS WITH THE LABELS CARRYING PATIENT PROJECT-ASSIGNED ID OBTAINED PRIOR TO SURGERY.**

**II. PROCEDURE:**

**A. BLOOD SAMPLES:**

- a. Collect 10 ml of blood according to standard procedures in tubes containing anticoagulant (recommended anticoagulant is sodium citrate or heparin).
- b. **Blood Separation:**
  1. Fractionate the whole blood by centrifuging at 1500-2000 X g for 10-15 min at room temperature. This will separate the blood into an upper plasma layer, a lower red blood cell (RBC) layer, and a thin interface containing the white blood cells (WBCs) / buffy coat (see Figure 1). Fractionate the blood as soon as possible after collection.
  - NOTE:** In a typical clinical centrifuge 1500-2000 X g is ~3000-3400 rpm. Check the appropriate settings for your centrifuge using the nomogram in your user's manual.
  2. Use a disposable, plastic transfer pipet (e.g. Falcon Cat #357524) to aspirate off the plasma (upper layer) down to ~1 mm from the buffy coat (see Figure). Discard the plasma. When removing the plasma do not disturb the WBC layer, also called the buffy coat, which forms a thin film between the upper plasma layer and the lower layer of packed RBCs. Samples with exceptionally high WBC counts will have a thicker buffy coat.
  3. Recover the WBCs in ≤0.5 ml by aspiration with a fresh disposable pipette or a Pasteur capillary pipette.
  4. Dispense the recovered buffy coat onto a cryovial labeled with a freezer-resistant label obtained prior to surgery from the Project Team (HIV+TMCP SOP #101). Screw on the cap **tightly** or else isopentane will seep into the vial during freezing and create a liquid in the vial upon thawing.



**c. Set Up Freezing Station**

1. Fill a small 100 ml metal beaker about 1/4 full with isopentane (2-methylbutane, certified grade).
2. Fill the Dewar thermo-flask about 1/3 full with liquid nitrogen.
3. Use extreme caution when dispensing liquid nitrogen

**d. Freezing Blood Cells in Cryovial**

1. Lower the 100 ml metal beaker containing isopentane half-way into the liquid nitrogen for cooling. The liquid nitrogen will boil as the beaker is lowered, when

## HTMCP SOP#103

the isopentane is reaching its freezing point the tone of the boiling will increase for 2-3 seconds.

2. Lift the beaker out of the liquid nitrogen once more than you see beads of solid isopentane at the bottom of the beaker (about 2 minutes).
3. Use long forceps to hold the cryovial down into the cooled isopentane. Keep submerged for at least 1 minute.
4. Use the long forceps to take out the cryovial/ frozen tissue.
5. Store frozen tissue vial(s) in liquid Nitrogen storage tanks or -80°C freezers.

**B. BUCCAL SWABS:**

1. To ensure adequate DNA collection, we recommend that the participant rub the inside of both cheeks firmly for a minimum of 1 minute with a minimum of three swabs.
2. Once swabbing is complete, the tips of each swab should be cut with a pair of scissors and placed into 1.5 ml microcentrifuge tube (one per tip). Add 1 ml TE buffer, close lid and vortex for 10s.
3. Remove the swab from the microcentrifuge tube using forceps. Squeeze as much liquid as possible out of the swab by pushing the swab against the side of the microcentrifuge tube. Combine all liquid from all tubes into a single microcentrifuge tube.
4. Centrifuge the microcentrifuge tube containing buccal cells at maximum speed for 10 s. Discard the supernatant and wash the buccal cells by resuspending the pellet in 1 ml TE and vortexing for 1 min.
5. Centrifuge the microcentrifuge tube containing buccal cells at maximum speed for 10 s. Discard the supernatant and resuspend the buccal cell pellet in 30 µl TE. Place suspension into a screw cap cryovial. Cell suspensions should then be frozen as described above.

Any questions regarding this protocol should be directed to the appropriate Pathology Coordinators listed in the contact list.

**THE FROZEN SPECIMENS SHOULD BE KEPT FROZEN ON DRY ICE AT ALL TIMES DURING TRANSPORT TO AND FROM STORAGE TANKS.**



## HTMCP SOP #104

Adopted: \_\_\_\_\_ 4/26/2010  
2<sup>nd</sup> Version : \_\_\_\_\_ 9/01/2010  
3<sup>rd</sup> Version : \_\_\_\_\_ 1/06/2011  
Reviewed: \_\_\_\_\_  
4<sup>th</sup> Version : \_\_\_\_\_

## **SHIPPING CRYOPORTS CONTAINING FROZEN BIOSAMPLES FOR PROCESSING AND EXTRACTION OF NUCLEIC ACIDS**

### **I. INTRODUCTION:**

Cryoports are shipped from the Genome Sciences Center at the British Columbia Cancer Agency (GSC-BC) to the Tissue Source Site (TSS). TSSs are instructed to use this SOP when shipping samples to the GSC-BC.

#### **A. SCOPE AND PURPOSE:**

1. To establish a procedure for personnel in shipping the cryoports.
2. This procedure applies to all laboratory personnel.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times and samples affected. This information should be given immediately to the project team by sending an e-mail to Dr. Jean C. Zenklusen ([jz44m@nih.gov](mailto:jz44m@nih.gov)) with the details.

#### **B. SAFETY PRECAUTIONS:**

1. Wear personal protective equipment (PPE) such as lab coats and gloves.
2. Liquid nitrogen is extremely cold and can cause ‘burns’. Wear gloves that are specially made to withstand liquid nitrogen, eye protection and a lab coat to protect skin from splashes and spills. Liquid nitrogen is an asphyxiant; be sure to use in a well-ventilated area.
3. Always keep the cryoport in the upright position.

#### **C. EQUIPMENT AND MATERIALS:**

1. Cryoport, obtained in 3 or 4 days in advance from the GSC-BC Coordinator (see below).
2. Personal protective equipment (PPE) to include heavy duty gloves, eye protection (preferably Face Shield), lab coat and closed-toe shoes
3. Shipping documents

## HTMCP SOP #104

**II. PROCEDURE:**

1. Request cryoport from GSC-BC shipping coordinator 3-4 days in advance of sectioning samples.
2. Complete the appropriate shipping forms needed for the sample(s).
3. Complete the sample shipping document with the project-assigned ID obtained prior to surgery, the sample type information and any comments. Sign and date the form and have a second individual verify the contents of the shipment and sign and date the form.
4. Don personal protection equipment.
5. To unlock the cryoport shipping carton, cut the zip ties securing the two twist latches on the outer lid, flip the butterfly handles outwards and turn counterclockwise to disengage the latches. Carefully open the cryoport shipping carton lid. The cryoport cork with attached data logger will be visible. It is not necessary to remove the cryoport from the shipping carton.
6. Extract the Allen key from the small pouch attached to the underside of the shipping carton lid. Leave the pouch attached to the lid.
7. Remove the large ziplock bag attached to the underside of the shipping carton lid. The bag contains the Cryoport Temperature Log sheet, an IATA shipping label, a FedEx Airbill Tie-On tag, a leak-proof biohazard bag, absorbent cloth sheets, and zip-ties.
8. Fill out the information on the “TSS Inbound” section of the Cryoport Temperature Log.
  - (1) The internal temperature of the cryoport is displayed on the data logger.
  - (2) If the cryoport will not be returned within 24 hours, please record the temperature each subsequent day after arrival in the “Temperature Records” section of the Cryoport Temperature Log.
  - (3) If the temperature is -180°C or colder, it can be used to ship the samples to the GSC-BC. **ALERT:** If the temperature is warmer than -180°C, please contact the GSC-BC coordinator for instructions before proceeding further.
9. Remove the zip tie securing the cryoport cork lid to the cryoport. Lift the cork up to gain internal access to the cryoport. The top of the inner, sealed, stainless steel canister will be visible. **NOTE:** Only remove the canister when you are ready to place your samples inside.
10. Carefully remove the stainless steel canister by grabbing the handle at the top and slowly lifting the canister up and out of the cryoport. **ATTENTION:** After removing the stainless steel canister from the cryoport, immediately lift the black lever of the relief valve on the top of the canister up into a vertical position to release any pressure/vacuum inside the canister.

## HTMCP SOP #104

11. Place the cork back in the cryoport while you perform the following steps. ATTENTION: Be careful that the temperature probe extending from the bottom of the cork goes into the cryoport and does not get trapped between the cork and the side of the cryoport.

- (a) Use the Allen key to remove the 6 Allen bolts securing the lid to the stainless steel canister. Be careful not to misplace any of the bolts. Ensure that the relief valve lever is still in the upright position, and lift the lid off the container. The top of the stainless steel rack will be visible.



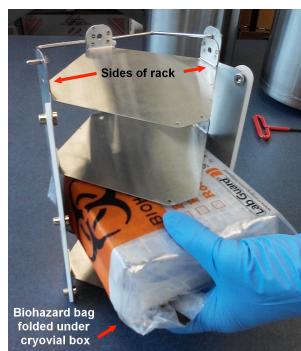
- (b) The rack has a hinged metal handle on top. Swing the handle upright (see photo at right) and then pull the rack up to lift it out of the canister.



- (c) To access the freezer box, slide the containment pin at the front of the rack (see photo at right) up and out of the guide holes, then slide the freezer box out of the rack.



- (d) Place the cryovials containing your samples into the cryovial box, then seal the cryovial box inside the supplied biohazard ziplock bag



along with 1 or more sheets (folded in half) of the absorbent cloth, as required. Each sheet is capable of absorbing 250mL of liquid. Ensure most of the air is pressed out of the bag before sealing. Fold the excess length of the biohazard bag under one edge of the freezer box (see photo at left).

- (e) Place the cryovial box back into a shelf on the rack, orienting the folded edge of the plastic bag to one side of the rack (see photo at right). Replace the containment pin by sliding it down through the top of the rack and the guide holes on each shelf. Ensure the top of the pin goes through the locking guide hole on the top of the rack (see photo at right).
- (f) Use the handles on top of the rack to carefully lower the apparatus back into the stainless steel canister. The fit is quite snug; you may need to slightly adjust the box position as you lower the rack into the canister in order for the box and bag to clear the edges of the canister.
- (g) Ensure that the top flange of the stainless steel canister and the underside of the canister lid are dry. Place the lid on the canister and align the holes in the lid with the screw holes in the canister. Ensure the relief valve lever is in the upright position, and use the Allen key to secure the lid with the 6 Allen bolts. Once all the bolts are secured, close the relief valve by flipping the lever downward into the horizontal position.



12. Carefully lower the stainless steel canister back into the cryoport, and replace the cork. **ATTENTION:** Be careful that the temperature probe extending from the bottom of the cork goes into the cryoport and does not get trapped between the cork and the side of the cryoport.
13. Align the openings in the side of the cork lid with the openings in the cryoport neck, and secure with one of the supplied zip ties. Cut most of the excess length off of the zip tie.
14. Allow the cryoport temperature to stabilize. When the datalogger displays a stable temperature reading, record the temperature in the “TSS Outbound” section of the Cryoport Temperature Log.
15. Place the Allen key back into the designated bag attached to the underside of the shipping carton lid. Ensure the enclosure is properly sealed so the Allen Key does not fall out during transport.
16. Carefully close the shipping carton lid. Engage both of the twist latches by interlocking the catches, turning the butterfly handles clockwise to close down the latch, and then folding handles down so they are flush with the body of the latch. Secure each latch with two zipties as illustrated by the image on the shipping carton.



## HTMCP SOP #104

17. Place the provided label with the IATA mark (UN 3373, Biological Substance, Category B) in the clear plastic envelope that is attached to the side of the shipping carton, such that the label is clearly visible and in the upright orientation. Ensure the plastic envelope is sealed.
18. Place all shipping documents, including the Sample Shipping Document, the Cryoport Temperature Log, and 5 copies of the Commercial Invoice, into the Airbill pouch on the Airbill Tie-On tag. Seal the pouch. Attach the Tie-On tag to the handle closest to the IATA mark on the shipping carton, and secure with a zip tie.
19. Notify the shipping carrier for pick-up. Under normal conditions, shipments should only be sent to GSC-BC on Monday through Wednesday. If an exception is needed, the GSC-BC must be contacted at 604-877-6088 for further instructions and to alert the GSC-BC personnel of any schedule changes.
20. TSS personnel will notify the coordinator by email stating the cryoport is being returned with tissue samples back to the GSC-BC, and providing the tracking number. Also provide an electronic copy of the Sample Shipping Document.
21. The GSC-BC Coordinator will track the cryoport in transit.
22. If there are any exceptions to the normal shipping schedule or in the event of an anticipated shipment delay, the Coordinator will notify the GSC-BC on-call personnel of the potential arrival of samples after normal working hours or on the weekend.
23. Upon receiving the cryoport, the temperature will be recorded and quality control verified by a second individual.
24. Any questions regarding shipments to the GSC-BC should be directed to the GSC-BC Coordinator at 604-877-6088.

**GSC-BC Coordinator:**

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## HTMCP SOP #105

Adopted: \_\_\_\_\_ 4/28/2010  
2<sup>nd</sup> Version : \_\_\_\_\_ 6/11/2010  
3<sup>rd</sup> Version : \_\_\_\_\_ 9/01/2010  
Reviewed: \_\_\_\_\_  
4<sup>th</sup> Version : \_\_\_\_\_

## SAMPLE SHIPPING GUIDELINES FOR THE HIV+ TUMOR MOLECULAR CHARACTERIZATION PROJECT

### I. INTRODUCTION:

Tumor samples from HIV+ patients are rare and they may be accrued at specific tumor source sites (TSS) at a rate of 3-5 per calendar year. Shipping costs for infectious labeled material in vapor phase liquid nitrogen containers (cryoports) are expensive.

#### A. SCOPE AND PURPOSE:

1. To establish a sample shipping guideline standard to be applied to all samples contributed to the HIV+ Tumor Molecular Characterization Project (HTMCP), that balances the need for expeditious transport while maintaining cost efficiency.
2. This procedure applies to all TSSs.

### II. ADOPTED STANDARD:

- Immediate requests for a cryoport will be made to the Genome Science Center at British Columbia (GSC-BC) coordinator when the contributing TSS has in its possession 3 or more matched tumor-normal tissues.
- However, if less than three cases are accrued, and the date of oldest sample resection is more than 4 months, shipment of this/these sample(s) is warranted.

Any questions regarding this protocol should be directed to Dr. Jean C. Zenklusen at 301-451-2144.

HTMCP SOP #105

**CONTACTS:**

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Phone: 604-877-6088

## HTMCP SOP #106

Adopted: \_\_\_\_\_ 4/28/2010  
 2<sup>nd</sup> Version : \_\_\_\_\_ 6/11/2010  
 3<sup>rd</sup> Version : \_\_\_\_\_ 9/01/2010  
 Reviewed: \_\_\_\_\_  
 4<sup>th</sup> Version : \_\_\_\_\_

## **SAMPLE IDENTIFIER STANDARDS FOR THE HIV+ TUMOR MOLECULAR CHARACTERIZATION PROJECT**

### **I. INTRODUCTION:**

To assure the privacy of all human subjects that have consented to donate their tissues and clinical data to the HIV+ Tumor Molecular Characterization Project (HTMCP), all the materials given to the project must be de-identified prior to shipment and study. This project-assigned ID must have a rational structure that permits tracking of which subproject, tissue source site (TSS) and case is labeled.

#### **A. SCOPE AND PURPOSE:**

- B. To establish a sample identifying standard to be applied to all samples and data contributed to the HTMCP.
- C. This procedure applies to all laboratory personnel.

### **II. ADOPTED STANDARD:**

- Samples contributed to the HTMCP must be labeled with a ID obtained from the Data Coordinating Center (DCC) by the TSS previous to shipment.
- These code must have the following form:

HTMCP - ##### - ##### - ##X - ##X

Where:

1. HTMCP stands for HIV+ Tumor Molecular Characterization Project
2. The next 4 digits
  - a. The first digit identifies the tumor type (1=DLBCL, 2=Lung, 3=Cervical, 4=Anal)
  - b. The next three digits identify the Tissue Source Site
3. The next five digits are the sample identifier
4. The last two digits specify sample type
5. First letter identifies the aliquote/section of the sample.
6. Next two digits and letter specify nucleic acid

## HTMCP SOP #106

**Tissue Codes:**

Sample Code	Description	Code
Primary Tumor	Primary Tumor	01
Recurrent Tumor	Recurrent Tumor	02
Primary Blood Cancer	Primary Blood Derived Cancer – Peripheral blood	03
Recurrent Blood Cancer	Blood derived Tumor Relapse - Bone Marrow	04
Addtl - New Primary	Additional - New Primary	05
Human Tumor Original	Human Tumor Original Cells	08
Primary Bld Cancer BM	Primary Blood Derived Cancer – Bone Marrow	09
Blood Derived Normal	Blood Derived Normal	10
Solid Tissue Normal	Solid Tissue Normal	11
Buccal Cell Normal	Buccal Cell Normal	12
EBV Normal	EBV Immortalized Normal	13
BM Normal	Bone Marrow Normal	14
Recurrent Blood Cancer	Blood derived Tumor Relapse – Peripheral blood	40

**Nuclei acid codes:**

- 01D=DNA, unamplified, from the first isolation of a tissue
- 01W=DNA, WGA'ed by Qiagen (1 of the 2 done)
- 01X=DNA, WGA'ed by Qiagen (2 of the 2 done)
- 01R=RNA

Note: If additional isolations are needed, the # would change to 02D, etc.

7.

Any questions regarding this protocol should be directed to Dr. Jean C. Zenklusen at 301-451-2144.

## HTMCP SOP #108

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2<sup>nd</sup> Version : \_\_\_\_\_ 9/01/20103<sup>rd</sup> Version : \_\_\_\_\_

Reviewed: \_\_\_\_\_

4<sup>th</sup> Version : \_\_\_\_\_

**DISPOSITION FORM FOR REMAINING MACROMOLECULES/TISSUES  
CONTRIBUTED TO THE HIV+ TUMOR MOLECULAR CHARACTERIZATION  
PROJECT**

**INTRODUCTION:**

The HIV+ Tumor Molecular Characterization Project (HTMCP) is an initiative to compare the cancer related alterations in HIV+ patients and HIV- patients. The project aims to generate large scale, high quality data on the cancers' genome and transcriptome using 2<sup>nd</sup> generation sequencing technology which means that the changes identified range from large genomic rearrangements, expression profile changes and detection of mutations. The characterization of the latter is mostly performed in other NCI-sponsored projects. The comparison of alterations in transcriptomes and genomes of tumors from HIV+ and HIV- individuals may or may not identify a) virus-associated genomic alterations (including mutations) which would indicate if the etiology of the illness is different; and/or b) novel non-human sequences which could suggest the presence of transcripts from known or hitherto undiscovered oncogenic viral agents.

Tissues to the HTMCP are contributed by a number of international investigators (tissue source site, TSS). A major contributor is the AIDS Malignancy Consortium (AMC), a National Cancer Institute-supported clinical trials group founded in 1995 to support innovative trials for AIDS-associated malignancies. The AMC is composed of 14 Clinical Trials Sites and their affiliates, and is committed to enhancing therapeutic options for patients with HIV-associated malignancies. All samples and macromolecules obtained from cases contributed by AMC members are sent to the AIDS and Cancer Specimen Resource (ACSR, <http://acsr.ucsf.edu/dotnetnuke/>) for banking.

ACSR is a resource for investigators working in the fields of HIV/AIDS, cancer, virology, immunology, pathology, epidemiology, tumor biology assay development, and many others. It is a biorepository for HIV-infected human biospecimens from a wide spectrum of HIV-related or associated diseases, including cancer, and from appropriate HIV-negative controls. ACSR was established by the NCI in 1994 to acquire, store, and equitably distribute tumor tissues, biological fluids, and associated clinical information from patients with HIV-associated malignancies to the scientific research community-at-large. Availability of such biospecimens facilitates efforts to identify therapeutic targets and gain further insights into the pathogenesis and treatment of cancer in the HIV-infected population.

The ACSR's public access and research facilitation function makes it an ideal location to bank any remaining tissue and/or derived macromolecule after the molecular characterization is completed by the HTMCP.

**SCOPE AND PURPOSE:**

- To establish a procedure to follow for the disposition of remaining macromolecules (DNA and/or RNA) and tissue after characterization is completed from cases submitted to the HTMCP.
- This form must be completed by every TSS and included along with the shipping documents at the time of tissue submission **if the default option of banking at the ACSR is not acceptable.**

**Remaining Material Disposition:**

**You only need to choose one of the options below if you do not want to send to ACSR for banking.**

Should after molecular characterization of case # \_\_\_\_\_ be any remaining material (tissue and or macromolecules); these remnants should be (choose one):

- Sent back to the TSS (at the TSS's expense).
- Destroyed.

Name:

Date:

Institution:

Signature:

**Should you have any question please contact Dr. Zenklusen at 301-451-2144**

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## HTMCP SOP #109

Adopted: \_\_\_\_\_  
2° Version: \_\_\_\_\_  
3° Version: \_\_\_\_\_  
4° Version: \_\_\_\_\_  
Under Revision: \_\_\_\_\_

**VERY USEFUL INSTRUCTIONS ON HOW TO COMPLETE A STUDY PROTOCOL  
REQUEST TO THE INSTITUTIONAL REVIEW BOARD (IRB)  
FOR THE HIV+ TUMOR MOLECULAR CHARACTERIZATION PROJECT**

**INTRODUCTION:**

The HIV+ Tumor Molecular Characterization Project's (HTMCP) goal is to develop a comprehensive database of the molecular changes in Human Immunodeficiency Virus (HIV)-associated cancers (from HIV-infected patients) that will be available to the research community world-wide. It will allow the comparison between the cancer related alterations in HIV+ patients and HIV- patients. The project aims to generate large scale, high quality data on the cancers' genomes and transcriptomes using 2<sup>nd</sup> generation sequencing technology which means that the changes identified range from large genomic rearrangements, expression profile changes and detection of mutations.

In order for cases to be included in the project, the patients must provide consent of participation in an approved IRB protocol specifying that the samples can be used for genomic characterization and that the data deposited in a publicly available, yet patient privacy designed database. The Office of Cancer Genomics of the National Cancer Institute has created a generic template that contains the appropriate language to help the Tissue Source Site (TSS) in producing the IRB document. This template lacks details that are Institution-specific and should not be considered complete.

**SCOPE AND PURPOSE:**

- To establish a set of instructions allowing each TSS to create their own IRB protocol to contribute samples to the HTMCP.
- These instructions should be useful to every TSS contributing samples to the HTMCP.

**INSTRUCTIONS:**

1. Obtain the IRB protocol template from either the SOP package sent when you agreed to participate in the HTMCP or the Sharepoint site ([https://ocg-sps.nci.nih.gov/HIV\\_Tumors/default.aspx](https://ocg-sps.nci.nih.gov/HIV_Tumors/default.aspx)). You may also request a copy from the project team (see address below).

## HTMCP SOP #109

2. Fill in your organization name, PI's name and other pertinent information in the form. The Project name should be "HIV+ Tumor Molecular Characterization Project" and it's acronym is HTMCP.
3. The project rationale can be found in the introduction section of SOP#101.
4. The total number of samples that will be analyzed for each tumor type is 100.
5. Details on amount of tissue requested is in SOP#101 under the sample requirement section (page 8)
6. Details on the blood collection for germline DNA extraction can be found in SOP#103.
7. Cheek swabs will not be used as a source of normal DNA in this project; please delete that language in the template.
8. All the operational details of the project are clearly specified in the SOPs sent to the TSSs. It is expected that all participating personnel will read the SOPs, be familiar with the project procedures and requirements and follow them in all instances.

**Should you have any question please contact Dr. Zenklusen at 301-451-2144**

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## **Data Release Policy for the HIV+ Tumor Molecular Characterization Project (HTMCP)**

### **Background:**

Rapidly evolving sequencing and informatics tools are substantially diminishing costs of comprehensive characterization of tumor transcriptomes and tumor genomes. These advances have resulted in detailed information on the repertoire of alterations in tumors. NCI already supports tumor genome characterization projects for several common cancers, as part of the Cancer Genome Characterization Initiative and the Cancer Genome Atlas (TCGA).

Comprehensive sequencing of genomes and transcriptomes in cancers that arise in HIV-infected individuals may provide a starting point for a systems biology approach towards understanding differences in etiologies among identical histological subtypes of cancers in HIV+ and HIV- patients. The results obtained could provide important clues to the pathways that either allow tumors to counteract immune surveillance mechanisms or are redundant in the presence of an extrinsic oncogenic influence such as viruses. It is also possible that the comparison of transcriptomes and genomes between tumors from HIV+ and HIV- individuals might identify novel non-human sequences that could suggest the presence of transcripts from hitherto undiscovered viral agents.

This is a “community resource project”, with rapid data release to enable accelerated translation to enhance clinical impact. Therefore patenting on the PRIMARY data is discouraged to allow easy access and encourage its use. There is an expectation of a rapid initial “summary” publication by the group once the data are generated.

Two data types will be produced; 1) raw sequences from the tumor/normal genomes and tumor transcriptome, 2) analyzed data from those raw sequences. It is important to acknowledge that algorithms for sequence analysis to identify tumor-specific calls are still in the development stage and thus the results obtained require confirmation. Confirmation is defined in two ways:

- Verification: assessment of sequence quality before data release (e.g. identifying Illumina artifacts, performing sample swaps, etc.).
- Validation: confirmation of variants identified by the current analytical algorithms by using orthogonal experimental methodology such as Sanger sequencing. Validation will be performed; the scope will depend on the costs and the accuracy of the sequence-calling algorithms available at the specific time. It may be performed either for a subset or all variants found (the details will be developed on real time basis to take advantage of the best approaches). The criteria for selection of a subset of variants for validation will be developed by the cancer-specific working group based on all empirical data available at decision time.

### **Policy:**

The data release policy should be consistent across all NCI-funded large-scale genomic characterization projects. The HIV+ cancers are hard to accrue and therefore the data generation

will span over a number of months or years. To best accomplish the goals of the project (generating and analyzing large enough data set to be able to draw statistically and biologically sound conclusions) and the Institute (to facilitate research and reduce redundancy by making primary data available to the scientific community in real time), the project members suggest the following policy:

- *Release of analyzed sequences (BAM files) will occur after a sample set (number to be determined) is complete, but not later than 4-6 month after they are generated.*
- *Table of the validated mutations (MAF) will be deposited to the Data Coordinating Center (DCC) after manuscript describing the findings of the dataset is submitted for publication.*

The DCC data portal (<http://cgap.nci.nih.gov/cgci.html>) will include a text about the philosophy of the rapid data release policy, “The Responsible use and publication of Data Generated by the Cancer Genome Characterization Initiative”. The language will be aligned as much as possible to the one used for TCGA and Therapeutically Applicable Research to Generate Effective Treatments (TARGET).

An HIV+ tumor project manuscript(s) could include:

1. Commentary detailing the scientific aims and organization of HIV+ tumor molecular characterization project.
2. Analysis of paired DNA sequencing data for the sample set.
3. Analysis of the RNA sequencing data for the sample set.
4. Validation of a subset of variant calls found by either DNA or RNA sequencing of the sample set.

To support the continued prompt public release of large-scale genomic data prior to publication, researchers who plan to prepare manuscripts that would be comparable to the analyses described above, and journal editors who receive such manuscripts, are encouraged to coordinate their independent reports with the project’s publication schedule described above. This may be done by contacting the Project Team (see below).

Once the first global analysis by the project members is in press, all other researchers are free, and indeed encouraged, to publish results based on integrating HIV+ tumor data with data from other sources. Researchers also are encouraged to use HTMCP data to publish on the development of novel methods to analyze genomic data related to cancer and genotype-phenotype relationships in cancer.

NCI does not consider that deposition of data from the HTMCP, like those from other large-scale genomic projects, into its own or public databases to be the equivalent of publication in a peer-reviewed journal. Therefore, although the data are available to others, the producers still consider them to be formally unpublished and expect that the data will be used in accord with standard scientific etiquette and practices concerning unpublished data.

Prior to the publication of the initial paper, the HTMCP project requests that authors who use

data acknowledge the H+TMCP as follows: “*The results published here are in whole or part based upon data generated by The HIV+ Tumor Molecular Characterization Project established by the Office of Cancer Genomics and Office of HIV and AIDS malignancies of the NCI. Information about project and the investigators and institutions that constitute the HIV+ Tumor workgroups can be found at <http://cgap.nci.nih.gov/cgci.html>*” . After initial publication, the paper and website should be referenced.

To ensure protection of genetic privacy for sample donors, data users will have to agree to certain conditions described in the HTMCP Patient Protection Policy and Controlled Access Policy as to how the data will be used. For example, users will have to agree that they will share these data only with others who have also completed a data access agreement and that they will not patent discoveries in a way that prevents others from using the data. This means that reviewers of a manuscript who need to see any controlled-access HTMCP data underlying a result must also agree to these user access conditions before they can see these data.

Meeting presentations of HTMCP data and analyses by project team members are possible and encouraged. , We would request that the project team members inform the NCI of public meeting oral and poster presentations. The HTMCP Project Team will develop two-three slides that should be used for oral presentations, posters, etc. They will provide a standard method of citing the HTMCP and its many contributors; it is critical that the HTMCP also be properly cited and identified in the meeting abstracts, and language will also be provided to accomplish this goal.

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**THE HIV+ TUMOR MOLECULAR CHARACTERIZATION PROJECT  
DIFFUSE LARGE B-CELL LYMPHOMA CONTACTS**

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## **HIV+ TUMOR MOLECULAR CHARACTERIZATION PROJECT LUNG TUMOR CONTACT PERSONS**

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**HIV+ TUMOR MOLECULAR CHARACTERIZATION PROJECT  
CAERVICAL/ANAL TUMOR CONTACT PERSONS**

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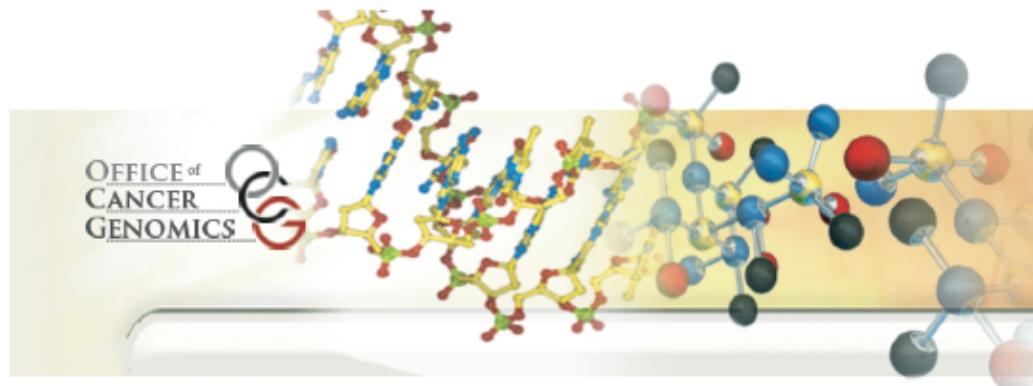
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National Cancer Institute



## HTMCP DLBCL-SPECIFIC PROTOCOLS

U.S. DEPARTMENT  
OF HEALTH AND  
HUMAN SERVICES

National Institutes  
of Health



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## **PROSPECTIVE CASE SUBMISSION PROCEDURE FOR THE HIV+DIFFUSE LARGE B-CELL LYMPHOMA PROJECT**

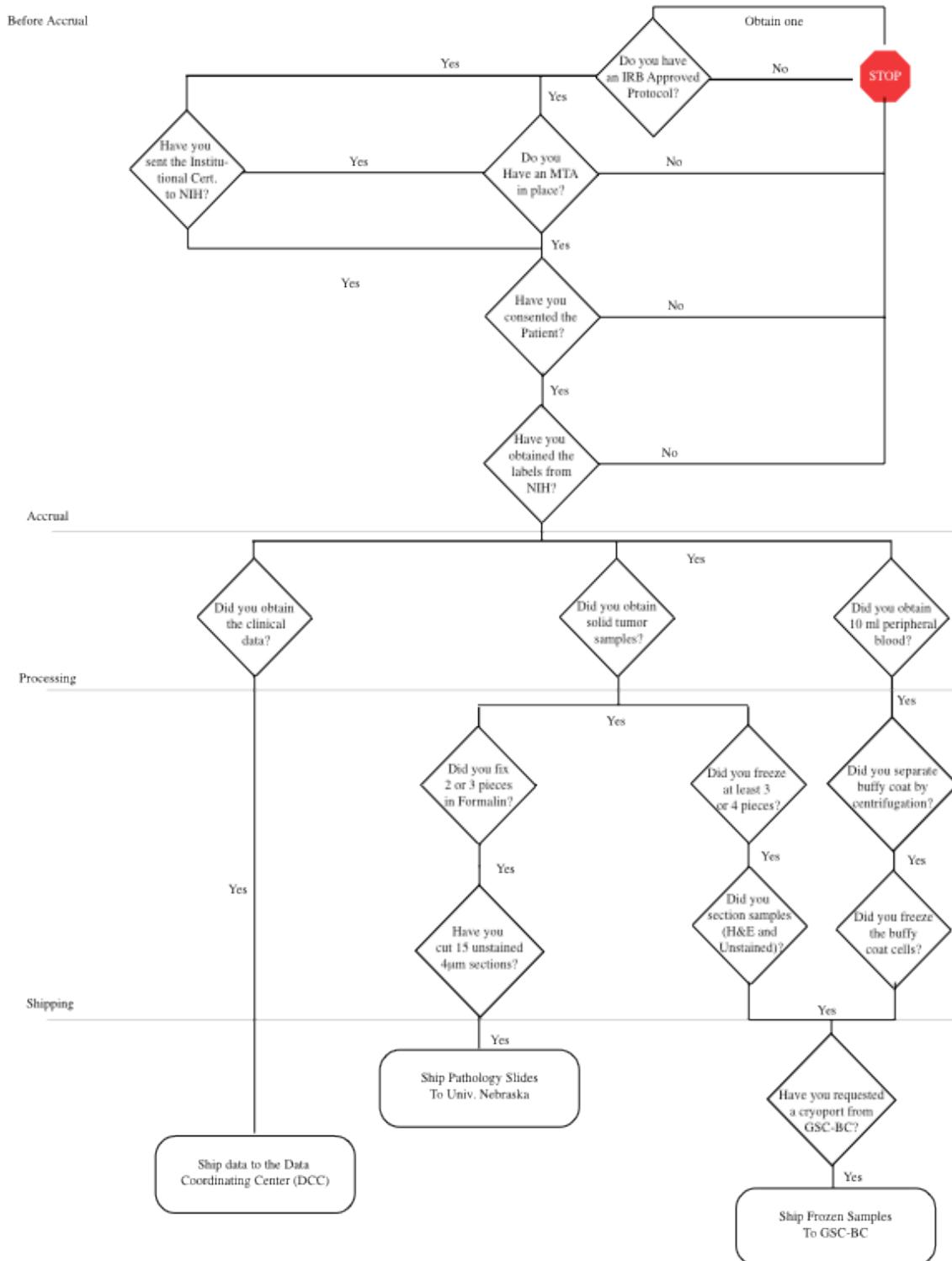
### **I. INTRODUCTION:**

The National Cancer Institute's Office of Cancer Genomics (OCG) and the Office of HIV and AIDS Malignancy (OHAM) have developed an initiative to compare the cancer related alterations in HIV+ patients and HIV- patients.

#### **A. SCOPE AND PURPOSE:**

1. To establish a general procedure to inform personnel of all the steps necessary for a successful submission of a sample to the HIV+ Tumor Molecular Characterization Project (HTMCP).
2. This protocol applies to all Tissue Source Sites (TSSs) providing tissues prospectively.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times and which samples were affected. This information should be given within 48 hours of the occurrence to the project team by sending an e-mail to Dr. Jean C. Zenklusen ([jz44m@nih.gov](mailto:jz44m@nih.gov)) with the details.

## HTMCP SOP #101A



## II. PROCEDURES:

### A. BEFORE PATIENT ACCRUAL BEGINS:

1. Make sure all the documents required for sample shipment as spelled out in SOP#100 are in place before you start case accruals.

### B. BEFORE PATIENT SURGERY:

1. Create a TSS-assigned ID for your patient. **The TSS is responsible for maintaining the link between project-assigned ID and TSS-assigned ID in order to retrieve clinical information when required.** In case that the original PI is no longer affiliated with the contributing Institution, it is the TSS's responsibility to be able to track the patient's records.
2. Contact the Data Coordinating Center (DCC, see address below) to obtain a project-assigned ID to use in all documents regarding the case and all materials shipped.
3. Contact the OCG Project Coordinator and obtain freezer-resistant labels that you should use to mark all containers/slides carrying materials for the project.
4. Make sure to prepare the tissue freezing station and have ready all the materials needed for tissue processing (HTMCP SOP #102).
5. Inform the research nurse that a 10 ml peripheral blood sample must be obtained from the patient to use as a non-tumor malignant control. Note that the buffy coat should be separated from the plasma within two hours of being obtained from the patient. The buffy coat sample should be moved immediately to a -80°C or Liquid Nitrogen (LN2) freezer for storage.

### C. DURING PATIENT SURGERY:

1. Inform the surgical staff of the tissue requirements for the project (see Appendix A).
2. Have a person ready to transport the ablated tissue to the processing station. It is generally accepted that for the best tissue preservation snap freezing should take place within 20 minutes after tissue is obtained from the patient.
3. Note the time between surgery and freezing in a notebook and send to the OCG Project Coordinator

### D. AFTER SURGERY:

1. Process solid tissue as described in the tissue processing protocol (HTMCP SOP #102). Timely processing is crucial.

2. Process blood sample according to protocol (see HTMCP SOP #103, blood processing). Store isolated cells in a -80°C freezer or liquid Nitrogen (LN<sub>2</sub>) storage until shipment.
3. Obtain a formalin-fixed, paraffin-embedded tissue block or, if not possible, **fifteen** unstained 4  $\mu$ m sections from the formalin-fixed block. Affix one of the provided freezer-resistant labels to each slide or block.

#### E. PREPARING SAMPLES AND SHIPMENT:

1. Section frozen tumor sample following the frozen tissue sectioning protocol (HTMCP SOP #201A).
2. Produce a 4 $\mu$ m frozen section of the top of the tumor sample for initial quality control as described in the tissue processing protocol (HTMCP SOP #102).
3. When tissue from at least three cases are accrued, or every quarter (See HTMCP SOP #105) contact the GSC-BC coordinator to obtain a cryoport transport vessel to ship the frozen tumor sample sections and frozen blood cells.
4. Follow the frozen sample shipment protocol (HTMCP SOP #104) and send the frozen samples to the GSC-BC. Provide both the GSC-BC and PT with tracking number the day of shipment.
5. Send a formalin-fixed, paraffin-embedded tissue block or, if not possible, **fifteen** unstained 4  $\mu$ m sections obtained from the formalin fixed blocks to the pathology coordinator. On shipment, provide both the pathology coordinator and OCG project coordinator with the tracking number of the parcel. For shipment use a closable box (such as Thermo Scientific\* Plastic Slide Box, capacity 25 slides, catalog# B1780).
6. Collect all the clinical data requested in the sample requirements (Appendix C). You will be requested by the OCG project coordinator to send the data electronically to the DCC using the appropriate TSS-assigned ID at a later date.

#### NOTES:

- A checklist is provided to help you track all the steps required by this process (Appendix B). Please use it!
- If any one of the required items (institutional certification, confirmation of informed consent, frozen tissue, frozen blood cells, unstained slides and clinical data) is not present, the submission is incomplete and reimbursement of costs cannot proceed.
- **At no point in the process can traditionally-used identifying information (such as the patient name, address, phone number, medical record number, or social security number) be used to label samples. Only use the project-assigned ID and labels provided by the OCG project coordinator.**

## APPENDIX A: Sample Requirements

### HIV+Tumor Molecular Characterization Project Tissue Sample Requirements for Accrual

#### Tissue Requirements:

To be accepted to the project, the following conditions have to be met at the tissue level.

- Tissue is from a patient who had not received neoadjuvant therapy for that tumor type or systemic treatment for other tumor.
- Paired tumor and normal tissue or plasma buffy coat must be available in sufficient quantities (see below).
- Tissues (both normal and tumor) need to be snap frozen. Time between tissue extraction and freezing must be recorded.
- Optimal storage of the tissues is in N<sub>2</sub>(liq), but -80°C or lower is acceptable. The form of tissue storage must be recorded.
- There must be 100 mg of tumor tissue with a minimum percent of tumor nuclei between 70-80% as assessed on an H&E section physically adjacent to the specimen that is candidate for generating the RNA and DNA.
- There must be enough tissue of both to produce a 4µm thick section from the top for H&E staining, then 10 sections of 20µm thickness, followed by another 4µm section to stain by H&E. **The number of sections needed is based on a block with a surface area of about one sq. cm. If the area is smaller, proportionately more sections will be required. See SOP #201A Tissue Sectioning Protocol for formula allowing calculation of number of sections needed.** A core biopsy obtained at the same time as the one produced for pathology might provide sufficient tissue if it is of high tumor content and low necrosis.
- Tumors need to have a minimum percent of tumor nuclei between 70-80% as assessed on an H&E section physically adjacent to the specimen that is candidate for generating the RNA and DNA.
- A paraffin embedded block for pathology consensus review must exist for the tumor.

**APPENDIX B: Checklist of Task Completion for Sample Submission****Date:****Institution:****Operator:**

- Do you have an IRB approved protocol?
- Have you consented the patient?
- Have you sent your Institutional Certification to the Project Team?
- Have you obtained the project-assigned ID and labels from the Project Team?
- Do you have frozen sections in cryovial and H&E stained slides? Are they labeled?
- Do you have frozen plasma derived white blood cells? Are they correctly labeled?
- Have you secured a cryoport from the Genome Science Center at British Columbia?
- Do you have a formalin-fixed, paraffin-embedded tissue block or, if not possible, (5) unstained 4  $\mu$ m sections from the Formalin-fixed block? Are they labeled?
- Do you have the clinical data elements required by the Project? (Appendix B)

**ONLY if all the above items check out, you are ready to ship the samples.**

**APPENDIX C: DLBCL Cancer Characterization Project from HIV+ Patients Clinical Data Requirements for Accrual****Clinical Data Requirements:**

To be accepted to the project, the following conditions must meet at the clinical data level. The case(s) must have available ALL the clinical data elements (CDEs) here listed, should some of the datafields be missing, please contact the OCG project coordinator to get approval for submission.

These clinical data elements must be reported to the DCC as an initial report within two weeks of enrolling the patient. Updates must be sent to the DCC as the patient returns for periodic visits (Annually).

- Patients need to be consented in such way that allows for the use of their tissues for genomic-scale molecular characterization

**Instructions:** The Enrollment Form should be completed for each qualified case in the HIV+ Tumor Characterization Project (HTMCP) study. The Tissue Source Site (TSS) should complete the form for qualified cases upon qualification notice from the Office of Cancer Genomics (OCG).

Questions regarding this form should be directed to the Clinical Data Collection Operation & Database (CDCOD) or OCG.

**Please note the following definitions for the “Unknown” and “Not Evaluated” answer options on this form.**

**Unknown:** This answer option should only be selected if the TSS does not know this information after all efforts to obtain the data have been exhausted. If this answer option is selected for a question that is part of the HTMCP required data set, the TSS must complete a discrepancy note providing a reason why the answer is unknown.

**Not Evaluated:** This answer option should only be selected by the TSS if it is known that the information being requested cannot be obtained. This could be because the test in question was never performed on the patient or the TSS knows that the information requested was never disclosed.

Tissue Source Site (TSS): \_\_\_\_\_ TSS Identifier: \_\_\_\_\_ TSS Unique Patient Identifier: \_\_\_\_\_

Completed By (Interviewer Name in OpenClinica): \_\_\_\_\_ Completed Date: \_\_\_\_\_

#	Data Element	Entry Alternatives	Working Instructions
<b>General Information</b>			
*1	Is this a prospective tissue collection?	<input type="checkbox"/> Yes <input type="checkbox"/> No	Indicate whether the TSS providing tissue is contracted for prospective tissue collection. If the submitted tissue was collected after the date the HTMCP contract was executed, the tissue has been collected prospectively. <a href="#">3088492</a>
*2	Is this a retrospective tissue collection?	<input type="checkbox"/> Yes <input type="checkbox"/> No	Indicate whether the TSS providing tissue is contracted for retrospective tissue collection. If the submitted tissue was collected prior to the date the HTMCP contract was executed, the tissue has been collected retrospectively. <a href="#">3088528</a>
<b>Patient Information</b>			
<b>Demographic Information</b>			
*3	Date of Birth	____ / ____ / ____ (month) (day) (year)	Provide the date the patient was born. <a href="#">2896950</a> (month), <a href="#">2896952</a> (day), <a href="#">2896954</a> (year) <i>Note: The day of Birth is not required.</i>
*4	Gender	<input type="checkbox"/> Female <input type="checkbox"/> Male	Provide the patient's gender using the provided categories. <a href="#">2200604</a>
*5	Race (check all that apply)	<input type="checkbox"/> American Indian or Alaska Native <input type="checkbox"/> Asian <input type="checkbox"/> White <input type="checkbox"/> Black or African American <input type="checkbox"/> Native Hawaiian or other Pacific Islander <input type="checkbox"/> Other (please specify) <input type="checkbox"/> Not Evaluated <input type="checkbox"/> Unknown	Provide the patient's race using the defined categories. <a href="#">2192199</a> <u>American Indian or Alaska Native:</u> A person having origins in any of the original peoples of North and South America (including Central America), and who maintains tribal affiliation or community attachment. <u>Asian:</u> A person having origins in any of the original peoples of the far East, Southeast Asia, or in the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. <u>White:</u> A person having origins in any of the original peoples of Europe, the Middle East, or North Africa. <u>Black or African American:</u> A person having origins in any of any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American." <u>Native Hawaiian or other Pacific Islander:</u> A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands. <u>Not Evaluated:</u> Not provided or available <u>Unknown:</u> Could not be determined or unsure
6	Other Race	_____	If the patient's race was not defined in the previous question, provide the patient's race. <a href="#">2192205</a>
7	Ethnicity	<input type="checkbox"/> Not Hispanic or Latino <input type="checkbox"/> Hispanic or Latino <input type="checkbox"/> Not Evaluated <input type="checkbox"/> Unknown	Provide the patient's ethnicity using the defined categories. <a href="#">2192217</a> <u>Not Hispanic or Latino:</u> A person not meeting the definition of Hispanic or Latino. <u>Hispanic or Latino:</u> A person of Mexican, Puerto Rican, Cuban, Central or South American, or other Spanish culture or origin, regardless of race. <u>Not Evaluated:</u> Not provided or available <u>Unknown:</u> Could not be determined or unsure
8	Height (at time of diagnosis)	_____ (cm)	Provide the patient's height (centimeters) at the time the patient was diagnosed with the tumor submitted for HTMCP. <a href="#">649</a>
9	Weight (at time of diagnosis)	_____ (kg)	Provide the patient's weight (kilograms) at the time the patient was diagnosed with the tumor submitted for HTMCP. <a href="#">651</a>

#	Data Element	Entry Alternatives	Working Instructions
<b>Survival Information</b>			
*10	Vital Status (at date of last contact)	<input type="checkbox"/> Living <input type="checkbox"/> Deceased	Indicate whether the patient was living or deceased at the date of last contact. <a href="#">2939553</a>
*11	Date of Last Contact	____ / ____ / ____ (month)      (day)      (year)	If the patient is living, provide the date of last contact with the patient (as reported by the patient, medical provider, family member, or caregiver). <a href="#">2897020</a> (month), <a href="#">2897022</a> (day), <a href="#">2897024</a> (year) <i>Do not answer if patient is deceased.</i> <i>Note: The day of Last Contact is not required.</i>
*12	Date of Last Known Alive	____ / ____ / ____ (month)      (day)      (year)	Indicate the last date the patient was known to be alive, regardless of whether the patient, medical provider, family member or caregiver was contacted. <a href="#">2975722</a> (month), <a href="#">2975724</a> (day), <a href="#">2975726</a> (year) <i>Note: The day of Last Known Alive is not required.</i>
*13	Date of Death	____ / ____ / ____ (month)      (day)      (year)	If the patient is deceased, provide the month of death. <a href="#">2897026</a> , (month) <a href="#">2897028</a> (day), <a href="#">2897030</a> (year) <i>Note: The day of Death is not required.</i>
<b>Patient Status (Regarding Submitted Tumor)</b>			
*14	Did the patient receive neo-adjuvant therapy for the tumor submitted for HTMCP?	<input type="checkbox"/> Yes (exclusion criterion) <input type="checkbox"/> No	Indicate whether the patient received treatment (radiation, pharmaceutical, or both) prior to the procurement of the sample submitted for TCGA. <a href="#">3382737</a> <i>If the answer to this question is "yes", the submitted case is excluded.</i>
*15	Tumor Status (at time of last contact or death)	<input type="checkbox"/> Tumor free <input type="checkbox"/> With tumor <input type="checkbox"/> Unknown	Indicate whether the patient was tumor/disease free (i.e. free of the malignancy that yielded the sample submitted for the HTMCP study) at the date of last contact or death. <a href="#">2759550</a>
16	Performance Status: Eastern Cooperative Oncology Group	<input type="checkbox"/> 0: Asymptomatic <input type="checkbox"/> 1: Symptomatic, but fully ambulatory <input type="checkbox"/> 2: Symptomatic, in bed less than 50% of day <input type="checkbox"/> 3: Symptomatic, in bed more than 50% of day, but not bed-ridden <input type="checkbox"/> 4: Bed-ridden <input type="checkbox"/> Unknown <input type="checkbox"/> Not Evaluated	Provide the Eastern Cooperative Oncology Group (ECOG) performance status of the patient at the time of diagnosis. <a href="#">2003853</a>
17	Performance Status: Eastern Cooperative Oncology Group	<input type="checkbox"/> 100: Normal, no complaints, no evidence of disease <input type="checkbox"/> 90: Able to carry on normal activity; minor signs or symptoms of disease <input type="checkbox"/> 80: Normal activity with effort; some signs or symptoms of disease <input type="checkbox"/> 70: Cares for self, unable to carry on normal activity or to do active work <input type="checkbox"/> 60: Requires occasional assistance <input type="checkbox"/> 50: Requires considerable assistance and frequent medical care <input type="checkbox"/> 40: Disabled, requires special care and assistance <input type="checkbox"/> 30: Severely disabled, hospitalization indicated. Death not imminent <input type="checkbox"/> 20: Very sick, hospitalization <input type="checkbox"/> 10: Moribund, fatal processes progressing rapidly <input type="checkbox"/> 0: Dead <input type="checkbox"/> Unknown <input type="checkbox"/> Not Evaluated	Provide the Eastern Cooperative Oncology Group (ECOG) performance status of the patient at the time of diagnosis. <a href="#">88</a>
18	Performance Status Score: Timing	<input type="checkbox"/> Preoperative <input type="checkbox"/> Pre-adjuvant Therapy <input type="checkbox"/> Post-adjuvant Therapy <input type="checkbox"/> Unknown	Indicate the timing of the performance status(es) provided in the previous question(s). <a href="#">2792763</a>
19	Tumor Response	<input type="checkbox"/> Progressive Disease <input type="checkbox"/> Stable Disease <input type="checkbox"/> Partial Response <input type="checkbox"/> Complete Response	Indicate the patient's measure of success after their primary treatment for the tumor submitted for HTMCP. Treatment includes surgery and adjuvant therapies. <a href="#">2786727</a>
<b>Patient History of Disease</b>			
<b>HIV Status</b>			
*20	Is this patient HIV positive?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	Indicate whether the patient is HIV positive. <a href="#">2180464</a>

#	Data Element	Entry Alternatives	Working Instructions
*21	Date of HIV Diagnosis (if known)	____ / ____ / ____ (month) (day) (year)	Provide the month the patient was diagnosed with HIV. <a href="#">3579640</a> (month), <a href="#">3579644</a> (day), <a href="#">3579643</a> (year) <i>Note: The day of HIV Diagnosis is not required.</i>
22	Nadir CD4 Counts	_____ (cells/mm <sup>3</sup> )	Provide the patient's Nadir CD4 counts, which are the lowest CD4 counts the patient has had. <a href="#">2684395</a>
*23	CD4 Counts at Diagnosis of the Submitted Malignancy	_____ (cells/mm <sup>3</sup> )	Provide the patient's CD4 Counts at the time the patient was diagnosed with the malignancy submitted for the HTMCP study. <a href="#">2922654</a>
*24	HIV RNA load at Diagnosis of Submitted Malignancy	_____	Provide the HIV RNA load (also known as the "viral load") at the time the patient was diagnosed with the malignancy submitted for the HTMCP study. <a href="#">2922674</a>
25	Prior AIDS Defining Co-Morbidities	_____	Prior to the malignancy submitted for the HTMCP study, provide any AIDS defining co-morbidities including, but not limited to the following: diabetes mellitus, cardiovascular disease, non-AIDS-defining malignancies, and osteoporosis. <a href="#">2970715</a>
26	Co-Infections ( <i>serology data/viral load if available</i> )	<b>Test</b> <b>Results</b>	Using the list provided, indicate whether the patient had any co-infections by providing the results of each of the tests listed.
		HBV	<a href="#">2180456</a>
		HCV	<a href="#">2695021</a>
		HPV	<a href="#">2230033</a>
		KSHV/HHV8	<a href="#">3335773</a>
*27	HAART Treatment Prior to Diagnosis of Submitted Malignancy	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	Indicate whether the patient received Highly Active Antiretroviral Therapy (HAART) treatment prior to the diagnosis of the malignancy submitted for the HTMCP study. <a href="#">3335156</a>
*28	HAART Treatment at Time of Diagnosis of Submitted Malignancy	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	Indicate whether the patient received Highly Active Antiretroviral Therapy (HAART) treatment at the time of the diagnosis of the malignancy submitted for the HTMCP study. <a href="#">2922679</a>
29	CDC HIV Risk Group(s)	<input type="checkbox"/> Homosexual or bisexual contact <input type="checkbox"/> Heterosexual contact <input type="checkbox"/> IV drug user <input type="checkbox"/> Transfusion recipient <input type="checkbox"/> Hemophiliac <input type="checkbox"/> Other	Indicate whether the patient has a history of any of the listed HIV Risk Groups as defined by the Center for Disease Control (CDC). <a href="#">2542215</a>
<b>Prior Malignancies</b>			
*30	Has this patient at any time in their life had a prior diagnosis of a malignant neoplasm?	<input type="checkbox"/> Yes ( <i>exclusion criterion</i> ) <input type="checkbox"/> No	Indicate whether the patient was, at any time in their life, diagnosed with a malignancy prior to the diagnosis of the specimen submitted for HTMCP. <a href="#">61396</a> <i>If the answer to this question is "yes", the submitted case is excluded. This exclusion does not apply if the patient only has a history of non-melanoma skin cancer OR in situ carcinoma.</i>
31	Type of Prior Malignancies	_____	If the patient has had a prior diagnosis of a malignant neoplasm, provide the type of prior malignancy. <a href="#">2718428</a>
<b>Prior Immunological Disease</b>			
32	Patient History of Prior Immunological Disease	<input type="checkbox"/> Rheumatoid Arthritis <input type="checkbox"/> Sjogren's Syndrome <input type="checkbox"/> Systemic Lupus Erythematosus <input type="checkbox"/> Crohn's Disease <input type="checkbox"/> Ulcerative Colitis <input type="checkbox"/> Hashimoto's Thyroiditis <input type="checkbox"/> Other <input type="checkbox"/> Unknown	Indicate whether the patient has a history of any of the listed immunological diseases. <a href="#">3233628</a>
33	Patient History of Prior Immunosuppressive Therapy for Immunological Disease	<input type="checkbox"/> Methotrexate <input type="checkbox"/> Cyclophosphamide <input type="checkbox"/> Azathioprine <input type="checkbox"/> Anti-TNF therapy <input type="checkbox"/> Other <input type="checkbox"/> Unknown	If the patient received immunosuppressive therapy for the immunological disease selected in the previous question, provide the type of immunosuppressive therapy given. <a href="#">3233638</a>

#	Data Element	Entry Alternatives	Working Instructions	
<b>Prior Infectious Disease</b>				
34	Patient History of Relevant Prior Infectious Disease	<input type="checkbox"/> Hepatitis B <input type="checkbox"/> Hepatitis C <input type="checkbox"/> H. Pylori <input type="checkbox"/> Other <input type="checkbox"/> Unknown	Indicate whether the patient has a history of any of the listed infectious disease. <a href="#">3233645</a>	
35	Patient History of Other Relevant Infectious Disease	_____	If the patient has a history of relevant prior disease that was not included in the list, provide the infectious disease. <a href="#">3233643</a>	
<b>Pathologic Information</b>				
*36	Histological Subtype	<input type="checkbox"/> Diffuse Large B-cell Lymphoma (DLBCL) NOS (any anatomic site, nodal or extra nodal) <input type="checkbox"/> Primary Mediastinal (thymic) Large B-cell Lymphoma <input type="checkbox"/> Primary DLBCL of the CNS <input type="checkbox"/> Primary cutaneous DLBCL, leg type <input type="checkbox"/> EBV Positive DLBCL of the Elderly <input type="checkbox"/> DLBCL Associated with Chronic Inflammation	Using the patient's final diagnostic pathology report, provide the most detailed histological subtype available. <a href="#">3081934</a>	
37	Percent Follicular Component	<input type="checkbox"/> <10% <input type="checkbox"/> >= 10%	Using the pathology report, indicate the percentage of the follicular component within the diffuse large B-cell lymphoma sample that was removed from the patient. <a href="#">3232840</a>	
*38	Site of Nodal Involvement at Diagnosis <i>(Please check all that apply)</i>	<input type="checkbox"/> Axillary <input type="checkbox"/> Cervical <input type="checkbox"/> Epitrochlear <input type="checkbox"/> Femoral <input type="checkbox"/> Iliilar <input type="checkbox"/> Iliac-common <input type="checkbox"/> Iliac-external <input type="checkbox"/> Mediastinal <input type="checkbox"/> Mesenteric	<input type="checkbox"/> Occipital <input type="checkbox"/> Paraaortic <input type="checkbox"/> Parotid <input type="checkbox"/> Popliteal <input type="checkbox"/> Retroperitoneal <input type="checkbox"/> Splenic <input type="checkbox"/> Supraclavicular <input type="checkbox"/> Submandibular	Using the patient's medical record check all applicable boxes to identify the lymph node chain(s) that were involved by diffuse large B-cell lymphoma at the time of initial diagnosis. <a href="#">2180591</a> <i>To select multiple sites of involvement, press the control button and select the sites of involvement. Your selections should be highlighted after you've selected.</i>
*39	Site(s) of Extranodal Involvement At Diagnosis <i>(Please check all that apply)</i>	<input type="checkbox"/> Adrenal <input type="checkbox"/> Bone <input type="checkbox"/> Bone Marrow <input type="checkbox"/> Breast <input type="checkbox"/> Peripheral Blood <input type="checkbox"/> Skin <input type="checkbox"/> Soft Tissue <i>(muscle, ligaments, subcutaneous)</i> <b>ENT &amp; Eye</b> <input type="checkbox"/> Intraocular <input type="checkbox"/> Larynx <input type="checkbox"/> Nasal Soft Tissue <input type="checkbox"/> Nasopharynx <input type="checkbox"/> Oropharynx <input type="checkbox"/> Parotid Gland <input type="checkbox"/> Peri-orbital Soft Tissue <input type="checkbox"/> Salivary Gland <input type="checkbox"/> Sinus <input type="checkbox"/> Thyroid <b>Central Nervous System</b> <input type="checkbox"/> Brain <input type="checkbox"/> Epidural <input type="checkbox"/> Lepomeninges	<b>Gastrointestinal/ Abdominal</b> <input type="checkbox"/> Ascites/ Peritoneum <input type="checkbox"/> Appendix <input type="checkbox"/> Colon <input type="checkbox"/> Esophagus <input type="checkbox"/> Liver <input type="checkbox"/> Pancreas <input type="checkbox"/> Rectum <input type="checkbox"/> Small Intestine <input type="checkbox"/> Stomach <b>Genito-urinary Tract</b> <input type="checkbox"/> Epididymis <input type="checkbox"/> Kidney <input type="checkbox"/> Ovary <input type="checkbox"/> Prostate <input type="checkbox"/> Testes <input type="checkbox"/> Uterus <b>Mediastinal/ Intra-thoracic</b> <input type="checkbox"/> Heart <input type="checkbox"/> Lung <input type="checkbox"/> Mediastinal Soft Tissue <input type="checkbox"/> Pericardium <input type="checkbox"/> Pleura <input type="checkbox"/> Other, please specify	Using the patient's medical record check all applicable boxes to identify the anatomic location of all site(s) of extranodal involvement by diffuse large B-cell lymphoma at the time of initial diagnosis. <a href="#">2735776</a> <i>To select multiple sites of involvement, press the control button and select the sites of involvement. Your selections should be highlighted after you've selected.</i>

#	Data Element	Entry Alternatives	Working Instructions		
40	Other Specified Site of Extranodal Involvement at Diagnosis (For Primary Clinical Involvement)	_____	If all extranodal sites of involvement are not included in the list provided, please indicate any sites of extranodal involvement. <a href="#">3234303</a>		
40	Number of Extranodal Sites of Involvement Above (to calculate the IPI)	_____	Provide the total number of extranodal sites with lymphoma involvement. Use the previous three questions to determine this number. This information, along with other data provided, will be used by the Analysis Working Group (AWG) to calculate the International Prognostic Index (IPI). <a href="#">3233242</a>		
41	Maximum Tumor Bulk (Dimension)	_____ (cm)	After review of the entire medical record, record the length of the largest dimension/ diameter of a tumor, regardless of anatomical plane. <a href="#">64215</a>		
*42	Anatomic Site of Maximum Tumor Bulk (Select one anatomic site from listing above)	_____	Using the list of sites in numbers 43 and 44, provide the anatomic site of the maximum tumor bulk. <a href="#">3233300</a>		
<b><i>Pathologic Diagnosis and Surgical Resection</i></b>					
*43	Date of Initial Pathologic Diagnosis	____ / ____ / ____ (month)      (day)      (year)	Provide the date the patient was initially diagnosed with the malignancy submitted for HTMCP. This may or may not be the date of the surgical resection that yielded the tumor sample submitted for HTMCP. <a href="#">2896956</a> (month), <a href="#">2896958</a> (day), <a href="#">2896960</a> (year) <b>Note: The day of Initial Pathologic Diagnosis is not required.</b>		
44	Method of Initial Pathologic Diagnosis	<input type="checkbox"/> Cytology <input type="checkbox"/> Biopsy <input type="checkbox"/> Surgical Resection <input type="checkbox"/> Other (please specify) <input type="checkbox"/> Unknown	Provide the method of the initial pathologic diagnosis. This is the method used on the date provided above. <a href="#">2757941</a>		
45	Other Method of Initial Pathologic Diagnosis	_____	If the method of initial pathologic diagnosis is not included in the list above, provide the method used. <a href="#">2757948</a>		
47	Date of Surgical Resection	____ / ____ / ____ (month)      (day)      (year)	Provide the date of the surgical resection that yielded the tumor sample submitted for HTMCP. <a href="#">3008197</a> (month), <a href="#">3008195</a> (day), <a href="#">3008199</a> (year)		
<b><i>Staging and Histology of Bone Marrow</i></b>					
*48	Tumor Stage	<b>Stage</b>	<b>Clinical (CS)</b>	<b>Pathologic (PS)</b>	Using the Ann Arbor criteria, provide the stage that was used to treat the patient. <a href="#">3065862</a> (pathologic), <a href="#">3440332</a> (clinical) <b>A:</b> Absence of the Ann Arbor staging system symptoms including fevers, night sweats, and weight loss. <b>B:</b> Presence of the Ann Arbor staging system symptoms including fevers, night sweats, and weight loss. <b>E:</b> Presence of lymphoma in extranodal sites.
		<input type="checkbox"/> Stage I	<input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> E	<input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> E	
		<input type="checkbox"/> Stage II	<input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> E	<input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> E	
		<input type="checkbox"/> Stage III	<input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> E	<input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> E	
		<input type="checkbox"/> Stage IV	<input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> E	<input type="checkbox"/> A <input type="checkbox"/> B <input type="checkbox"/> E	
49	Presence of Malignant Cells in Bone Marrow by Histology	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	Indicate if malignant cells are histologically Confirmed in the patient's bone marrow. <a href="#">2180550</a>		
50	Histology of Bone Marrow Samples	<input type="checkbox"/> Concordant Histology <input type="checkbox"/> Disconcordant Histology <input type="checkbox"/> Unknown	If malignant cells are present in the bone marrow at the time of initial staging workup, determine if the histologic diagnosis of the bone marrow is concordant with the previously diagnosed DLBCL. <a href="#">3233401</a>		
<b><i>Tests Performed</i></b>					
<b><i>LDH Level (at the time of staging)</i></b>					
*51	LDH Level	_____	(IU)	Record the result of the LDH lab test performed during the staging workup. <a href="#">298766</a>	

#	Data Element	Entry Alternatives	Working Instructions
*52	LDH Level Upper Limit for Normal at Facility	_____ (IU)	Record the upper limit of the normal range of the LDH lab test performed at the reporting facility. <a href="#">2953115</a>
<b>Genetic Testing</b>			
53	B-cell Immunophenotype Methodology	<input type="checkbox"/> IHC <input type="checkbox"/> Flow Cytometry <input type="checkbox"/> Unknown	If B-cell genotype was performed, indicate the testing method used. <a href="#">64540</a>
54	Immunophenotyping	(+)      (-)      Indeterminant	Indicate all tests performed for immunophenotypic analysis in order to classify clonal subgroups. <a href="#">3234614</a>
		CD19 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		CD10 > 30% <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		BCL2 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		P53 > 20% <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		CD20 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		MUM1 > 30% <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		CD138 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		CD22 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		BCL6 > 30% <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		CD23 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		CD79a <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		PAX5 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		CD5 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		HHV8 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		CD30 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		Cytoplasmic Ig <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		CD15 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		Surface Ig <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		EBER <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		Cyclin D1 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		ALK <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
55	Immunophenotyping MIB-1 (Percent Positive; 4+ Scale)	<input type="checkbox"/> 0-25% <input type="checkbox"/> 26-50% <input type="checkbox"/> 51-75% <input type="checkbox"/> 76-100%	Provide the percentage range of MIB-1 positive cells identified through immunophenotypic analysis. <a href="#">3233414</a>
56	B-Cell Genotype: IgH	<input type="checkbox"/> Clonal <input type="checkbox"/> Non-Clonal <input type="checkbox"/> Not Performed	If B-cell genotype was performed, indicate the results of the IgH. <a href="#">3233560</a>
57	B-Cell Genotype: IgK	<input type="checkbox"/> Clonal <input type="checkbox"/> Non-Clonal <input type="checkbox"/> Not Performed	If B-cell genotype was performed, indicate the results of the IgK. <a href="#">3233565</a>
58	Methodology Used to Determine B-Cell Genotype	<input type="checkbox"/> IgH PCR <input type="checkbox"/> IgL PCR <input type="checkbox"/> IgH Southern <input type="checkbox"/> IgK Southern	If B-cell genotype was performed, indicate the testing method used. <a href="#">3233449</a>
<b>Genetic Abnormalities</b>			
59	Genetic Abnormalities	N      T      G      A      L      O	Indicate all genetic abnormalities for which the patient was tested. <a href="#">3234675</a>
		<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
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		<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
60	Other Genetic Abnormalities (please specify)	N      T      G      A      L      O	Specify any other genetic abnormalities not in the provided list for which the patient was tested. <a href="#">3234685</a>
		<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	

#	Data Element	Entry Alternatives	Working Instructions
61	Methodology Used to Identify Genetic Abnormalities	1      2      3      4	If the patient was tested for a specific genetic abnormality, indicate the testing method used to perform each analysis. <a href="#">3234684</a>
		C-MYC <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		BLC2 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		BCL6 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		ALK <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		C-REL <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		9p21 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		CCND1 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		MALT1 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
			Methodology Code: 1 = PCR 2 = Southern Blot 3 = FISH 4 = Cytogenetic
62	EBV Status of Malignant Cells	<input type="checkbox"/> Positive <input type="checkbox"/> Negative <input type="checkbox"/> Not Performed	Provide the result of the lab test to detect the presence of Epstein/Barr Virus antibody in the patient. <a href="#">2003961</a>
63	If EBV status is positive, provide the percent positive. <i>(does not include background positives)</i>	_____ (%)	If the patient's EBV status was positive, provide the percentage of EBV positive malignant cells. Do not include the number of background positives. <a href="#">3233649</a>
64	Methodology Used to Determine EBV Status of Malignant Cells	<input type="checkbox"/> EBER in situ Hybridization <input type="checkbox"/> LMP Immunohistochemistry <input type="checkbox"/> EBV PCR	If the patient's EBV status was positive, provide the testing method used to determine the EBV status of the malignant cells. <a href="#">3233656</a>
<b>General Comments</b>			

Principal Investigator Signature

Date

*I acknowledge that the above information provided by my institution is true and correct and has been quality controlled.*

## HTMCP SOP #107A

Adopted: \_\_\_\_\_ 09/01/2010  
 2<sup>nd</sup> Version : \_\_\_\_\_ 04/07/2011  
 3<sup>rd</sup> Version : \_\_\_\_\_ 05/25/2012  
 Reviewed: \_\_\_\_\_  
 4<sup>th</sup> Version : \_\_\_\_\_

## **CENTRALIZED PATHOLOGY REVIEW PROCESS FOR HIV+ DIFFUSE LARGE B-CELL LYMPHOMA**

### **INTRODUCTION:**

Pathological diagnosis of tumor can be impacted by the subjective nature of the process as well as the subjective definition of the criteria used in the assessment. To assure that samples entering the sequencing pipeline of the HIV+ Tumor Molecular Characterization Project meet the tissue requirements (set forth in SOP#101A) and are diagnosed as Diffuse Large B-cell Lymphoma, DLBCL, a central pathology review panel of five board-certified pathologists is established. The review of tissues by a group minimizes the subjectivity encountered in pathology practice.

#### **A. SCOPE AND PURPOSE:**

1. To establish a standard procedure to follow for the centralized pathology review of tissue submitted to the HIV+ Tumor Molecular Characterization Project.

#### **B. EQUIPMENT AND MATERIALS:**

1. De-identified pathology report provided by the tissue source site (TSS) contributing the sample.
2. Fifteen (15) unstained 4  $\mu$ m thick sections from the formalin-fixed paraffin-embedded (FFPE) diagnostic block (or whole FFPE block). These sections will be provided by the tissue source site (TSS) contributing the sample labeled with the project-assigned ID (as specified in SOP #101 and 102).
3. BioImagene Slide Scanner

### **II. PROCEDURE:**

#### **A. Preparation for review:**

1. All members of a centralized pathology board obtain their PathXchange credentials by going to the following website: <http://www.pathxchange.org/user/register>
2. Once the credentials are secured, they should be communicated to the appropriate OCG project manager.
3. Upon arrival at the pathology core, the pathology coordinator will verify that all slides and reports submitted are labeled with the same project-assigned ID.
  - The report will be scanned and uploaded to the PathXchange website (<http://www.pathxchange.org>).

## HTMCP SOP #107A

4. Pathology coordinator will send the appropriate number of slides to the histology service to perform hematoxylin & eosin (H&E) as well as necessary immunohistochemical (IHC) and in situ hybridization protocols.
  - IHC to be performed include: **CD3, CD10, CD20, BCL6, MUM1, BCL2, Ki67, TP53 , CD79a.**
  - In situ hybridization will be performed: **EBER.**
  - The processing should take no longer than 5 days.
5. After the H&E and IHC processing is completed, the pathology coordinator will scan the whole slide on the Aperio system and deposit them, together w/ a blank review form on the PathXchange website (<http://www.pathxchange.org>).
6. The coordinator will send an e-mail to the members of the pathology review core (PRC) (with copy to the OCG project manager) informing them that materials for review have been deposited in a folder. This communication must specify the number and name of files, as well as the project-assigned ID(s) for the case(s) under review.

**B. Review:**

1. Within three days of receipt of the e-mail from the coordinator, all members of the PRC will return their pathology review form to the pathology coordinator via e-mail.
2. The tumors will be classified using the WHO classification
3. If a consensus is reached, 3 out of 5 reviewers agree, and the case passes the specified criteria the pathology coordinator will create a final pathology report and submit it to the Data Coordinating Center and the Genome Science Center at British Columbia (GSC-BC) no later than 4 days. Steps 1-3 will take 2 weeks total.
4. Cases considered inadequate for diagnosis due to low quality of FFPE sections will be labeled as such and taken out of the study.
5. Discrepant cases (cases in which the 2 out of 3 majority consensus is not reached) will be submitted for a web-based consensus review, to be convened by Dr. John Chan. The schedule of such consensus reviews will be dictated by the number of discordant cases accrued as follows:
  - When 6 or more discordant cases have been accrued, a consensus review panel must be convened.
  - If there are less than 6 discordant cases, but the oldest accrued case is more than six months old, a consensus review panel must be convened.

**CONTACTS:****Project Team Representative:**

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**Data Coordinating Center:**

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**HIV+TMCP Pathology Coordinators:**

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HTMCP SOP #107A

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## HTMCP SOP#201A

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2<sup>nd</sup> Version : \_\_\_\_\_ 6/11/2010  
3<sup>rd</sup> Version : \_\_\_\_\_ 9/01/2010  
Reviewed: \_\_\_\_\_  
4<sup>th</sup> Version : \_\_\_\_\_

## **SECTIONING TISSUE FOR THE HIV+ TUMOR MOLECULAR CHARACTERIZATION PROJECT**

### I. INTRODUCTION:

#### A. SCOPE AND PURPOSE:

1. To establish a common procedure for tissue sectioning previous to shipment to the Genome Science Center at British Columbia (GSC-BC) across tissue source sites (TSS).
2. This protocol applies to all TSSs providing tissues prospectively.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times and which samples were affected. This information should be given within 48 hours of occurrence to the project team by sending an e-mail to Dr. Jean C. Zenklusen (**jz44m@nih.gov**) with the details.

#### B. SAFETY PRECAUTIONS:

1. Wear personal protective equipment (PPE) such as lab coats and gloves.

#### C. EQUIPMENT AND MATERIALS:

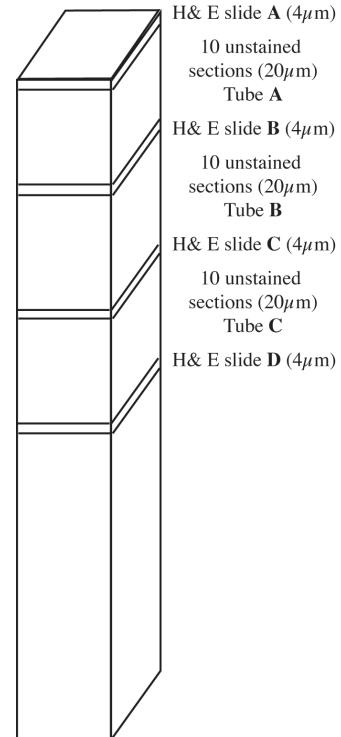
1. Personal protective equipment (PPE) to include heavy duty gloves, eye protection (preferably Face Shield), lab coat and closed-toe shoes
2. Frozen sample
3. OCT Freezing Compound
4. Cryostat
5. Glass slide(s) (such as Corning Glass Slides, 3 x 1" frosted end, # 26003)
6. Cryovials (2mL vials, e. g., ChartBiomed, Part Number 10778828)
7. Freezer resistant labels with project-assigned ID (obtained from Project Team, see HTMCP SOP #101A)

**MARK ALL CONTAINERS WITH THE LABELS CARRYING PATIENT PROJECT-ASSIGNED ID OBTAINED PRIOR TO SURGERY.**

**II. PROCEDURE:**

- A. When preparing a tissue block for shipment to the GSC-BC the following steps must be followed:

1. All tube(s) must be kept in dry ice at all times and be stored in liquid nitrogen storage tanks until shipment to the GSC-BC can be arranged following the HTMCP Shipping Protocol (HTMCP SOP #105)
2. Transport the cryovial containing the sample on dry ice to the cryostat.
3. Remove Frozen tissue from cryovial with a clean pair of forceps (e.g., by washing them in 70% Ethanol).
4. Coat the top of the tissue piece with a thin layer of OCT by dipping into the compound held in a small weighing boat or similar container.
5. Obtain a 4um section and stain with H&E to assess the quality of the tissue (i.e, is the tumor percent nuclei greater than 70%). Label with the project-assigned ID and a capital letter A (e.g., HTCP-###-#####-##-A) and save the section for shipment.  
**No sample should be shipped if the preliminary % tumor nuclei assessment at the TSS is below the 70% cut-off.**
6. Label a cryovial with the project-assigned ID and a capital letter A (e.g., HTCP-###-#####-##-A). Cut ten 20um thick sections and put into a cryovial in a beaker of dry ice inside the cryostat. **The number of sections needed is based on a tissue with a surface area of about one sq. cm. If the area is smaller, proportionately more sections will be required and vice versa (see calculation formula below to estimate the number of sections needed)**
7. Coat the top of the tissue piece with a thin layer of OCT by dipping into the compound held in a small weighing boat or similar container.
8. Obtain a 4um section and stain with H&E to assess the quality of the tissue. Label with the project-assigned ID and a capital letter B (e.g., HTCP-###-#####-##-B) and save the section for shipment (see figure).
9. Additional sections (10/tube) may be cut into tubes B, C, etc. depending on the anticipated future research needs. A 4 um section must be obtained and stain with H&E to assess the quality of the tissue in between each series of thick sections. These H&E slides must be shipped to the appropriate location.
10. Return the remaining tissue to the liquid nitrogen storage tank.
- 11. The blade should be cleaned with alcohol after each case and different parts of the blade used for different cases.**



## HTMCP SOP#201A

12. Note that excess OCT must be carefully trimmed away before sectioning as its inclusion will interfere with subsequent RNA extraction.
  13. Shipping institutions for the cryovials containing the frozen sections as well as the H&E sections are in the HIV+ Tumor Molecular Characterization Project Protocol (HTMCP SOP #101A)
- B. Any questions regarding this protocol should be directed to the HTMCP Pathology Coordinators at 402-559-7689 or 402-559-7526.

**THE FROZEN SPECIMENS SHOULD BE KEPT FROZEN ON DRY ICE AT ALL TIMES DURING TRANSPORT TO AND FROM STORAGE TANKS.**

**Estimating the number of 20 $\mu$ m sections needed:**

- 1) Measure, in millimeters, the length and width of the tissue in the block.
- 2) Use the formula below to estimate the number of 20 $\mu$ m sections needed per cryovial to fulfill tissue requirements. Use that number of sections in step 6 of this protocol.

$$\text{Number of sections} = [\text{Length (mm)} \times \text{width (mm)}] \times 10 / 100 \text{ mm}^2$$

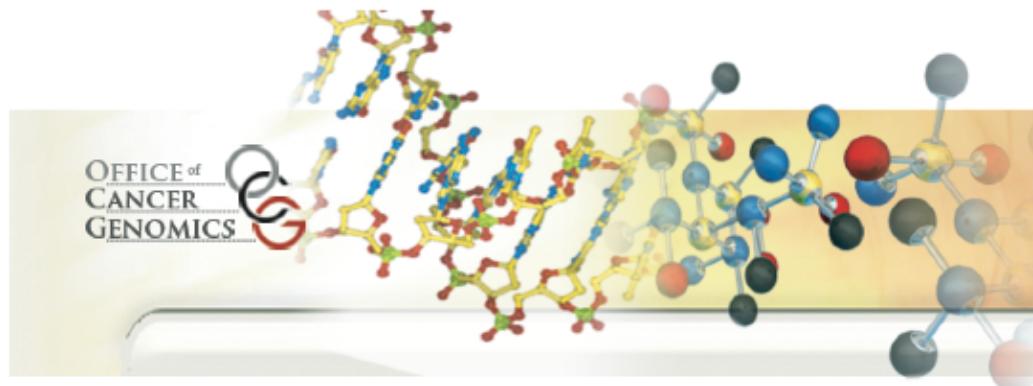
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National Cancer Institute



## HTMCP LUNG-SPECIFIC PROTOCOLS

U.S. DEPARTMENT  
OF HEALTH AND  
HUMAN SERVICES

National Institutes  
of Health



Adopted: \_\_\_\_\_ 09/14/2010  
2° Version: \_\_\_\_\_ 05/25/2012  
3° Version: \_\_\_\_\_  
4° Version: \_\_\_\_\_  
Under Revision: \_\_\_\_\_

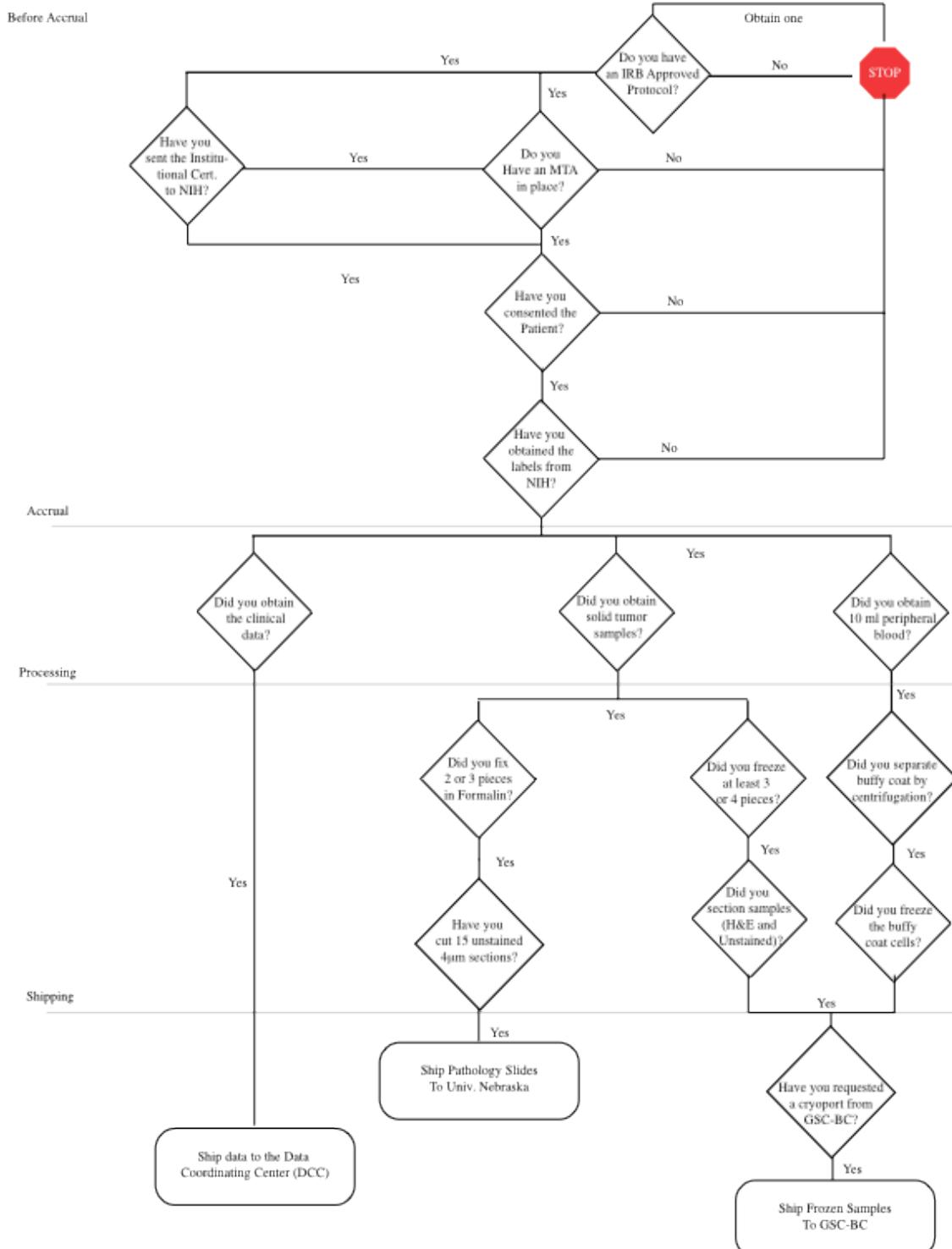
## **PROSPECTIVE CASE SUBMISSION PROCEDURE FOR THE HIV+ LUNG PROJECT**

### **I. INTRODUCTION:**

The National Cancer Institute's Office of Cancer Genomics (OCG) and the Office of HIV and AIDS Malignancy (OHAM) have developed an initiative to compare the cancer related alterations in HIV+ patients and HIV- patients.

#### **A. SCOPE AND PURPOSE:**

1. To establish a general procedure to inform personnel of all the steps necessary for a successful submission of a sample to the HIV+ Tumor Molecular Characterization Project (HTMCP).
2. This protocol applies to all Tissue Source Sites (TSSs) providing tissues prospectively.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times and which samples were affected. This information should be given within 48 hours of the occurrence to the project team by sending an e-mail to Dr. Jean C. Zenklusen (**jz44m@nih.gov**) with the details.



## II. PROCEDURES:

### A. BEFORE PATIENT ACCRUAL BEGINS:

1. Make sure all the documents required for sample shipment as spelled out in SOP#100 are in place before you start case accruals.

### B. BEFORE PATIENT SURGERY:

1. Create a TSS-assigned ID for your patient. **The TSS is responsible for maintaining the link between project-assigned ID and TSS-assigned ID in order to retrieve clinical information when required.** In case that the original PI is no longer affiliated with the contributing Institution, it is the TSS's responsibility to be able to track the patient's records.
2. Contact the Data Coordinating Center (DCC, see address below) to obtain a project-assigned ID to use in all documents regarding the case and all materials shipped.
3. Contact the OCG Project Coordinator and obtain freezer-resistant labels that you should use to mark all containers/slides carrying materials for the project.
4. Make sure to prepare the tissue freezing station and have ready all the materials needed for tissue processing (HTMCP SOP #102).
5. Inform the research nurse that a 10 ml peripheral blood sample must be obtained from the patient to use as a non-tumor malignant control. Note that the buffy coat should be separated from the plasma within two hours of being obtained from the patient. The buffy coat sample should be moved immediately to a -80°C or Liquid Nitrogen (LN<sub>2</sub>) freezer for storage.

### C. DURING PATIENT SURGERY:

1. Inform the surgical staff of the tissue requirements for the project (see Appendix A).
2. Have a person ready to transport the ablated tissue to the processing station. It is generally accepted that for the best tissue preservation snap freezing should take place within 20 minutes after tissue is obtained from the patient.
3. Note the time between surgery and freezing in a notebook and send to the OCG Project Coordinator

### D. AFTER SURGERY:

1. Process solid tissue as described in the tissue processing protocol (HTMCP SOP #102). Timely processing is crucial.
2. Process blood sample according to protocol (see HTMCP SOP #103, blood processing). Store isolated cells in a -80°C freezer or liquid Nitrogen (LN<sub>2</sub>) storage until shipment.

3. Obtain a formalin-fixed, paraffin-embedded tissue block or, if not possible, **five** unstained 4  $\mu\text{m}$  sections from the formalin-fixed block. Affix one of the provided freezer-resistant labels to each slide or block.

#### E. PREPARING SAMPLES AND SHIPMENT:

1. Produce a 4 $\mu\text{m}$  frozen section of the top of the tumor sample for initial quality control as described in the tissue processing protocol (HTMCP SOP #102).
2. When tissue from at least three cases are accrued, or every quarter (See HTMCP SOP #105) contact the GSC-BC coordinator to obtain a cryoport transport vessel to ship the frozen tumor sample sections and frozen blood cells.
3. Follow the frozen sample shipment protocol (HTMCP SOP #104) and send the frozen samples to the GSC-BC. Provide both the GSC-BC and PT with tracking number the day of shipment.
4. Send a formalin-fixed, paraffin-embedded tissue block or, if not possible, **five** unstained 4  $\mu\text{m}$  sections obtained from the formalin fixed blocks to the pathology coordinator. On shipment, provide both the pathology coordinator and OCG project coordinator with the tracking number of the parcel. For shipment use a closable box (such as Thermo Scientific\* Plastic Slide Box, capacity 25 slides, catalog# B1780).
5. Collect all the clinical data requested in the sample requirements (Appendix C). You will be requested by the OCG project coordinator to send the data electronically to the DCC using the appropriate TSS-assigned ID at a later date.

#### NOTES:

- A checklist is provided to help you track all the steps required by this process (Appendix C). Please use it!
- If any one of the required items (institutional certification, confirmation of informed consent, frozen tissue, frozen blood cells, unstained slides and clinical data) is not present, the submission is incomplete.
- **At no point in the process can traditionally-used identifying information (such as the patient name, address, phone number, medical record number, or social security number) be used to label samples. Only use the project-assigned ID and labels provided by the OCG project coordinator.**

**APPENDIX A: Sample Requirements****HIV+ Lung Cancer Characterization Project  
Tissue Sample Requirements for Accrual****Tissue Requirements:**

To be accepted to the project, the following conditions have to be met at the tissue level.

- Paired tumor and normal tissue or plasma buffy coat must be available in sufficient quantities (see below).
- Tissues (both normal and tumor) need to be snap frozen. Time between tissue extraction and freezing must be recorded.
- Optimal storage of the tissues is in N<sub>2</sub>(liq), but -80°C or lower is acceptable. The form of tissue storage must be recorded.
- There must be 100 mg of tumor tissue with a minimum percent of tumor nuclei between 70-80% as assessed on an H&E section physically adjacent to the specimen that is candidate for generating the RNA and DNA.
- A paraffin embedded block for pathology consensus review must exist for the tumor.

**APPENDIX B: Checklist of Task Completion for Sample Submission****Date:****Institution:****Operator:**

- Do you have an IRB approved protocol?
- Have you consented the patient?
- Have you sent your Institutional Certification to the Project Team?
- Have you obtained the project-assigned ID and labels from the Project Team?
- Do you have frozen sections in cryovial and H&E stained slides? Are they labeled?
- Do you have frozen plasma derived white blood cells? Are they correctly labeled?
- Have you secured a cryoport from the Genome Science Center at British Columbia?
- Do you have a formalin-fixed, paraffin-embedded tissue block or, if not possible, (5) unstained 4  $\mu$ m sections from the Formalin-fixed block? Are they labeled?
- Do you have the clinical data elements required by the Project? (Appendix C)

**ONLY if all the above items check out, you are ready to ship the samples.**

**APPENDIX C: Lung Cancer Characterization Project from HIV+ Patients Clinical Data Requirements for Accrual****Clinical Data Requirements:**

To be accepted to the project, the following conditions must meet at the clinical data level. The case(s) must have available ALL the clinical data elements (CDEs) here listed, should some of the datafields be missing, please contact the OCG project coordinator to get approval for submission.

These clinical data elements must be reported to the DCC as an initial report within two weeks of enrolling the patient. Updates must be sent to the DCC as the patient returns for periodic visits (Annually).

- Patients need to be consented in such way that allows for the use of their tissues for genomic-scale molecular characterization.

**Instructions:** The Enrollment Form should be completed for each qualified case in the HIV+ Tumor Characterization Project (HTMCP) study. The Tissue Source Site (TSS) should complete the form for qualified cases upon qualification notice from the Office of Cancer Genomics (OCG).

Questions regarding this form should be directed to the Clinical Data Collection Operation & Database (CDCOD) or OCG.

**Please note the following definitions for the “Unknown” and “Not Evaluated” answer options on this form.**

**Unknown:** This answer option should only be selected if the TSS does not know this information after all efforts to obtain the data have been exhausted. If this answer option is selected for a question that is part of the HTMCP required data set, the TSS must complete a discrepancy note providing a reason why the answer is unknown.

**Not Evaluated:** This answer option should only be selected by the TSS if it is known that the information being requested cannot be obtained. This could be because the test in question was never performed on the patient or the TSS knows that the information requested was never disclosed.

Tissue Source Site (TSS): \_\_\_\_\_ TSS Identifier: \_\_\_\_\_ TSS Unique Patient Identifier: \_\_\_\_\_

Completed By (Interviewer Name in OpenClinica): \_\_\_\_\_ Completed Date: \_\_\_\_\_

#	Data Element	Entry Alternatives	Working Instructions
<b>General Information</b>			
*1	Is this a prospective tissue collection?	<input type="checkbox"/> Yes <input type="checkbox"/> No	Indicate whether the TSS providing tissue is contracted for prospective tissue collection. If the submitted tissue was collected after the date the HTMCP contract was executed, the tissue has been collected prospectively. <a href="#">3088492</a>
*2	Is this a retrospective tissue collection?	<input type="checkbox"/> Yes <input type="checkbox"/> No	Indicate whether the TSS providing tissue is contracted for retrospective tissue collection. If the submitted tissue was collected prior to the date the HTMCP contract was executed, the tissue has been collected retrospectively. <a href="#">3088528</a>
<b>Patient Information</b>			
<b>Demographic Information</b>			
*3	Date of Birth	____ / ____ / ____ (month) (day) (year)	Provide the date the patient was born. <a href="#">2896950</a> (month), <a href="#">2896952</a> (day), <a href="#">2896954</a> (year) <i>Note: The day of Birth is not required.</i>
*4	Gender	<input type="checkbox"/> Female <input type="checkbox"/> Male	Provide the patient's gender using the provided categories. <a href="#">2200604</a>
*5	Race (check all that apply)	<input type="checkbox"/> American Indian or Alaska Native <input type="checkbox"/> Asian <input type="checkbox"/> White <input type="checkbox"/> Black or African American <input type="checkbox"/> Native Hawaiian or other Pacific Islander <input type="checkbox"/> Other (please specify) <input type="checkbox"/> Not Evaluated <input type="checkbox"/> Unknown	Provide the patient's race using the defined categories. <a href="#">2192199</a> <b>American Indian or Alaska Native:</b> A person having origins in any of the original peoples of North and South America (including Central America), and who maintains tribal affiliation or community attachment. <b>Asian:</b> A person having origins in any of the original peoples of the far East, Southeast Asia, or in the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. <b>White:</b> A person having origins in any of the original peoples of Europe, the Middle East, or North Africa. <b>Black or African American:</b> A person having origins in any of any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American." <b>Native Hawaiian or other Pacific Islander:</b> A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands. <b>Not Evaluated:</b> Not provided or available <b>Unknown:</b> Could not be determined or unsure
6	Other Race	_____	If the patient's race was not defined in the previous question, provide the patient's race. <a href="#">2192205</a>
7	Ethnicity	<input type="checkbox"/> Not Hispanic or Latino <input type="checkbox"/> Hispanic or Latino <input type="checkbox"/> Not Evaluated <input type="checkbox"/> Unknown	Provide the patient's ethnicity using the defined categories. <a href="#">2192217</a> <b>Not Hispanic or Latino:</b> A person not meeting the definition of Hispanic or Latino. <b>Hispanic or Latino:</b> A person of Mexican, Puerto Rican, Cuban, Central or South American, or other Spanish culture or origin, regardless of race. <b>Not Evaluated:</b> Not provided or available <b>Unknown:</b> Could not be determined or unsure
8	Height (at time of diagnosis)	_____ (cm)	Provide the patient's height (centimeters) at the time the patient was diagnosed with the tumor submitted for HTMCP. <a href="#">649</a>
9	Weight (at time of diagnosis)	_____ (kg)	Provide the patient's weight (kilograms) at the time the patient was diagnosed with the tumor submitted for HTMCP. <a href="#">651</a>

#	Data Element	Entry Alternatives	Working Instructions
*10	Tobacco Smoking History Indicator	<input type="checkbox"/> 1: Lifelong Non-Smoker <input type="checkbox"/> 2: Current Smoker <input type="checkbox"/> 3: Current Reformed Smoker for > 15 years <input type="checkbox"/> 4: Current Reformed Smoker for <= 15 years <input type="checkbox"/> 5: Current Reformed Smoker (duration not specified) <input type="checkbox"/> Smoking Status not Documented	Indicate the patient's history of tobacco smoking as well as their current smoking status using the defined categories. If the patient is a lifelong non-smoker, skip the additional smoking questions. <a href="#">2181650</a>
11	Age of Onset Tobacco History Indicator	____ years	Provide the age in years when the patient began smoking cigarettes. <a href="#">2178045</a>
12	Year of Quitting Tobacco Smoking	____ (YYYY)	Provide the year the patient quit smoking. <a href="#">2228610</a>
13	Number of Pack Years Smoked	____ pack years	Provide the number of pack years the patient smoked. This is calculated using the number of cigarettes smoked per day times the number of years smoked, divided by 20. For example, if the patient smoked 5 cigarettes per day times 10 years divided by 20, the patient would have 2.5 pack years (e.g. 5x10/20=2.5). <a href="#">2955385</a>
<b>Survival Information</b>			
*14	Vital Status (at date of last contact)	<input type="checkbox"/> Living <input type="checkbox"/> Deceased	Indicate whether the patient was living or deceased at the date of last contact. <a href="#">2939553</a>
*15	Date of Last Contact	____ / ____ / ____ (month) (day) (year)	If the patient is living, provide the date of last contact with the patient (as reported by the patient, medical provider, family member, or caregiver). <a href="#">2897020</a> (month), <a href="#">2897022</a> (day), <a href="#">2897024</a> (year) <i>Note: The day of Last Contact is not required.</i>
*16	Date of Last Known Alive	____ / ____ / ____ (month) (day) (year)	Indicate the last date the patient was known to be alive, regardless of whether the patient, medical provider, family member or caregiver was contacted. <a href="#">2975722</a> (month), <a href="#">2975724</a> (day), <a href="#">2975726</a> (year) <i>Note: The day of Last Known Alive is not required.</i>
*17	Date of Death	____ / ____ / ____ (month) (day) (year)	If the patient is deceased, provide the date of death. <a href="#">2897026</a> , (month) <a href="#">2897028</a> (day), <a href="#">2897030</a> (year) <i>Note: The day of Death is not required.</i>
<b>Patient Status (Regarding Submitted Tumor)</b>			
18	Did the patient receive neo-adjuvant therapy for the tumor submitted for HTMCP?	<input type="checkbox"/> Yes (exclusion criterion) <input type="checkbox"/> No	Indicate whether the patient received treatment (radiation, pharmaceutical, or both) prior to the procurement of the sample submitted for TCGA. <a href="#">3382737</a> <i>If the answer to this question is "yes", the submitted case is excluded.</i>
*19	Tumor Status (at time of last contact or death)	<input type="checkbox"/> Tumor free <input type="checkbox"/> With tumor <input type="checkbox"/> Unknown	Indicate whether the patient was tumor/disease free (i.e. free of the malignancy that yielded the sample submitted for the HTMCP study) at the date of last contact or death. <a href="#">2759550</a>
20	Performance Status: Eastern Cooperative Oncology Group Score	<input type="checkbox"/> 0: Asymptomatic <input type="checkbox"/> 1: Symptomatic, but fully ambulatory <input type="checkbox"/> 2: Symptomatic, in bed less than 50% of day <input type="checkbox"/> 3: Symptomatic, in bed more than 50% of day, but no bed-ridden <input type="checkbox"/> 4: Bed-ridden <input type="checkbox"/> Unknown <input type="checkbox"/> Not Evaluated	Provide the Eastern Cooperative Oncology Group (ECOG) performance status of the patient at the time it was evaluated, as selected in the "Performance Status: Timing" question below. <a href="#">88</a>
21	Performance Status: Karnofsky Score	<input type="checkbox"/> 100: Normal, no complaints, no evidence of disease <input type="checkbox"/> 90: Able to carry on normal activity; minor signs or symptoms of disease <input type="checkbox"/> 80: Normal activity with effort; some signs or symptoms of disease <input type="checkbox"/> 70: Cares for self, unable to carry on normal activity or to do active work <input type="checkbox"/> 60: Requires occasional assistance <input type="checkbox"/> 50: Requires considerable assistance and frequent medical care <input type="checkbox"/> 40: Disabled, requires special care and assistance <input type="checkbox"/> 30: Severely disabled, hospitalization indicated. Death not imminent <input type="checkbox"/> 20: Very sick, hospitalization <input type="checkbox"/> 10: Moribund, fatal processes progressing rapidly <input type="checkbox"/> 0: Dead <input type="checkbox"/> Unknown <input type="checkbox"/> Not Evaluated	Provide the Karnofsky performance status of the patient at the time it was evaluated, as selected in the "Performance Status: Timing" question below. <a href="#">2003853</a>

#	Data Element	Entry Alternatives	Working Instructions
22	Performance Status: Timing	<input type="checkbox"/> Preoperative <input type="checkbox"/> Pre-adjuvant Therapy <input type="checkbox"/> Post-adjuvant Therapy <input type="checkbox"/> Unknown	Indicate the timeing of the performance status(es) provided in the previous question(s). <a href="#">2792763</a>
<b>Patient History of Disease</b>			
<b>HIV Status</b>			
*23	Is this patient HIV positive?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	Indicate whether the patient is HIV positive. <a href="#">2180464</a>
*24	Date of HIV Diagnosis (if known)	____ / ____ / ____ (month)      (day)      (year)	Provide the month the patient was diagnosed with HIV. <a href="#">3579640</a> (month), <a href="#">3579644</a> (day), <a href="#">3579643</a> (year) <i>Note: The day of HIV Diagnosis is not required.</i>
25	Nadir CD4 Counts	_____ (cells/mm <sup>3</sup> )	Provide the patient's Nadir CD4 counts, which are the lowest CD4 counts the patient has had. <a href="#">2684395</a>
26	CD4 Counts at Diagnosis of the Submitted Malignancy	_____ (cells/mm <sup>3</sup> )	Provide the patient's CD4 Counts at the time the patient was diagnosed with the malignancy submitted for the HTMCP study. <a href="#">2922654</a>
*27	HIV RNA load at Diagnosis of Submitted Malignancy	_____	Provide the HIV RNA load (also known as the "viral load") at the time the patient was diagnosed with the malignancy submitted for the HTMCP study. <a href="#">2922674</a>
28	Prior AIDS Defining Co-Morbidities	_____	Prior to the malignancy submitted for the HTMCP study, provide any AIDS defining co-morbidities including, but not limited to the following: diabetes mellitus, cardiovascular disease, non-AIDS-defining malignancies, and osteoporosis. <a href="#">2970715</a>
29	Co-Infections ( <i>serology data/viral load if available</i> )	Test	Results
		HBV	<a href="#">2180456</a>
		HCV	<a href="#">2695021</a>
		HPV	<a href="#">2230033</a>
		KSHV/HHV8	<a href="#">3335773</a>
*30	HAART Treatment Prior to Diagnosis of Submitted Malignancy	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	Indicate whether the patient received Highly Active Antiretroviral Therapy (HAART) treatment prior to the diagnosis of the malignancy submitted for the HTMCP study. <a href="#">3335156</a>
*31	HAART Treatment at Time of Diagnosis of Submitted Malignancy	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	Indicate whether the patient received Highly Active Antiretroviral Therapy (HAART) treatment at the time of the diagnosis of the malignancy submitted for the HTMCP study. <a href="#">2922679</a>
32	CDC HIV Risk Group(s)	<input type="checkbox"/> Homosexual or bisexual contact <input type="checkbox"/> Heterosexual contact <input type="checkbox"/> IV drug user <input type="checkbox"/> Transfusion recipient <input type="checkbox"/> Hemophiliac <input type="checkbox"/> Other	Indicate whether the patient has a history of any of the listed HIV Risk Groups as defined by the Center for Disease Control (CDC). <a href="#">2542215</a>
<b>Prior Malignancies</b>			
*33	Has this patient at any time in their life had a prior diagnosis of a malignant neoplasm?	<input type="checkbox"/> Yes ( <i>exclusion criterion</i> ) <input type="checkbox"/> No	Indicate whether the patient was, at any time in their life, diagnosed with a malignancy prior to the diagnosis of the specimen submitted for HTMCP. <a href="#">61396</a> <i>If the answer to this question is "yes", the submitted case is excluded. This exclusion does not apply if the patient only has a history of non-melanoma skin cancer OR in situ carcinoma.</i>
34	Type of Prior Malignancies	_____	If the patient has had a prior diagnosis of a malignant neoplasm, provide the type of prior malignancy. <a href="#">2718428</a>
<b>Prior Immunological Disease</b>			
35	Patient History of Prior Immunological Disease	<input type="checkbox"/> Rheumatoid Arthritis <input type="checkbox"/> Sjogren's Syndrome <input type="checkbox"/> Systemic Lupus Erythematosus <input type="checkbox"/> Crohn's Disease <input type="checkbox"/> Ulcerative Colitis <input type="checkbox"/> Hashimoto's Thyroiditis <input type="checkbox"/> Other <input type="checkbox"/> Unknown	Indicate whether the patient has a history of any of the listed immunological diseases. <a href="#">3233628</a>

#	Data Element	Entry Alternatives	Working Instructions
36	Patient History of Prior Immunosuppressive Therapy for Immunological Disease	<input type="checkbox"/> Methotrexate <input type="checkbox"/> Anti-TNF therapy <input type="checkbox"/> Cyclophosphamide <input type="checkbox"/> Other <input type="checkbox"/> Azathioprine <input type="checkbox"/> Unknown	If the patient received immunosuppressive therapy for the immunological disease selected in the previous question, provide the type of immunosuppressive therapy given. <a href="#">3233638</a>
<b>Prior Infectious Disease</b>			
37	Patient History of Relevant Prior Infectious Disease	<input type="checkbox"/> Hepatitis B <input type="checkbox"/> Other <input type="checkbox"/> Hepatitis C <input type="checkbox"/> Unknown <input type="checkbox"/> H. Pylori	Indicate whether the patient has a history of any of the listed infectious disease. <a href="#">3233645</a>
38	Patient History of Other Relevant Infectious Disease	_____	If the patient has a history of relevant prior disease that was not included in the list, provide the infectious disease. <a href="#">3233643</a>
<b>Pathologic Information</b>			
*39	Histological Subtype	<b>Squamous Cell Carcinoma</b> <input type="checkbox"/> Papillary Squamous Cell Carcinoma <input type="checkbox"/> Clear Cell Squamous Cell Carcinoma <input type="checkbox"/> Small Cell Squamous Cell Carcinoma <input type="checkbox"/> Basaloid Squamous Cell Carcinoma <input type="checkbox"/> Squamous Cell Carcinoma (NOS) <b>Adenocarcinoma</b> <input type="checkbox"/> Adenocarcinoma, Mixed Subtype <input type="checkbox"/> Acinar Adenocarcinoma <input type="checkbox"/> Papillary Adenocarcinoma <input type="checkbox"/> Bronchioloalveolar Carcinoma, Mucinous <input type="checkbox"/> Bronchioloalveolar Carcinoma, Non-Mucinous <input type="checkbox"/> Solid Pattern Predominant Adenocarcinoma <input type="checkbox"/> Micropapillary Adenocarcinoma <input type="checkbox"/> Fetal Adenocarcinoma <input type="checkbox"/> Mucinous Cytadenocarcinoma <input type="checkbox"/> Mucinous (Colloid) Adenocarcinoma <input type="checkbox"/> Signet Ring Adenocarcinoma <input type="checkbox"/> Clear Cell Adenocarcinoma <input type="checkbox"/> Adenocarcinoma (NOS)	Using the patient's final diagnostic pathology report, provide the most detailed histological subtype available. <a href="#">3081934</a>
*40	Organ of Origin	<input type="checkbox"/> Lung	Using the patient's pathology/laboratory report, select the organ where the disease originated. <a href="#">2735776</a>
*41	Laterality	<input type="checkbox"/> Right <input type="checkbox"/> Left <input type="checkbox"/> Bilateral	Using the patient's pathology/laboratory report, select the laterality of the disease. Include all areas of invasion. <a href="#">827</a>
*42	Anatomic Organ Subdivision <i>(Check all that apply)</i>	<input type="checkbox"/> Upper Lobe <input type="checkbox"/> Middle Lobe ( <i>right only</i> ) <input type="checkbox"/> Lower Lobe <input type="checkbox"/> Bronchus <input type="checkbox"/> Mediastinal <input type="checkbox"/> Other (please specify)	Using the patient's pathology/laboratory report, select the anatomic organ subdivision(s) of the disease. Include all areas of invasion. <a href="#">2008006</a>
43	Other Anatomic Organ Subdivision	_____	If the anatomic organ subdivision was not included in the provided, indicate the anatomic organ subdivision of the disease. <a href="#">3407703</a>
<b>Pathologic Diagnosis and Surgical Resection</b>			
*44	Date of Initial Pathologic Diagnosis	____ / ____ / ____ (month)      (day)      (year)	Provide the date the patient was initially diagnosed with the malignancy submitted for HTMCP. This may or may not be the date of the surgical resection that yielded the tumor sample submitted for HTMCP. <a href="#">2896956</a> <i>Note: The day of Initial Pathologic Diagnosis is not required.</i>
*45	Method of Initial Pathologic Diagnosis	<input type="checkbox"/> Cytology <input type="checkbox"/> Fine Needle Aspiration Biopsy <input type="checkbox"/> Incisional Biopsy <input type="checkbox"/> Excisional Biopsy <input type="checkbox"/> Tumor Resection <input type="checkbox"/> Other (please specify) <input type="checkbox"/> Unknown	Provide the method of the initial pathologic diagnosis. This is the method used on the date provided above. <a href="#">2757941</a>
46	Other Method of Initial Pathologic Diagnosis	_____	If the method of initial pathologic diagnosis is not included in the list above, provide the method used. <a href="#">2757948</a>

#	Data Element	Entry Alternatives		Working Instructions
47	Date of Surgical Resection	<u>  </u> / <u>  </u> / <u>  </u> (month)      (day)      (year)		Provide the date of the surgical resection that yielded the tumor sample submitted for HTMCP. <a href="#">3008197</a> (month), <a href="#">3008195</a> (day), <a href="#">3008199</a> (year)
48	Residual Tumor	<input type="checkbox"/> RX: Margins not assessed <input type="checkbox"/> R0: Negative margins <input type="checkbox"/> R1: Microscopic positive margins <input type="checkbox"/> R2: Macroscopic positive margins <input type="checkbox"/> Unknown		Using the defined categories, indicate the patient's residual tumor margins after their final surgery. <a href="#">2608702</a>
<b>AJCC Staging</b>				
*49	Primary Tumor (pT)	<b>Clinical</b> <input type="checkbox"/> TX <input type="checkbox"/> T0 <input type="checkbox"/> T1 <input type="checkbox"/> T1a <input type="checkbox"/> T1b <input type="checkbox"/> T2 <input type="checkbox"/> T2a <input type="checkbox"/> T2b <input type="checkbox"/> T3 <input type="checkbox"/> T4	<b>Pathologic</b> <input type="checkbox"/> TX <input type="checkbox"/> T0 <input type="checkbox"/> T1 <input type="checkbox"/> T1a <input type="checkbox"/> T1b <input type="checkbox"/> T2 <input type="checkbox"/> T2a <input type="checkbox"/> T2b <input type="checkbox"/> T3 <input type="checkbox"/> T4	Using the patient's medical records, select the primary tumor category (T) used to determine the patient's final AJCC stage. <a href="#">3440328</a> (clinical), <a href="#">3045435</a> (pathologic) Clinical and/or pathologic staging can be selected, but pathologic staging is preferred.
*50	Regional Lymph Nodes (pN)	<b>Clinical</b> <input type="checkbox"/> NX <input type="checkbox"/> N0 <input type="checkbox"/> N1 <input type="checkbox"/> N2 <input type="checkbox"/> N3	<b>Pathologic</b> <input type="checkbox"/> NX <input type="checkbox"/> N0 <input type="checkbox"/> N1 <input type="checkbox"/> N2 <input type="checkbox"/> N3	Using the patient's medical records, select the patient's regional lymph node category (N) used to determine the patient's final AJCC stage. <a href="#">3440330</a> (clinical), <a href="#">3203106</a> (pathologic) Clinical and/or pathologic staging can be selected, but pathologic staging is preferred.
*51	Distant Metastasis (M)	<b>Clinical</b> <input type="checkbox"/> MX <input type="checkbox"/> M0 <input type="checkbox"/> M1 <input type="checkbox"/> M1a <input type="checkbox"/> M1b	<b>Pathologic</b> <input type="checkbox"/> MX <input type="checkbox"/> M0 <input type="checkbox"/> M1 <input type="checkbox"/> M1a <input type="checkbox"/> M1b	Using the patient's medical records, select the patient's distant metastasis category (M) used to determine the patient's final AJCC stage. <a href="#">3440331</a> (clinical), <a href="#">3045439</a> (pathologic) Clinical and/or pathologic staging can be selected, but pathologic staging is preferred.
*52	Overall Stage	<b>Clinical</b> <input type="checkbox"/> Stage I <input type="checkbox"/> Stage IA <input type="checkbox"/> Stage IB <input type="checkbox"/> Stage II <input type="checkbox"/> Stage IIIA <input type="checkbox"/> Stage IIB <input type="checkbox"/> Stage III <input type="checkbox"/> Stage IIIA <input type="checkbox"/> Stage IIIB <input type="checkbox"/> Stage IV	<b>Pathologic</b> <input type="checkbox"/> Stage I <input type="checkbox"/> Stage IA <input type="checkbox"/> Stage IB <input type="checkbox"/> Stage II <input type="checkbox"/> Stage IIIA <input type="checkbox"/> Stage IIB <input type="checkbox"/> Stage III <input type="checkbox"/> Stage IIIB <input type="checkbox"/> Stage IV	Using the patient's medical records, select the final AJCC stage. <a href="#">3440332</a> (clinical), <a href="#">3203222</a> (pathologic) Clinical and/or pathologic staging can be selected, but pathologic staging is preferred.
*53	AJCC Staging Edition Used to Stage the Patient	<input type="checkbox"/> 1 <sup>st</sup> Edition (1978-1983) <input type="checkbox"/> 2 <sup>nd</sup> Edition (1984-1988) <input type="checkbox"/> 3 <sup>rd</sup> Edition (1989-1992) <input type="checkbox"/> 4 <sup>th</sup> Edition (1993-1997) <input type="checkbox"/> 5 <sup>th</sup> Edition (1998-2002) <input type="checkbox"/> 6 <sup>th</sup> Edition (2003-2009) <input type="checkbox"/> 7 <sup>th</sup> Edition (2010-present) <input type="checkbox"/> Unknown		Please selected the AJCCC cancer staging edition used to determine the T, N, M, and stage provided. <a href="#">279876</a>

Principal Investigator Signature

Date

*I acknowledge that the above information provided by my institution is true and correct and has been quality controlled.*

## HTMCP SOP #107B

Adopted: \_\_\_\_\_ 09/14/2010  
 2<sup>o</sup> Version: \_\_\_\_\_ 04/07/2011  
 3<sup>o</sup> Version: \_\_\_\_\_ 05/25/2012  
 4<sup>o</sup> Version: \_\_\_\_\_  
 Under Revision: \_\_\_\_\_

## **CENTRALIZED PATHOLOGY REVIEW PROCESS FOR HIV+ LUNG TUMORS**

### **INTRODUCTION:**

Pathological diagnosis of tumor can be impacted by the subjective nature of the process as well as the subjective definition of the criteria used in the assessment. To ensure that samples entering the sequencing pipeline of the HIV+ Tumor Characterization Project meet the tissue requirements (set forth in SOP#101) and are diagnosed as Lung Cancer, a central pathology review panel of three board-certified pathologists will be established. The review of tissues by a group minimizes the subjectivity encountered in pathology practice.

#### **A. SCOPE AND PURPOSE:**

1. To establish a standard procedure to follow for the centralized pathology review of tissue submitted to the HIV+ Tumor Characterization Project.

#### **B. EQUIPMENT AND MATERIALS:**

1. De-identified pathology reports provided by the tissue source site (TSS) contributing the sample.
2. Five unstained 4  $\mu\text{m}$  thick sections from the formalin-fixed paraffin-embedded (FFPE) diagnostic block (or the whole FFPE block). These sections will be provided by the tissue source site (TSS) contributing the sample labeled with the project-assigned ID (as specified in SOP #101B and 102).
3. Aperio Slide Scanner

### **II. PROCEDURE:**

#### **A. Preparation for review:**

1. All members of a centralized pathology board obtain their PathXchange credentials by going to the following website: <http://www.pathxchange.org/user/register>
2. Once the credentials are secured, they should be communicated to the appropriate OCG project manager.
3. Immediately upon arrival to the pathology core, the pathology coordinator will verify that all slides and reports submitted are labeled with the same project-assigned ID.
  - The report will be scanned and PathXchange website (<http://www.pathxchange.org>).

4. Pathology coordinator will send the appropriate number of slides to the histology service to perform hematoxylin & eosin (H&E) as well as necessary immunohistochemical (IHC) and in situ hybridization procedures.
  - IHC to be performed include: **TTF-1, p63**
  - In situ hybridization will be performed: **ALK FISH/HPV**.
  - The processing should take no longer than 5 days.
5. After the H&E and IHC processing is completed, the pathology coordinator will scan the whole slide on the Aperio system and deposit them, together w/ a blank review form on the PathXchange website (<http://www.pathxchange.org>).
6. The coordinator will send an e-mail to the members of the pathology review core (PRC) (with copy to the OCG project manager) informing them that materials for review have been deposited in a folder. This communication must specify the number and name of files, as well as the project-assigned ID(s) for the case(s) under review.

B. Review:

1. Within a three days of receipt of the e-mail from the coordinator, all members of the PRC wil return their pathology review form to the pathology coordinator via e-mail.
2. The tumors will be classified using to the WHO classification
3. If a consensus is reached, 2 out of 3 reviewers agree, and the case passes the specified criteria the pathology coordinator will create a final pathology report and submit it to the Data Coordinating Center and the Genome Science Center at British Columbia (GSC-BC) no later than 4 days. Steps 1-3 will take 2 weeks total.
4. Cases considered inadequate for diagnosis due to low quality of FFPE sections will be labeled as such and taken out of the study.
5. Discrepant cases (cases in which the 2 out of 3 majority consensus is not reached) will be submitted for a web-based consensus review, to be convened by Dr. Teresa Darragh. The schedule of such consensus reviews will be dictated by the number of discordant cases accrued as follows:
  - When 6 or more discordant cases have been accrued, a consensus review panel must be convened.
  - If there are less than 6 discordant cases, but the oldest accrued case is more than six months old, a consensus review panel must be convened.

**CONTACTS:****Project Team Representative:**

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Scientific Programs Director  
Office of Cancer Genomics  
National Cancer Institute  
31 Center Drive, Suite 10A07  
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Fax: 301-480-4368  
e-mail: [jz44m@nih.gov](mailto:jz44m@nih.gov)

**Data Coordinating Center:**

Patee Gesuwan  
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Rockville, MD 20892  
Phone: 301-443-6147  
E-mail: [gesuwanp@mail.nih.gov](mailto:gesuwanp@mail.nih.gov)

**HTMCP Pathology Coordinator:**

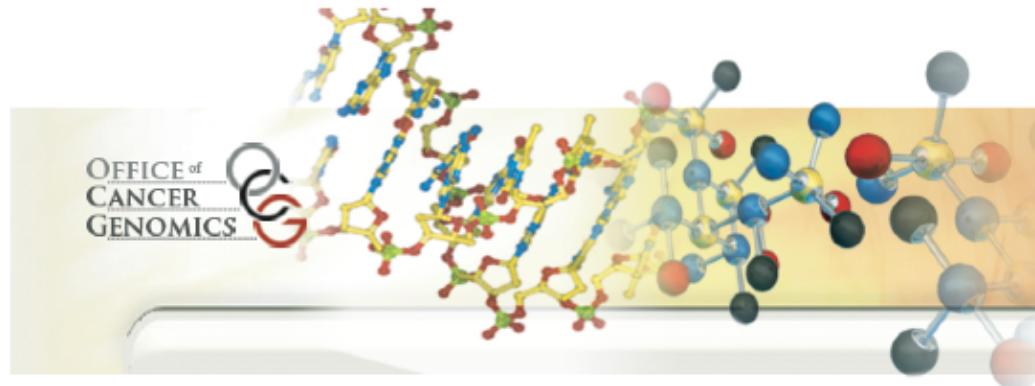
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Phone: 604-877-6088



National Cancer Institute



# HTMCP 7Yfj MW-SPECIFIC PROTOCOLS

U.S. DEPARTMENT  
OF HEALTH AND  
HUMAN SERVICES  
National Institutes  
of Health



Adopted: \_\_\_\_\_ 05/25/2012  
2° Version: \_\_\_\_\_  
3° Version: \_\_\_\_\_  
4° Version: \_\_\_\_\_  
Under Revision: \_\_\_\_\_

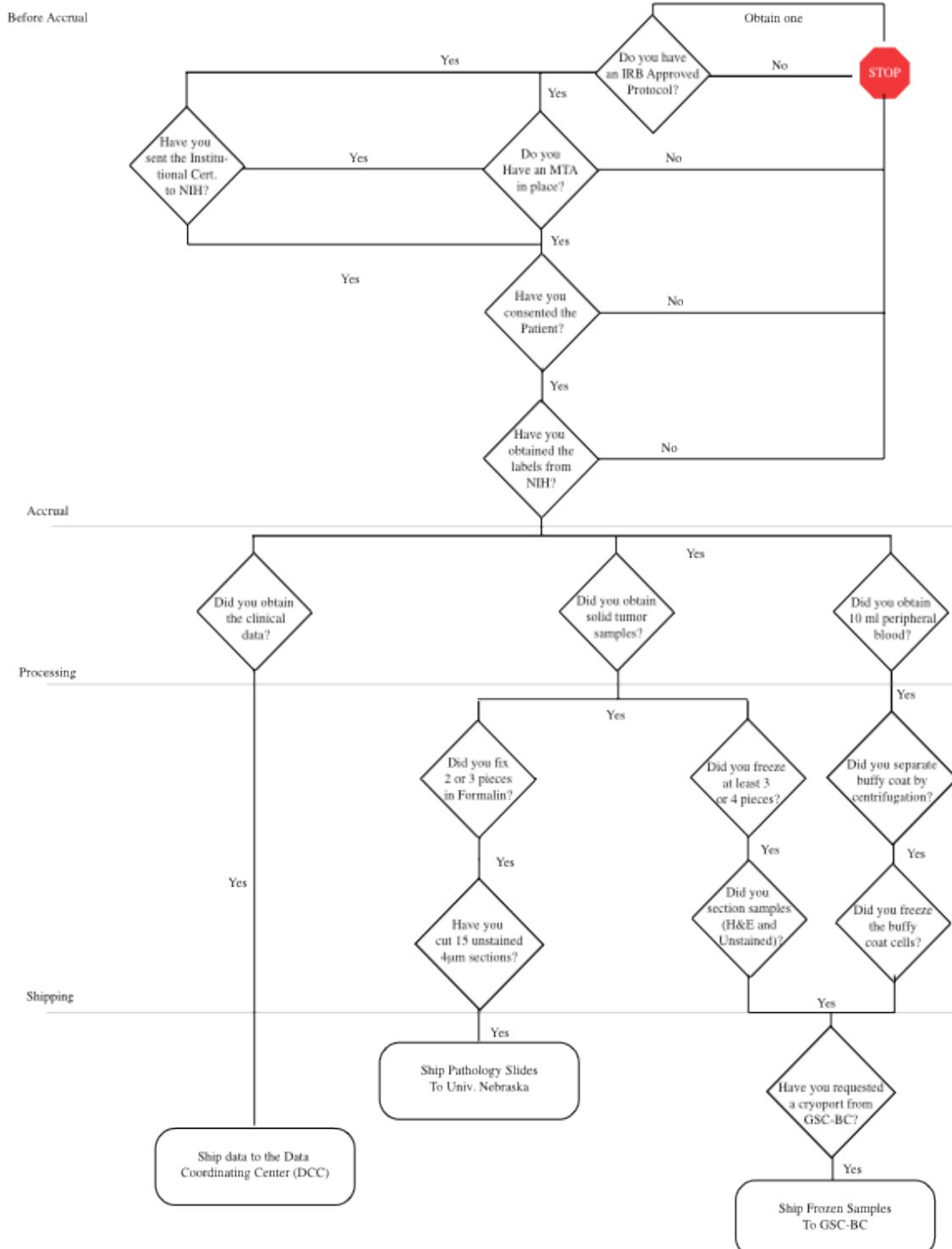
## **PROSPECTIVE CASE SUBMISSION PROCEDURE FOR THE HIV+ CERVICAL PROJECT**

### **I. INTRODUCTION:**

The National Cancer Institute's Office of Cancer Genomics (OCG) and the Office of HIV and AIDS Malignancy (OHAM) have developed an initiative to compare the cancer related alterations in HIV+ patients and HIV- patients

#### **A. SCOPE AND PURPOSE:**

1. To establish a general procedure to inform personnel of all the steps necessary for a successful submission of a sample to the HIV+ Tumor Molecular Characterization Project (HTMCP).
2. This protocol applies to all Tissue Source Sites (TSSs) providing tissues prospectively.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times and which samples were affected. This information should be given within 48 hours of the occurrence to the project team by sending an e-mail to Dr. Jean C. Zenklusen (**jz44m@nih.gov**) with the details.



## II. PROCEDURES:

### A. BEFORE PATIENT ACCRUAL BEGINS:

1. Make sure all the documents required for sample shipment as spelled out in SOP#100 are in place before you start case accruals.

### B. BEFORE PATIENT SURGERY:

1. Create a TSS-assigned ID for your patient. **The TSS is responsible for maintaining the link between project-assigned ID and TSS-assigned ID in order to retrieve clinical information when required.** In case that the original PI is no longer affiliated with the contributing Institution, it is the TSS's responsibility to be able to track the patient's records.
2. Contact the Data Coordinating Center (DCC, see address below) to obtain a project-assigned ID to use in all documents regarding the case and all materials shipped.
3. Contact the OCG Project Coordinator and obtain freezer-resistant labels that you should use to mark all containers/slides carrying materials for the project.
4. Make sure to prepare the tissue freezing station and have ready all the materials needed for tissue processing (HTMCP SOP #102).
5. Inform the research nurse that a 10 ml peripheral blood sample must be obtained from the patient to use as a non-tumor malignant control. Note that the buffy coat should be separated from the plasma within two hours of being obtained from the patient. The buffy coat sample should be moved immediately to a -80°C or Liquid Nitrogen (LN<sub>2</sub>) freezer for storage.

### C. DURING PATIENT SURGERY:

1. Inform the surgical staff of the tissue requirements for the project (see Appendix A).
2. Have a person ready to transport the ablated tissue to the processing station. It is generally accepted that for the best tissue preservation snap freezing should take place within 20 minutes after tissue is obtained from the patient.
3. Note the time between surgery and freezing in a notebook and send to the OCG Project Coordinator

### D. AFTER SURGERY:

1. Process solid tissue as described in the tissue processing protocol (HTMCP SOP #102). Timely processing is crucial.
2. Process blood sample according to protocol (see HTMCP SOP #103, blood processing). Store isolated cells in a -80°C freezer or liquid Nitrogen (LN<sub>2</sub>) storage until shipment.

## HTMCP SOP #101C

3. Obtain a formalin-fixed, paraffin-embedded tissue block or, if not possible, **five** unstained 4  $\mu\text{m}$  sections from the formalin-fixed block. Affix one of the provided freezer-resistant labels to each slide or block.

**E. PREPARING SAMPLES AND SHIPMENT:**

1. Produce a 4 $\mu\text{m}$  frozen section of the top of the tumor sample for initial quality control as described in the tissue processing protocol (HTMCP SOP #102).
2. When tissue from at least three cases are accrued, or every quarter (See HTMCP SOP #105) contact the GSC-BC coordinator to obtain a cryoport transport vessel to ship the frozen tumor sample sections and frozen blood cells.
3. Follow the frozen sample shipment protocol (HTMCP SOP #104) and send the frozen samples to the GSC-BC. Provide both the GSC-BC and PT with tracking number the day of shipment.
4. Send a formalin-fixed, paraffin-embedded tissue block or, if not possible, **five** unstained 4  $\mu\text{m}$  sections obtained from the formalin fixed blocks to the pathology coordinator. On shipment, provide both the pathology coordinator and OCG project coordinator with the tracking number of the parcel. For shipment use a closable box (such as Thermo Scientific\* Plastic Slide Box, capacity 25 slides, catalog# B1780).
5. Collect all the clinical data requested in the sample requirements (Appendix C). You will be requested by the OCG project coordinator to send the data electronically to the DCC using the appropriate TSS-assigned ID at a later date.

**NOTES:**

- A checklist is provided to help you track all the steps required by this process (Appendix B). Please use it!
- If any one of the required items (institutional certification, confirmation of informed consent, frozen tissue, frozen blood cells, unstained slides and clinical data) is not present, the HTMCP cannot accept the case.
- **At no point in the process can traditionally-used identifying information (such as the patient name, address, phone number, medical record number, or social security number) be used to label samples. Only use the project-assigned ID and labels provided by the OCG project coordinator.**

**APPENDIX A: Sample Requirements****HIV+ Cervical Cancer Characterization Project  
Tissue Sample Requirements for Accrual****Tissue Requirements:**

To be accepted to the project, the following conditions have to be met at the tissue level.

- Tissue is from a patient who had not received neoadjuvant therapy for that tumor type or systemic treatment for other tumor.
- Paired tumor and normal tissue or plasma buffy coat must be available in sufficient quantities (see below).
- Tissues (both normal and tumor) need to be snap frozen. Time between tissue extraction and freezing must be recorded.
- Optimal storage of the tissues is in N<sub>2</sub>(liq), but -80°C or lower is acceptable. The form of tissue storage must be recorded.
- There must be 100 mg of tumor tissue with a minimum percent of tumor nuclei between 70-80% as assessed on an H&E section physically adjacent to the specimen that is candidate for generating the RNA and DNA.
- A paraffin embedded block for pathology consensus review must exist for the tumor.

**APPENDIX B: Checklist of Task Completion for Sample Submission****Date:****Institution:****Operator:**

- Do you have an IRB approved protocol?
- Have you consented the patient?
- Have you sent your Institutional Certification to the Project Team?
- Have you obtained the project-assigned ID and labels from the Project Team?
- Do you have frozen sections in cryovial and H&E stained slides? Are they labeled?
- Do you have frozen plasma derived white blood cells? Are they correctly labeled?
- Have you secured a cryoport from the Genome Science Center at British Columbia?
- Do you have a formalin-fixed, paraffin-embedded tissue block or, if not possible, (5) unstained 4  $\mu\text{m}$  sections from the Formalin-fixed block? Are they labeled?
- Do you have the clinical data elements required by the Project? (Appendix B)

**ONLY if all the above items check out, you are ready to ship the samples.**

**APPENDIX C: Cervical Cancer Characterization Project from HIV+ Patients Clinical Data Requirements for Accrual****Clinical Data Requirements:**

To be accepted to the project, the following conditions must meet at the clinical data level. The case(s) must have available ALL the clinical data elements (CDEs) here listed, should some of the datafields be missing, please contact the OCG project coordinator to get approval for submission.

These clinical data elements must be reported to the DCC as an initial report within two weeks of enrolling the patient. Updates must be sent to the DCC as the patient returns for periodic visits (Annually).

- Patients need to be consented in such way that allows for the use of their tissues for genomic-scale molecular characterization

**Instructions:** The Enrollment Form should be completed for each qualified case in the HIV+ Tumor Characterization Project (HTMCP) study. The Tissue Source Site (TSS) should complete the form for qualified cases upon qualification notice from the Office of Cancer Genomics (OCG).

Questions regarding this form should be directed to the Clinical Data Collection Operation & Database (CDCOD) or OCG.

**Please note the following definitions for the “Unknown” and “Not Evaluated” answer options on this form.**

**Unknown:** This answer option should only be selected if the TSS does not know this information after all efforts to obtain the data have been exhausted. If this answer option is selected for a question that is part of the HTMCP required data set, the TSS must complete a discrepancy note providing a reason why the answer is unknown.

**Not Evaluated:** This answer option should only be selected by the TSS if it is known that the information being requested cannot be obtained. This could be because the test in question was never performed on the patient or the TSS knows that the information requested was never disclosed.

Tissue Source Site (TSS): \_\_\_\_\_ TSS Identifier: \_\_\_\_\_ TSS Unique Patient Identifier: \_\_\_\_\_

Completed By (Interviewer Name in OpenClinica): \_\_\_\_\_ Completed Date: \_\_\_\_\_

#	Data Element	Entry Alternatives	Working Instructions
<b>General Information</b>			
*1	Is this a prospective tissue collection?	<input type="checkbox"/> Yes <input type="checkbox"/> No	Indicate whether the TSS providing tissue is contracted for prospective tissue collection. If the submitted tissue was collected after the date the HTMCP contract was executed, the tissue has been collected prospectively. <a href="#">3088492</a>
*2	Is this a retrospective tissue collection?	<input type="checkbox"/> Yes <input type="checkbox"/> No	Indicate whether the TSS providing tissue is contracted for retrospective tissue collection. If the submitted tissue was collected prior to the date the HTMCP contract was executed, the tissue has been collected retrospectively. <a href="#">3088528</a>
<b>Patient Information</b>			
<i>Demographic Information</i>			
*3	Date of Birth	____ / ____ / ____ (month)      (day)      (year)	Provide the date the patient was born. <a href="#">2896950</a> (month), <a href="#">2896952</a> (day), <a href="#">2896954</a> (year)
*4	Gender	<input type="checkbox"/> Female <input type="checkbox"/> Male	Provide the patient's gender using the provided categories. <a href="#">2200604</a>
5	Menopause Status (at time of diagnosis)	<input type="checkbox"/> Premenopausal <i>&lt;6 months since LMP AND no prior bilateral oophorectomy AND not on estrogen replacement</i> <input type="checkbox"/> Perimenopausal <i>6-12 months since last menstrual period</i> <input type="checkbox"/> Postmenopausal <i>Prior bilateral oophorectomy OR &gt; 12 months since LMP with no prior ooporectomy</i> <input type="checkbox"/> Indeterminate or Unknown <input type="checkbox"/> Not Evaluated	Using the patient's medical records, indicate their menopause status at the time the patient was diagnosed with the malignancy submitted for HTMCP. <a href="#">2957270</a>
*6	Race (check all that apply)	<input type="checkbox"/> American Indian or Alaska Native <input type="checkbox"/> Asian <input type="checkbox"/> White <input type="checkbox"/> Black or African American <input type="checkbox"/> Native Hawaiian or other Pacific Islander <input type="checkbox"/> Other (please specify) <input type="checkbox"/> Not Evaluated <input type="checkbox"/> Unknown	Provide the patient's race using the defined categories. <a href="#">2192199</a> <u>American Indian or Alaska Native:</u> A person having origins in any of the original peoples of North and South America (including Central America), and who maintains tribal affiliation or community attachment. <u>Asian:</u> A person having origins in any of the original peoples of the far East, Southeast Asia, or in the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. <u>White:</u> A person having origins in any of the original peoples of Europe, the Middle East, or North Africa. <u>Black or African American:</u> A person having origins in any of any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American." <u>Native Hawaiian or other Pacific Islander:</u> A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands. <u>Not Evaluated:</u> Not provided or available <u>Unknown:</u> Could not be determined or unsure
7	Other Race	_____	If the patient's race was not defined in the previous question, provide the patient's race. <a href="#">2192205</a>
8	Ethnicity	<input type="checkbox"/> Not Hispanic or Latino <input type="checkbox"/> Hispanic or Latino <input type="checkbox"/> Not Evaluated <input type="checkbox"/> Unknown	Provide the patient's ethnicity using the defined categories. <a href="#">2192217</a> <u>Not Hispanic or Latino:</u> A person not meeting the definition of Hispanic or Latino. <u>Hispanic or Latino:</u> A person of Mexican, Puerto Rican, Cuban, Central or South American, or other Spanish culture or origin, regardless of race. <u>Not Evaluated:</u> Not provided or available <u>Unknown:</u> Could not be determined or unsure

#	Data Element	Entry Alternatives	Working Instructions
9	Height (at time of diagnosis)	_____ (cm)	Provide the patient's height (centimeters) at the time the patient was diagnosed with the tumor submitted for HTMCP. <a href="#">649</a>
10	Weight (at time of diagnosis)	_____ (kg)	Provide the patient's weight (kilograms) at the time the patient was diagnosed with the tumor submitted for HTMCP. <a href="#">651</a>
11	Tobacco Smoking History Indicator (at time of diagnosis)	<input type="checkbox"/> 1: Lifelong Non-Smoker <input type="checkbox"/> 2: Current Smoker <input type="checkbox"/> 3: Current Reformed Smoker for > 15 years <input type="checkbox"/> 4: Current Reformed Smoker for <= 15 years <input type="checkbox"/> 5: Current Reformed Smoker (duration not specified) <input type="checkbox"/> Smoking Status not Documented	Indicate the patient's history of tobacco smoking as well as their current smoking status using the defined categories. If the patient is a lifelong non-smoker, skip the additional smoking questions. <a href="#">2181650</a>
12	Age of Onset Tobacco History Indicator	_____ years	Provide the age in years when the patient began smoking cigarettes. <a href="#">2178045</a>
13	Year of Quitting Tobacco Smoking	_____ (YYYY)	Provide the year the patient quit smoking, if applicable. <a href="#">2228610</a>
14	Number of Pack Years Smoked (at time of diagnosis)	_____ pack years	Provide the number of pack years the patient smoked. This is calculated using the number of cigarettes smoked per day times the number of years smoked, divided by 20. For example, if the patient smoked 5 cigarettes per day times 10 years divided by 20, the patient would have 2.5 pack years (e.g. 5x10/20=2.5). <a href="#">2955385</a>
<b>History of Pregnancies and Contraceptive Use</b>			
15	Hormonal Contraceptive Use	<input type="checkbox"/> Current User <input type="checkbox"/> Never Used <input type="checkbox"/> Former User <input type="checkbox"/> Unknown	Indicate whether the patient has used or is currently using hormonal contraceptives. <a href="#">3104217</a>
16	Total Number of Pregnancies	_____	Provide the total number of times the patient conceived and became pregnant. This should include all of the pregnancies under the question "Number of Pregnancies by Outcome Type" and current pregnancies. <a href="#">2005341</a>
17	Number of Pregnancies by Outcome Type (Complete all that apply)	Pregnancy Type      Number of Pregnancies	
		Live Birth (single or multiple births) _____	Provide the number of times the patient had successful pregnancies that resulted in the live birth of at least one child. <a href="#">2005342</a>
		Miscarriage _____	Provide the number of times the patient conceived and became pregnant, but did not carry fetus to term due to natural occurrences or problems during the pregnancy. <a href="#">2180637</a>
		Induced Abortion _____	Provide the number of times the patient conceived and became pregnant, but did not carry fetus to term due to medical intervention to end the pregnancy. <a href="#">2180648</a>
		Ectopic Pregnancy _____	Provide the number of times the patient conceived and became pregnant, but did not carry the fetus to term due to an ectopic pregnancy. <a href="#">2261915</a>
		Stillbirth (early fetal death) _____	Indicate the number of times the patient conceived and became pregnant, but the pregnancy ended with stillbirth. <a href="#">2183304</a>
		Unknown _____	Provide the number of times the patient was known to be pregnant, but the outcome of the pregnancy was unknown.
18	Pregnant at Time of Diagnosis	<input type="checkbox"/> Yes <input type="checkbox"/> No	Indicate whether the patient was pregnant at the time of initial diagnosis. <a href="#">3012573</a>
<b>Survival Information</b>			
*19	Vital Status (at date of last contact)	<input type="checkbox"/> Living <input type="checkbox"/> Deceased	Indicate whether the patient was living or deceased at the date of last contact. <a href="#">2939553</a>
*20	Date of Last Contact	____ / ____ / ____ (month)      (day)      (year)	If the patient is living, provide the date of last contact with the patient (as reported by the patient, medical provider, family member, or caregiver). <a href="#">2897020</a> (month), <a href="#">2897022</a> (day), <a href="#">2897024</a> (year) <b><i>Do not answer if patient is deceased.</i></b>
*21	Date of Last Known Alive	____ / ____ / ____ (month)      (day)      (year)	Indicate the last date the patient was known to be alive, regardless of whether the patient, medical provider, family member or caregiver was contacted. <a href="#">2975722</a> (month), <a href="#">2975724</a> (day), <a href="#">2975726</a> (year)
*22	Date of Death	____ / ____ / ____ (month)      (day)      (year)	If the patient is deceased, provide the month of death. <a href="#">2897026</a> , (month) <a href="#">2897028</a> (day), <a href="#">2897030</a> (year)

#	Data Element	Entry Alternatives	Working Instructions
<b>Patient Status (Regarding Submitted Tumor)</b>			
*23	Did the patient receive neo-adjuvant therapy for the tumor submitted for HTMCP?	<input type="checkbox"/> Yes ( <i>exclusion criterion</i> ) <input type="checkbox"/> No	Indicate whether the patient received treatment (radiation, pharmaceutical, or both) prior to the procurement of the sample submitted for TCGA. <a href="#">3382737</a> <i>If the answer to this question is "yes", the submitted case is excluded.</i>
*24	Tumor Status (at time of last contact)	<input type="checkbox"/> Tumor free <input type="checkbox"/> With tumor <input type="checkbox"/> Unknown	Indicate whether the patient was tumor/disease free (i.e. free of the malignancy that yielded the sample submitted for the HTMCP study) at the date of last contact or death. <a href="#">2759550</a>
25	Performance Status: Eastern Cooperative Oncology Group	<input type="checkbox"/> 0: Asymptomatic <input type="checkbox"/> 1: Symptomatic, but fully ambulatory <input type="checkbox"/> 2: Symptomatic, in bed less than 50% of day <input type="checkbox"/> 3: Symptomatic, in bed more than 50% of day, but no bed-ridden <input type="checkbox"/> 4: Bed-ridden <input type="checkbox"/> Unknown <input type="checkbox"/> Not Evaluated	Provide the Eastern Cooperative Oncology Group (ECOG) performance status of the patient at the time of diagnosis. <a href="#">2003853</a>
26	Performance Status: Eastern Cooperative Oncology Group	<input type="checkbox"/> 100: Normal, no complaints, no evidence of disease <input type="checkbox"/> 90: Able to carry on normal activity; minor signs or symptoms of disease <input type="checkbox"/> 80: Normal activity with effort; some signs or symptoms of disease <input type="checkbox"/> 70: Cares for self, unable to carry on normal activity or to do active work <input type="checkbox"/> 60: Requires occasional assistance <input type="checkbox"/> 50: Requires considerable assistance and frequent medical care <input type="checkbox"/> 40: Disabled, requires special care and assistance <input type="checkbox"/> 30: Severely disabled, hospitalization indicated. Death not imminent <input type="checkbox"/> 20: Very sick, hospitalization <input type="checkbox"/> 10: Moribund, fatal processes progressing rapidly <input type="checkbox"/> 0: Dead <input type="checkbox"/> Unknown <input type="checkbox"/> Not Evaluated	Provide the Eastern Cooperative Oncology Group (ECOG) performance status of the patient at the time of diagnosis. <a href="#">88</a>
27	Performance Status Score: Timing	<input type="checkbox"/> Preoperative <input type="checkbox"/> Pre-adjuvant Therapy <input type="checkbox"/> Post-adjuvant Therapy <input type="checkbox"/> Unknown	Indicate the timing of the performance status(es) provided in the previous question(s). <a href="#">2792763</a>
28	Tumor Response	<input type="checkbox"/> Progressive Disease <input type="checkbox"/> Stable Disease <input type="checkbox"/> Partial Response <input type="checkbox"/> Complete Response	Indicate the patient's measure of success after their primary treatment including surgery and adjuvant therapies. <a href="#">2786727</a>
<b>Patient History of Disease</b>			
<b>HIV Status</b>			
*29	Is this patient HIV positive?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	Indicate whether the patient is HIV positive. <a href="#">2180464</a>
*30	Date of HIV Diagnosis (if known)	____ / ____ / ____ (month)      (day)      (year)	Provide the month the patient was diagnosed with HIV. <a href="#">3579640</a> (month), <a href="#">3579644</a> (day), <a href="#">3579643</a> (year)
31	Nadir CD4 Counts	_____ (cells/mm <sup>3</sup> )	Provide the patient's Nadir CD4 counts, which are the lowest CD4 counts the patient has had. <a href="#">2684395</a>
*32	CD4 Counts at Diagnosis of the Submitted Malignancy	_____ (cells/mm <sup>3</sup> )	Provide the patient's CD4 Counts at the time the patient was diagnosed with the malignancy submitted for the HTMCP study. <a href="#">2922654</a>
*33	HIV RNA load at Diagnosis of Submitted Malignancy	_____	Provide the HIV RNA load (also known as the "viral load") at the time the patient was diagnosed with the malignancy submitted for the HTMCP study. <a href="#">2922674</a>
34	Prior AIDS Defining Co-Morbidities	_____	Prior to the malignancy submitted for the HTMCP study, provide any AIDS defining co-morbidities including, but not limited to the following: diabetes mellitus, cardiovascular disease, non-AIDS-defining malignancies, and osteoporosis. <a href="#">2970715</a>

#	Data Element	Entry Alternatives	Working Instructions
	Co-Infections (serology data/viral load if available)	Test Results	Using the list provided, indicate whether the patient had any co-infections by providing the results of each of the tests listed.
35		HBV	<a href="#">2180456</a>
		HCV	<a href="#">2695021</a>
		HPV	<a href="#">2230033</a>
		KSHV/HHV8	<a href="#">3335773</a>
*36	HAART Treatment Prior to Diagnosis of Submitted Malignancy	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	Indicate whether the patient received Highly Active Antiretroviral Therapy (HAART) treatment prior to the diagnosis of the malignancy submitted for the HTMCP study. <a href="#">3335156</a>
*37	HAART Treatment at Time of Diagnosis of Submitted Malignancy	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	Indicate whether the patient received Highly Active Antiretroviral Therapy (HAART) treatment at the time of the diagnosis of the malignancy submitted for the HTMCP study. <a href="#">2922679</a>
38	CDC HIV Risk Group(s)	<input type="checkbox"/> Homosexual or bisexual contact <input type="checkbox"/> Heterosexual contact <input type="checkbox"/> IV drug user <input type="checkbox"/> Transfusion recipient <input type="checkbox"/> Hemophiliac <input type="checkbox"/> Other	Indicate whether the patient has a history of any of the listed HIV Risk Groups as defined by the Center for Disease Control (CDC). <a href="#">2542215</a>
<b>Prior Malignancies</b>			
*39	Has this patient at any time in their life had a prior diagnosis of a malignant neoplasm?	<input type="checkbox"/> Yes (exclusion criterion) <input type="checkbox"/> No	Indicate whether the patient was, at any time in their life, diagnosed with a malignancy prior to the diagnosis of the specimen submitted for HTMCP. <a href="#">61396</a> <i>If the answer to this question is "yes", the submitted case is excluded. This exclusion does not apply if the patient only has a history of non-melanoma skin cancer OR in situ carcinoma.</i>
40	Type of Prior Malignancies	_____	If the patient has had a prior diagnosis of a malignant neoplasm, provide the type of prior malignancy. <a href="#">2718428</a>
<b>Prior Immunological Disease</b>			
41	Patient History of Prior Immunological Disease	<input type="checkbox"/> Rheumatoid Arthritis <input type="checkbox"/> Sjogren's Syndrome <input type="checkbox"/> Systemic Lupus Erythematosus <input type="checkbox"/> Crohn's Disease <input type="checkbox"/> Ulcerative Colitis <input type="checkbox"/> Hashimoto's Thyroiditis <input type="checkbox"/> Other <input type="checkbox"/> Unknown	Indicate whether the patient has a history of any of the listed immunological diseases. <a href="#">3233628</a>
42	Patient History of Prior Immunosuppressive Therapy for Immunological Disease	<input type="checkbox"/> Methotrexate <input type="checkbox"/> Anti-TNF therapy <input type="checkbox"/> Cyclophosphamide <input type="checkbox"/> Other <input type="checkbox"/> Azathioprine <input type="checkbox"/> Unknown	If the patient received immunosuppressive therapy for the immunological disease selected in the previous question, provide the type of immunosuppressive therapy given. <a href="#">3233638</a>
<b>Prior Infectious Disease</b>			
43	Patient History of Relevant Prior Infectious Disease	<input type="checkbox"/> Hepatitis B <input type="checkbox"/> Other <input type="checkbox"/> Hepatitis C <input type="checkbox"/> Unknown <input type="checkbox"/> H. Pylori	Indicate whether the patient has a history of any of the listed infectious disease. <a href="#">3233645</a>
44	Patient History of Other Relevant Infectious Disease	_____	If the patient has a history of relevant prior disease that was not included in the list, provide the infectious disease. <a href="#">3233643</a>
<b>Pathologic Information</b>			
*45	Histological Subtype	<input type="checkbox"/> Cervical Squamous Cell Carcinoma <input type="checkbox"/> Endocervical type of Adenocarcinoma <input type="checkbox"/> Endocervical Adenocarcinoma of the Usual Type <input type="checkbox"/> Mucin-depleted Adenocarcinoma <input type="checkbox"/> Endometrioid Adenocarcinoma of Endocervix	Using the patient's final diagnostic pathology report, provide the most detailed histological subtype available. <a href="#">3081934</a>
46	Keratinization in Squamous Cell Carcinoma	<input type="checkbox"/> Keratinizing squamous cell carcinoma <input type="checkbox"/> Non-keratinizing squamous cell carcinoma	If the patient had squamous cell carcinoma, indicate whether the tumor has any keratinizing squamous cell carcinoma using the patient's pathology/laboratory report. Keratinizing tumors have at least one well-formed keratin pearl. All other patterns are non-keratinizing. <a href="#">3151599</a>
*47	Primary Site of Disease	<input type="checkbox"/> Cervix	Using the patient's pathology/laboratory report, select the organ where the disease originated. <a href="#">2735776</a>

#	Data Element	Entry Alternatives	Working Instructions
48	Tumor Grade	<input type="checkbox"/> G1 Well Differentiated <input type="checkbox"/> G2 Moderately Differentiated <input type="checkbox"/> G3 Poorly Differentiated <input type="checkbox"/> G4 Undifferentiated <input type="checkbox"/> GX Grade cannot be assessed	Using the patient's pathology/laboratory report, select the tumor grade. <a href="#">2785839</a>
<b><i>Pathologic Diagnosis and Surgical Resection</i></b>			
*49	Date of Initial Pathologic Diagnosis	____ / ____ / ____ (month) (day) (year)	Provide the date the patient was initially diagnosed with the malignancy submitted for HTMCP. This may or may not be the date of the surgical resection that yielded the tumor sample submitted for HTMCP. <a href="#">2896956</a>
*50	Method of Initial Pathologic Diagnosis	<input type="checkbox"/> Cytology <input type="checkbox"/> Biopsy (cervical, CT-guided or other) <input type="checkbox"/> Cone Biopsy / LEEP <input type="checkbox"/> Lymph Node Sampling or Dissection <input type="checkbox"/> Other (please specify) <input type="checkbox"/> Unknown	Provide the method of the initial pathologic diagnosis. This is the method used on the date provided above. <a href="#">2757941</a>
51	Other Method of Initial Pathologic Diagnosis	_____	If the method of initial pathologic diagnosis is not included in the list above, provide the method used. <a href="#">2757948</a>
52	Date of Surgical Resection	____ / ____ / ____ (month) (day) (year)	Provide the date of the surgical resection that yielded the tumor sample submitted for HTMCP. <a href="#">3008197</a> (month), <a href="#">3008195</a> (day), <a href="#">3008199</a> (year)
53	If hysterectomy was performed, what type was it?	<input type="checkbox"/> Hysterectomy not performed <input type="checkbox"/> Simple <input type="checkbox"/> Radical (modified or not modified) <input type="checkbox"/> Other, specify	Indicate whether a hysterectomy was performed at diagnosis. If a hysterectomy was performed, indicate the type. <a href="#">2647164</a>
54	Other Type of Hysterectomy	_____	If the type of hysterectomy performed was not included in the list provided, please provide the type of hysterectomy performed. <a href="#">3151506</a>
55	If hysterectomy was performed, were there involved pathologic margins?	<input type="checkbox"/> Macroscopic parametrial involvement <input type="checkbox"/> Microscopic parametrial involvement <input type="checkbox"/> Positive bladder margin <input type="checkbox"/> Positive vaginal margin <input type="checkbox"/> Unknown <input type="checkbox"/> Other, specify	If a hysterectomy was performed, provide the patient's margin involvement after surgery. <a href="#">3151541</a>
56	Other Involved Pathologic Margins	_____	If the margin involvement was not included in the provided list, describe the pathologic margins. <a href="#">3151544</a>
57	Pelvic Extension Comment	_____	Using the patient's pathology/laboratory report, provide comments regarding any tumor extension to the pelvic wall. <a href="#">3151605</a>
58	Pathologic Lymphovascular Invasion	<input type="checkbox"/> Present <input type="checkbox"/> Absent <input type="checkbox"/> Unknown	Using the patient's pathology/laboratory report, indicate the presence or absents of pathologic lymphovascular invasion. <a href="#">2008052</a>
59	Corpus Involvement	<input type="checkbox"/> Present <input type="checkbox"/> Absent <input type="checkbox"/> Unknown	The corpus uteri is the part of the uterus above the isthmus, comprising about two thirds of the non-pregnant organ. To have a connection by participation or association or use; sharing in an activity or process. <a href="#">3151610</a>
<b><i>Lymph Node Status</i></b>			
60	Were Lymph Nodes Examined at the Time of Primary Resection?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	Indicate whether any lymph nodes were examined at the time of the primary resection. <a href="#">2200396</a>
61	Number of Lymph Nodes Examined	_____	Provide the number of lymph nodes examined, if one or more lymph nodes were removed. <a href="#">3</a>
62	Number of Lymph Nodes Positive by H&E light microscopy	_____	Provide the number of lymph nodes positive through hematoxylin and eosin (H&E) staining and light microscopy. <a href="#">3086388</a>
63	Number of Lymph Nodes Positive by IHC Keratin Staining only	_____	Provide the number of lymph nodes positive through keratin immunohistochemistry (IHC) staining. <a href="#">3086383</a>

#	Data Element	Entry Alternatives	Working Instructions
64	Pathologic Positive Lymph Node Location(s) <i>(Check all that apply)</i>	<input type="checkbox"/> Pelvic (external iliac, internal iliac, obturator) <input type="checkbox"/> Common iliac <input type="checkbox"/> Paraaortic <input type="checkbox"/> Supraclavicular <input type="checkbox"/> Unknown <input type="checkbox"/> Other, specify _____	Using the patient's pathology/laboratory report, provide the location(s) of any positive lymph nodes. <a href="#">3151519</a>
65	Other Positive Lymph Node	_____	If the location of positive lymph nodes was not included in the list provide, please provide the location of positive lymph nodes. <a href="#">3151522</a>
<b>AJCC and FIGO Staging</b>			
*66	Primary Tumor (T)	<b>Clinical</b> <input type="checkbox"/> TX <input type="checkbox"/> T0 <input type="checkbox"/> Tis <input type="checkbox"/> T1 <input type="checkbox"/> T1a <input type="checkbox"/> T1a1 <input type="checkbox"/> T1a2 <input type="checkbox"/> T1b <input type="checkbox"/> T1b1 <input type="checkbox"/> T1b2  <b>Pathologic</b> <input type="checkbox"/> T2 <input type="checkbox"/> T2a <input type="checkbox"/> T2a1 <input type="checkbox"/> T2a2 <input type="checkbox"/> T2b <input type="checkbox"/> T3 <input type="checkbox"/> T3a <input type="checkbox"/> T3b <input type="checkbox"/> T4 <input type="checkbox"/> T1 <input type="checkbox"/> T1a <input type="checkbox"/> T1a1 <input type="checkbox"/> T1a2 <input type="checkbox"/> T1b <input type="checkbox"/> T1b1 <input type="checkbox"/> T1b2	Using the patient's medical records, select the primary tumor category (T) used to determine the patient's final AJCC stage. <a href="#">3440328</a> (clinical), <a href="#">3045435</a> (pathologic)
*67	Regional Lymph Nodes (N)	<b>Clinical</b> <input type="checkbox"/> NX <input type="checkbox"/> N0 <input type="checkbox"/> N1  <b>Pathologic</b> <input type="checkbox"/> NX <input type="checkbox"/> N0 <input type="checkbox"/> N1	Using the patient's medical records, select the patient's regional lymph node category (N) used to determine the patient's final AJCC stage. <a href="#">3440330</a> (clinical), <a href="#">3203106</a> (pathologic)
*68	Distant Metastasis (M)	<b>Clinical</b> <input type="checkbox"/> MX <input type="checkbox"/> M0 <input type="checkbox"/> M1  <b>Pathologic</b> <input type="checkbox"/> MX <input type="checkbox"/> M0 <input type="checkbox"/> M1	Using the patient's medical records, select the patient's distant metastasis category (M) used to determine the patient's final AJCC stage. <a href="#">3440331</a> (clinical), <a href="#">3045439</a> (pathologic)
*69	AJCC Staging Edition Used to Determine the T, N, and M values	<input type="checkbox"/> 1 <sup>st</sup> Edition (1978-1983) <input type="checkbox"/> 2 <sup>nd</sup> Edition (1984-1988) <input type="checkbox"/> 3 <sup>rd</sup> Edition (1989-1992) <input type="checkbox"/> 4 <sup>th</sup> Edition (1993-1997) <input type="checkbox"/> 5 <sup>th</sup> Edition (1998-2002) <input type="checkbox"/> 6 <sup>th</sup> Edition (2003-2009) <input type="checkbox"/> 7 <sup>th</sup> Edition (2010-present) <input type="checkbox"/> Unknown	Please selected the AJCCC cancer staging edition used to determine the T, N, M, and stage provided. <a href="#">2798766</a>
*70	FIGO Stage	<input type="checkbox"/> Stage I <input type="checkbox"/> Stage IA <input type="checkbox"/> Stage IA1 <input type="checkbox"/> Stage IA2 <input type="checkbox"/> Stage IB <input type="checkbox"/> Stage IB1  <input type="checkbox"/> Stage IB2 <input type="checkbox"/> Stage II <input type="checkbox"/> Stage IIA <input type="checkbox"/> Stage IIA1 <input type="checkbox"/> Stage IIA2 <input type="checkbox"/> Stage IIB  <input type="checkbox"/> Stage III <input type="checkbox"/> Stage IIIA <input type="checkbox"/> Stage IIIB <input type="checkbox"/> Stage IV <input type="checkbox"/> Stage IVA <input type="checkbox"/> Stage IVB	Using the patient's pathology/laboratory report, provide the FIGO stage given to the patient at the time of diagnosis. <a href="#">3225684</a>
*71	FIGO Staging System <i>(Publication Date Used for Staging)</i>	<input type="checkbox"/> 1988 <input type="checkbox"/> 1995 <input type="checkbox"/> 2009	Using the patient's pathology/laboratory report, provide the FIGO staging system used to stage the patient. <a href="#">3114049</a>
<b>Tests Performed</b>			
<b>FED-PET or PET/CT</b>			
72	Date of FED-PET or PET/CT	____ / ____ / ____ (month)      (day)      (year)	If the patient's medical records indicate the patient had a FED-PT or PET/CT, provide the date of the procedure. <a href="#">3151498</a> (month), <a href="#">3151499</a> (day), <a href="#">3151500</a> (year)
73	Cervix Standardized Update Value (SUV)	_____	If the patient's medical records indicate the patient had a FED-PT or PET/CT, provide the patient's cervix SUV. <a href="#">3151615</a>
74	FED-PET or PET/CT Results <i>Check all that apply</i>	<b>Test</b>	<b>Outcome</b>
			<i>Present</i> <i>Absent</i> <i>Unknown</i>
		Pelvic Nodes	
		Paraortic Nodes	
		Supraclavicular Nodes	
		Parametrium	
		Bladder	
		Extra-Pelvic Meastatic Disease	
If the patient's medical records indicate the patient had a FED-PT or PET/CT, provide the results for each applicable anatomic site. <a href="#">3151497</a>			

#	Data Element	Entry Alternatives			Working Instructions	
<b>Magnetic Resonance Imaging (MRI)</b>						
75	Date of MRI	____ / ____ / ____	(month)	(day)	(year)	If the patient's medical records indicate the patient had an MRI, provide the date of the MRI. <a href="#">3151491</a> (month), <a href="#">3151492</a> (day), <a href="#">3151493</a> (year)
76	MRI Results <i>Check all that apply</i>	<b>Test</b>	<b>Outcome</b>			If the patient's medical records indicate the patient had an MRI, provide the results for each applicable anatomic site. <a href="#">3151441</a>
			<i>Present</i>	<i>Absent</i>	<i>Unknown</i>	
		Pelvic Nodes				
		Paraortic Nodes				
		Supraclavicular Nodes				
		Parametrium				
		Bladder				
		Extra-Pelvic Meastatic Disease				
<b>X-ray Computed Tomography (CT Scan)</b>						
77	Date of CT Scan	____ / ____ / ____	(month)	(day)	(year)	If the patient's medical records indicate the patient had a CT scan, provide the date of the CT scan. <a href="#">3151134</a> (month), <a href="#">3151132</a> (day), <a href="#">3151133</a> (year)
78	CT Scan Results <i>Check all that apply</i>	<b>Test</b>	<b>Outcome</b>			If the patient's medical records indicate the patient had an CT scan, provide the results for each applicable anatomic site. <a href="#">2932340</a>
			<i>Present</i>	<i>Absent</i>	<i>Unknown</i>	
		Pelvic Nodes				
		Paraortic Nodes				
		Supraclavicular Nodes				
		Parametrium				
		Bladder				
		Extra-Pelvic Meastatic Disease				
<b>Tumor Marker Analysis</b>						
79	HPV Positive Type <i>Check all that apply</i>	<input type="checkbox"/> HPV 16 <input type="checkbox"/> Other HPV Type (please specify) <input type="checkbox"/> HPV 18 <input type="checkbox"/> None				If the patient's medical records indicate a positive diagnosis of the human papillomavirus (HPV), provide the HPV type found to be positive for this patient. <a href="#">2922649</a>
80	Other HPV Type	_____			If the patient's medical records indicate a positive diagnosis of the human papillomavirus (HPV) and the type is not included in the provided list, describe the HPV type found to be positive for this patient. <a href="#">3166168</a>	
81	Method of HPV Typing	<input type="checkbox"/> PCR <input type="checkbox"/> Qiagen - digene #2 <input type="checkbox"/> Roche - linear array <input type="checkbox"/> Other (please specify)			Indicate the method used for HPV typing. <a href="#">3151457</a>	
82	Other Method of HPV Typing	_____			If the method used for HPV typing is not included in the provided list, describe the HPV typing method used. <a href="#">3151460</a>	
83	PCR Primer Pairs	<input type="checkbox"/> MY09/MY11 <input type="checkbox"/> SPF10-LiPA <input type="checkbox"/> PGMY09/PGMY11 <input type="checkbox"/> GP5+/GP6+ <input type="checkbox"/> Roche - linear array <input type="checkbox"/> Other (please specify)			Indicate the PCR primar pairs used. <a href="#">3151487</a>	
84	Other PCR Primer Pairs	_____			If the method used for PCR primer pairs used are not included in the provided list, describe the PCR primer pairs used. <a href="#">3151490</a>	
85	Squamous Cellular Carcinoma Antigen (SCCA) Tumor Marker	_____			Provide the patient's squamous cellular carcinoma antigen (SCCA) tumor marker results. <a href="#">3151234</a>	
86	Date of SCCA Performed	____ / ____ / ____	(month)	(day)	(year)	Provide the date SCCA was performed. <a href="#">3151235</a> (month), <a href="#">3151236</a> (day), <a href="#">3151237</a> (year)
<b>General Comments</b>						

Principal Investigator Signature

Date

*I acknowledge that the above information provided by my institution is true and correct and has been quality controlled.*

Adopted: 05/25/2012

2<sup>o</sup> Version: \_\_\_\_\_

3<sup>o</sup> Version: \_\_\_\_\_

4<sup>o</sup> Version: \_\_\_\_\_

Under Revision: \_\_\_\_\_

## **CENTRALIZED PATHOLOGY REVIEW PROCESS FOR HIV+ CERVICAL TUMORS**

### **INTRODUCTION:**

Pathological diagnosis of tumor can be impacted by the subjective nature of the process as well as the subjective definition of the criteria used in the assessment. To ensure that samples entering the sequencing pipeline of the HIV+ Tumor Characterization Project meet the tissue requirements (set forth in SOP#101) and are diagnosed as Cervical Cancer, a central pathology review panel of three board-certified pathologists will be established. The review of tissues by a group minimizes the subjectivity encountered in pathology practice.

#### **A. SCOPE AND PURPOSE:**

1. To establish a standard procedure to follow for the centralized pathology review of tissue submitted to the HIV+ Tumor Characterization Project.

#### **B. EQUIPMENT AND MATERIALS:**

1. De-identified pathology reports provided by the tissue source site (TSS) which contributes the sample.
2. Five unstained slides with 4  $\mu$ m thick sections from the formalin-fixed paraffin-embedded (FFPE) diagnostic block (or the whole FFPE block). These slides or blocks will be provided by the tissue source site (TSS) contributing the sample with each labeled with the project-assigned ID (as specified in SOP #101C and 102).
3. Aperio Slide Scanner

### **II. PROCEDURE:**

#### **A. Preparation for review:**

1. All members of a centralized pathology board obtain their PathXchange credentials by going to the following website: <http://www.pathxchange.org/user/register>
2. Once the credentials are secured, they should be communicated to the appropriate OCG project manager.
3. Upon arrival at the pathology core, the pathology coordinator will verify that all slides and reports submitted are labeled with the same project-assigned ID.
  - The report will be scanned and uploaded to the PathXchange website (<http://www.pathxchange.org>).

4. Pathology coordinator will send the appropriate number of slides or block to the histology service to perform hematoxylin & eosin (H&E) as well as necessary immunohistochemical (IHC) and in situ hybridization procedures.
  - IHC to be performed include: **p16**. In cases of adenocarcinoma where an endometrial origin is suspected, **Vimentin, Estrogen Receptor, Carcinoembryonic Antigen (CEA) levels will be assessed by IHC.**
  - The processing should take typically 14 days.
5. After all the processing is completed, the pathology coordinator will facilitate whole slide scanning on the Aperio system.
6. Digital files from the whole slide scanning of the H&E and IHC slides from each case and a blank review form will be deposited in the PathXchange website (<http://www.pathxchange.org>) by the pathology coordinator.
7. The coordinator will send an e-mail to the members of the pathology review core (PRC) (with copy to the OCG project manager) informing them that materials for review have been deposited in a folder. This communication must specify the number and name of files, as well as the project-assigned ID for the case(s) under review.

**B. Review:**

1. Within ten days of receipt of the e-mail from the coordinator, all members of the PRC will return their pathology review form to the pathology coordinator via e-mail.
2. The tumors will be classified using the WHO classification
3. If a consensus is reached, 2 out of 3 reviewers agree, and the case passes the specified criteria the pathology coordinator will create a final pathology report and submit it to the Data Coordinating Center and the Genome Science Center at British Columbia (GSC-BC) no later than 4 days. Steps 1-3 will take 2 weeks total.
4. Cases considered inadequate for diagnosis due to low quality of FFPE sections will be labeled as such and taken out of the study.
5. Discrepant cases (cases in which the 2 out of 3 majority consensus is not reached) will be submitted for a web-based consensus review, to be convened by Dr. Teresa Darragh. The schedule of such consensus reviews will be dictated by the number of discordant cases accrued as follows:
  - When 6 or more discordant cases have been accrued, a consensus review panel must be convened.
  - If there are less than 6 discordant cases, but the oldest accrued case is more than six months old, a consensus review panel must be convened.

Any questions regarding this protocol should be directed to the HTMCP Pathology Coordinator

**CONTACTS:****Project Team Representative:**

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Scientific Programs Director  
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**Data Coordinating Center:**

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E-mail: [gesuwanp@mail.nih.gov](mailto:gesuwanp@mail.nih.gov)

**HTMCP Pathology Coordinator:**

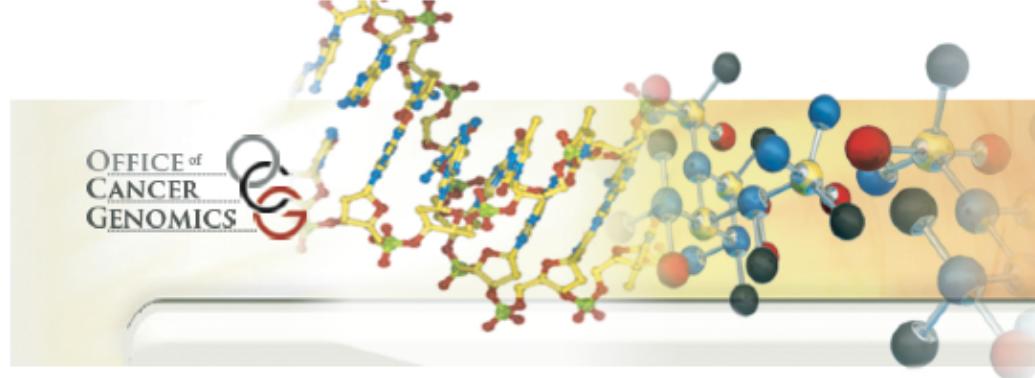
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National Cancer Institute



## HTMCP Anal-SPECIFIC PROTOCOLS

U.S. DEPARTMENT  
OF HEALTH AND  
HUMAN SERVICES  
National Institutes  
of Health



## HTMCP SOP #101D

Adopted: 05/25/2012  
2<sup>o</sup> Version: \_\_\_\_\_  
3<sup>o</sup> Version: \_\_\_\_\_  
4<sup>o</sup> Version: \_\_\_\_\_  
Under Revision: \_\_\_\_\_

## **PROSPECTIVE CASE SUBMISSION PROCEDURE FOR THE HIV+ ANAL PROJECT**

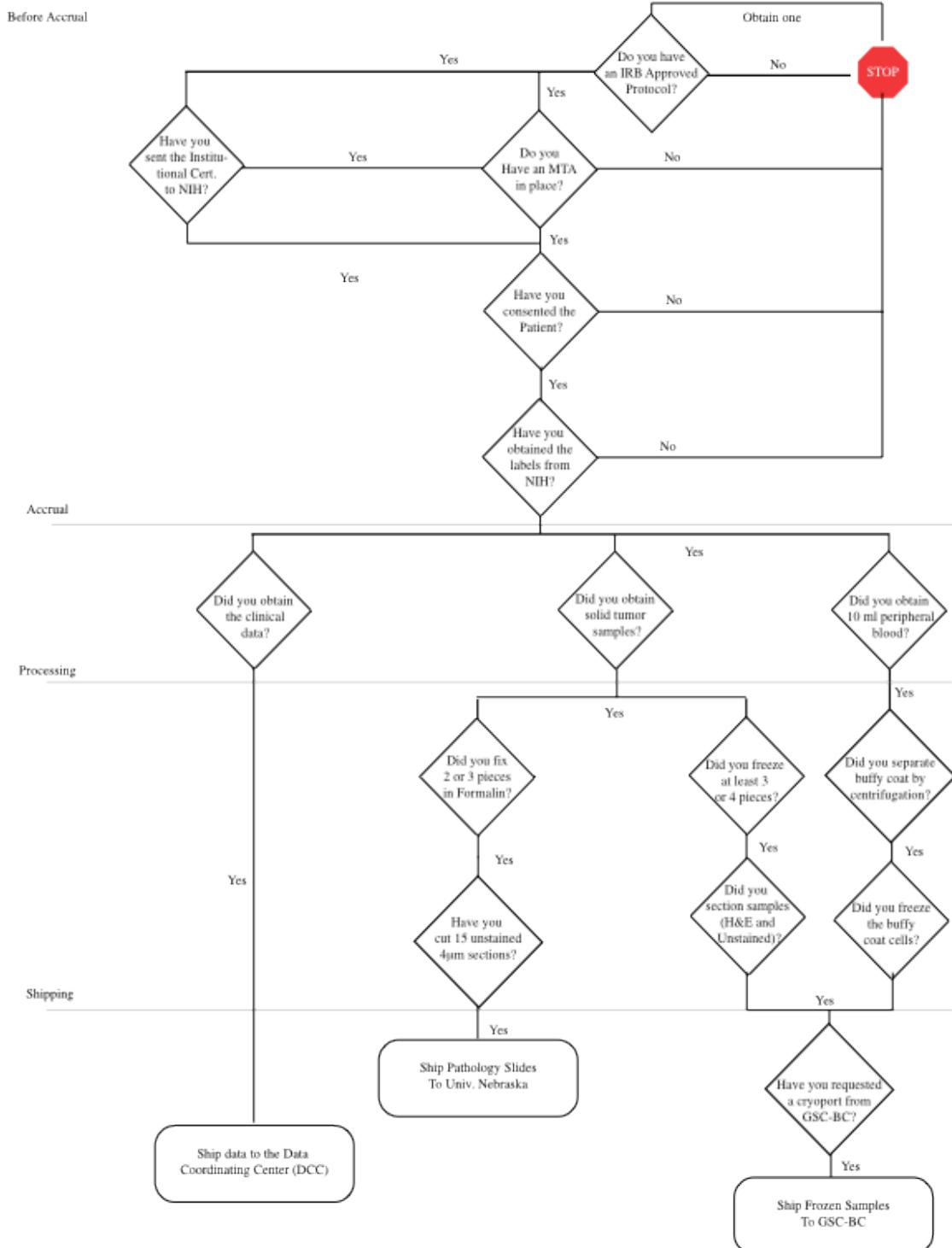
### **I. INTRODUCTION:**

The National Cancer Institute's Office of Cancer Genomics (OCG) and the Office of HIV and AIDS Malignancy (OHAM) have developed an initiative to compare the cancer related alterations in HIV+ patients and HIV- patients.

#### **A. SCOPE AND PURPOSE:**

1. To establish a general procedure to inform personnel of all the steps necessary for a successful submission of a sample to the HIV+ Tumor Molecular Characterization Project (HTMCP).
2. This protocol applies to all Tissue Source Sites (TSSs) providing tissues prospectively.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times and which samples were affected. This information should be given within 48 hours of the occurrence to the project team by sending an e-mail to Dr. Jean C. Zenklusen (**jz44m@nih.gov**) with the details.

## HTMCP SOP #101D



## II. PROCEDURES:

### A. BEFORE PATIENT ACCRUAL BEGINS:

1. Make sure all the documents required for sample shipment as spelled out in SOP#100 are in place before you start case accruals.

### B. BEFORE PATIENT SURGERY:

1. Create a TSS-assigned ID for your patient. Your institution will be the keeper of the key as described in your approved IRB protocol. **The TSS is responsible for maintaining the link between project-assigned ID and TSS-assigned ID in order to retrieve clinical information when required.** In case that the original PI is no longer affiliated with the contributing Institution, it is the TSS's responsibility to be able to track the patient's records.
2. Contact the Data Coordinating Center (DCC, see address below) to obtain a project-assigned ID to use in all documents regarding the case and all materials shipped.
3. Contact the OCG Project Coordinator and obtain freezer-resistant labels that you should use to mark all containers/slides carrying materials for the project.
4. Make sure to prepare the tissue freezing station and have ready all the materials needed for tissue processing (HTMCP SOP #102).
5. Inform the research nurse that a 10 ml peripheral blood sample must be obtained from the patient to use as a non-tumoral malignant control (see Appendix A). Note that the plasma should be processed and the buffy coat should be separated from the plasma within two hours of being obtained from the patient. Storage of the buffy coat sample should then occur in a -80°C or Liquid Nitrogen (LN2) freezer.

### C. DURING PATIENT SURGERY:

1. Inform the surgical staff of the tissue requirements for the project (see Appendix A).
2. Have a person ready to transport the ablated tissue to the processing station. It is generally accepted that for the best tissue preservation snap freezing should take place within 20 minutes after tissue is obtained from the patient.
3. Note the time between surgery and freezing in a notebook and send to the OCG Project Coordinator

### D. AFTER SURGERY:

1. Process solid tissue as described in the tissue processing protocol (HTMCP SOP #102). Timely processing is crucial, it is generally accepted that for the best tissue preservation snap freezing should take place within 20 minutes after tissue is obtained from the patient.

## HTMCP SOP #101D

2. Process blood sample according to protocol (see HTMCP SOP #103, blood processing). Store isolated cells in a -80°C freezer or liquid Nitrogen (LN<sub>2</sub>) storage until shipment.
3. Obtain a formalin-fixed, paraffin-embedded tissue block or, if not possible, **five** unstained 4  $\mu\text{m}$  sections from the formalin-fixed block (or the whole block). Affix one of the provided freezer-resistant labels to each slide or block.

## E. PREPARING SAMPLES AND SHIPMENT:

1. Produce a 4  $\mu\text{m}$  frozen section of the top of the tumor sample for initial quality control as described in the tissue processing protocol (HTMCP SOP #102).
2. When tissue from at least three cases are accrued, or every quarter (See HTMCP SOP #105), contact the GSC-BC coordinator to obtain a cryoport transport vessel to ship the cryovials containing frozen tumor sample sections and frozen blood cells.
3. Follow the frozen sample shipment protocol (HTMCP SOP #104) and send the frozen samples to the GSC-BC. Provide both the GSC-BC and PT with tracking number the day of shipment.
4. Send a formalin-fixed, paraffin-embedded tissue block or, if not possible, **five** unstained 4  $\mu\text{m}$  sections obtained from the formalin fixed blocks to the pathology coordinator. On shipment, provide both the pathology coordinator and OCG project coordinator with the tracking number of the parcel. For shipment use a closable box (such as Thermo Scientific\* Plastic Slide Box, capacity 25 slides, catalog# B1780).
5. Collect all the clinical data requested in the sample requirements (Appendix C). You will be requested by the OCG project coordinator to send the data electronically to the DCC using the appropriate TSS-assigned ID at a later date.

## NOTES:

- A checklist is provided to help you track all the steps required by this process (Appendix B). Please use it!
- If any one of the required items (institutional certification, confirmation of informed consent, frozen tissue, frozen blood cells, unstained slides and clinical data) is not present, the submission is incomplete.
- **At no point in the process can traditionally-used identifying information (such as the patient name, address, phone number, medical record number, or social security number) be used to label samples. Only use the project-assigned ID and labels provided by the OCG project coordinator.**

**APPENDIX A: Sample Requirements****HIV+ Anal Cancer Characterization Project  
Tissue Sample Requirements for Accrual****Tissue Requirements:**

To be accepted to the project, the following conditions have to be met at the tissue level.

- Paired tumor and normal tissue or plasma buffy coat must be available in sufficient quantities (see below).
- Tissues (both normal and tumor) need to be snap frozen. Time between tissue extraction and freezing must be recorded.
- Optimal storage of the tissues is in N<sub>2</sub>(liq), but -80°C or lower is acceptable. The form of tissue storage must be recorded.
- There must be 100 mg of tumor tissue with a minimum percent of tumor nuclei between 70-80% as assessed on an H&E section physically adjacent to the specimen that is candidate for generating the RNA and DNA.
- A paraffin embedded block for pathology consensus review must exist for the tumor.

**APPENDIX B: Checklist of Task Completion for Sample Submission****Date:****Institution:****Operator:**

- ❖ Do you have an IRB approved protocol?
- ❖ Have you consented the patient?
- ❖ Have you sent your Institutional Certification to the Project Team?
- ❖ Have you obtained the project-assigned ID and labels from the Project Team?
- ❖ Do you have frozen sections in cryovial and H&E stained slides? Are they labeled?
- ❖ Do you have frozen plasma derived white blood cells? Are they correctly labeled?
- ❖ Have you secured a cryoport from the Genome Science Center at British Columbia?
- ❖ Do you have (5) unstained 4  $\mu\text{m}$  sections from the Formalin-fixed block? Are they labeled?
- ❖ Do you have the clinical data elements required by the Project? (Appendix B)

**ONLY if all the above items check out, you are ready to ship the samples.**

**APPENDIX C: Anal Cancer Characterization Project from HIV+ Patients Clinical Data Requirements for Accrual**

**Clinical Data Requirements:**

To be accepted to the project, the following conditions must meet at the clinical data level. The case(s) must have available ALL the clinical data elements (CDEs) here listed, should some of the datafields be missing, please contact the OCG project coordinator to get approval for submission.

These clinical data elements must be reported to the DCC as an initial report within two weeks of enrolling the patient. Updates must be sent to the DCC as the patient returns for periodic visits (Annually).

- Patients need to be consented in such way that allows for the use of their tissues for genomic-scale molecular characterization

Month of Birth	_____
Day of Birth	_____
Year of Birth	_____
Gender	<input type="checkbox"/> Female <input type="checkbox"/> Male
Height (at time of diagnosis)	_____ (cm)
Weight (at time of diagnosis)	_____ (kg)

## HTMCP SOP #101D

Race	<input type="checkbox"/> American Indian or Alaska Native <i>A person having origins in any of the original peoples of North and South America (including Central America), and who maintains tribal affiliation or community attachment.</i> <input type="checkbox"/> Asian <i>A person having origins in any of the original peoples of the far East, Southeast Asia, or in the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.</i> <input type="checkbox"/> White <i>A person having origins in any of the original peoples of the far Europe, the Middle East, or North Africa.</i> <input type="checkbox"/> Black or African American <i>A person having origins in any of any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."</i> <input type="checkbox"/> Native Hawaiian or other Pacific Islander: <i>A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.</i> <input type="checkbox"/> Not Reported: <i>Not provided or available.</i> <input type="checkbox"/> Unknown: <i>Could not be determined or unsure.</i>
Ethnicity	<input type="checkbox"/> Not Hispanic or Latino: <i>A person not meeting the definition of Hispanic or Latino.</i> <input type="checkbox"/> Hispanic or Latino: <i>A person of Mexican, Puerto Rican, Cuban, Central or South American or other Spanish culture or origin, regardless of race.</i> <input type="checkbox"/> Not Reported: <i>Not provided or available.</i> <input type="checkbox"/> Unknown: <i>Could not be determined or unsure.</i>
History of Other Malignancy	<input type="checkbox"/> Yes <input type="checkbox"/> No
Neo-adjuvant (pre-operative) therapy	<input type="checkbox"/> Yes <input type="checkbox"/> No <i>Note: Systemic therapy and certain localized therapies (those administered to the same site as the submitted tissue) given prior to the resection of the sample submitted for is exclusionary.</i>
Vital Status (at date of last contact)	<input type="checkbox"/> Living <input type="checkbox"/> Deceased
Month of Last Contact	_____
Day of Last Contact	_____

## HTMCP SOP #101D

Year of Last Contact	_____
Month of Death	_____
Day of Death	_____
Year of Death	_____
Tumor Status <i>(at time of last contact or death)</i>	<input type="checkbox"/> Tumor free <input type="checkbox"/> With tumor
Tobacco Smoking History	<input type="checkbox"/> 1 - Lifelong non-smoker (<100 cigarettes smoked in lifetime) <input type="checkbox"/> 2 - Current smoker (includes daily and non-daily smokers) <input type="checkbox"/> 3 - Current reformed smoker for > 15 years <input type="checkbox"/> 4 - Current reformed smoker for < 15 years
Age at Onset of Tobacco Smoking	______ Years of Age
Year Tobacco Smoking Ceased	______ (YYYY)
Number of Pack Years Smoked	______ Years of Age
History if Immunosuppressive Disease <i>(Check all that apply)</i>	<input type="checkbox"/> HIV <input type="checkbox"/> Organ Transplant <input type="checkbox"/> Chronic Systemic Steroid Use <input type="checkbox"/> Other, specify _____
Other Immunosuppressive Disease	_____
Performance Status Scale: Eastern Cooperative Oncology Group (ECOG)	<input type="checkbox"/> 0 – Asymptomatic <input type="checkbox"/> 1 – Symptomatic but fully ambulatory <input type="checkbox"/> 2 – Symptomatic but in bed less than 50% of day <input type="checkbox"/> 3 – Symptomatic and in bed more than 50% of day <input type="checkbox"/> 4 – Bedridden
Performance Status Scale: Timing	<input type="checkbox"/> Preoperative <input type="checkbox"/> Pre-adjuvant therapy <input type="checkbox"/> Post-adjuvant therapy <input type="checkbox"/> Other
Other Performance Status Scale: Timing	_____
Month of Performance Status	_____
Day of Performance Status	_____

## HTMCP SOP #101D

Year of Performance Status	_____
Tumor Response	<input type="checkbox"/> Progressive Disease <input type="checkbox"/> Stable Disease <input type="checkbox"/> Partial Response <input type="checkbox"/> Complete Response
Primary Site of Disease	<input type="checkbox"/> Anus
Histological Subtype	<input type="checkbox"/> Anal Squamous Cell Carcinoma <input type="checkbox"/> Anal Adenocarcinoma
Keratinization in Squamous Cell Carcinoma	<input type="checkbox"/> Keratinizing squamous cell carcinoma <input type="checkbox"/> Non-keratinizing squamous cell carcinoma
Tumor Grade	<input type="checkbox"/> G1 Well Differentiated <input type="checkbox"/> G2 Moderately Differentiated <input type="checkbox"/> G3 Poorly Differentiated <input type="checkbox"/> G4 Undifferentiated <input type="checkbox"/> GX Grade cannot be assessed
Month of Initial Pathological Diagnosis	_____
Day of Initial Pathological Diagnosis	_____
Year of Initial Pathological Diagnosis	_____
Method of Initial Pathologic Diagnosis	<input type="checkbox"/> Cytology <input type="checkbox"/> Biopsy (CT-guided or other) <input type="checkbox"/> Lymph node sampling or dissection <input type="checkbox"/> Other, specify _____
Other Method of Pathologic Diagnosis	_____
Pathologic Positive Lymph Node Location(s) <i>(Check all that apply)</i>	<input type="checkbox"/> Pelvic (external iliac, internal iliac, obturator) <input type="checkbox"/> Common iliac <input type="checkbox"/> Paraaortic <input type="checkbox"/> Supraclavicular <input type="checkbox"/> Other, specify _____
Other Positive Lymph Node	_____
Other Involved Pathologic Margins	_____
Were Lymph Nodes Examined at the Time of Primary Resection?	<input type="checkbox"/> Yes <input type="checkbox"/> No
Number of Lymph Nodes	_____

## HTMCP SOP #101D

Examined				
Number of Lymph Nodes Positive by H&E light microscopy	<hr/>			
Number of Lymph Nodes Positive by IHC Keratin Staining only	<hr/>			
Pathologic Lymphovascular Invasion	<input type="checkbox"/> Present <input type="checkbox"/> Absent			
Pathologic Spread: Primary Tumor (pT)	<input type="checkbox"/> TX <input type="checkbox"/> T0 <input type="checkbox"/> Tis <input type="checkbox"/> T1 <input type="checkbox"/> T1a <input type="checkbox"/> T1a1 <input type="checkbox"/> T1a2	<input type="checkbox"/> T1b <input type="checkbox"/> T1b1 <input type="checkbox"/> T1b2 <input type="checkbox"/> T2 <input type="checkbox"/> T2a <input type="checkbox"/> T2a1	<input type="checkbox"/> T2a2 <input type="checkbox"/> T2b <input type="checkbox"/> T3 <input type="checkbox"/> T3a <input type="checkbox"/> T3b <input type="checkbox"/> T4	
Pathologic Spread: Regional Nodes (pN)	<input type="checkbox"/> NX <input type="checkbox"/> N0 <input type="checkbox"/> N1			
Pathologic Distant Spread: Distant Metastasis (M)	<input type="checkbox"/> MX <input type="checkbox"/> M0 <input type="checkbox"/> M1			
Month of FED-PET OR PET/CT	<hr/>			
Day of FED-PET OR PET/CT	<hr/>			
Year of FED-PET OR PET/CT	<hr/>			
Cervix SUV Results	<hr/>			
FED-PET OR PET/CT Results <i>(Check all that apply)</i>	Test	Outcome		
		<i>Present</i>	<i>Absent</i>	<i>Unknown</i>
	Pelvic Nodes			
	Paraortic Nodes			
	Supraclavicular Nodes			
	Parametrium			
	Bladder			
	Extra-Pelvic Metastatic Disease			
Month of MRI	<hr/>			
Day of MRI	<hr/>			
Year of MRI	<hr/>			
MRI Results <i>(Check all that apply)</i>	Test	Outcome		
		<i>Present</i>	<i>Absent</i>	<i>Unknown</i>
	Pelvic Nodes			
	Paraortic Nodes			

## HTMCP SOP #101D

	Supraclavicular Nodes			
	Parametrium			
	Bladder			
	Extra-Pelvic Metastatic Disease			
Month of CT Scan				
Day of CT Scan				
Year of CT Scan				
CT Results <i>(Check all that apply)</i>	Test	Outcome		
		Present	Absent	Unknown
	Pelvic Nodes			
	Paraortic Nodes			
	Supraclavicular Nodes			
	Parametrium			
	Bladder			
Extra-Pelvic Metastatic Disease				
HPV Type <i>(List all types)</i>	<input type="checkbox"/> HPV 16 <input type="checkbox"/> HPV 18 <input type="checkbox"/> Other HPV Type <input type="checkbox"/> None			
Other HPV Type(s)				
Method of HPV Typing	<input type="checkbox"/> PCR <input type="checkbox"/> Qiagen – digene #C2 <input type="checkbox"/> Roche – linear array <input type="checkbox"/> Other, specify			
Other Method of HPV Typing				
PCR Primer Pairs	<input type="checkbox"/> MY09/MY11 <input type="checkbox"/> PGMY09/PGMY11 <input type="checkbox"/> Roche – linear array <input type="checkbox"/> SPF10-LiPA <input type="checkbox"/> GP5+/GP6+ <input type="checkbox"/> Other, specify			
Other PCR Primer Pairs				
Squamous Cellular Carcinoma Antigen (SCCA) Tumor Marker	_____ $\mu\text{g}/\mu\text{L}$			

## HTMCP SOP #101D

Month of SCCA Performed	_____
Day of SCCA Performed	_____
Year of SCCA Performed	_____

<b>HIV Related Data</b>	
Date of HIV diagnosis, if known	Text mm/yyyy
Nadir CD4 counts	number cells/mm <sup>3</sup>
CD4 counts at lung cancer diagnosis	number cells/mm <sup>3</sup>
HIV RNA load at lung cancer diagnosis	copies/ml
Prior AIDS defining comorbidities	Text Y/N
Co-infections- serology data/viral load if available HBV HCV KSHV	Text Y/N
HAART treatment prior to lung cancer diagnosis	Text Y/N
HAART treatment at time of lung cancer diagnosis	Y/N Drugs usedText
HIV risk group(s)	Text Y/N
History of other malignancies (by definition, this should be none, but I think it is good to keep this question here)	



Adopted: 05/25/2012  
2<sup>o</sup> Version: \_\_\_\_\_  
3<sup>o</sup> Version: \_\_\_\_\_  
4<sup>o</sup> Version: \_\_\_\_\_  
Under Revision: \_\_\_\_\_

## **CENTRALIZED PATHOLOGY REVIEW PROCESS FOR HIV+ ANAL TUMORS**

### **INTRODUCTION:**

Pathological diagnosis of tumor can be impacted by the subjective nature of the process as well as the subjective definition of the criteria used in the assessment. To ensure that samples that are part of the HIV+ Tumor Characterization Project meet the tissue requirements (set forth in SOP#101) and are diagnosed as Anal Cancer, a central pathology review panel of three board-certified pathologists is be established. The review of tissues by a group minimizes the subjectivity encountered in pathology practice.

#### **A. SCOPE AND PURPOSE:**

1. To establish a standard procedure to follow for the centralized pathology review of tissue submitted to the HIV+ Tumor Characterization Project.

#### **B. EQUIPMENT AND MATERIALS:**

1. De-identified pathology reports provided by the tissue source site (TSS) which contributes the sample.
2. Five unstained slides with 4  $\mu\text{m}$  thick sections from the formalin-fixed paraffin-embedded (FFPE) diagnostic block (or the whole FFPE block). These slides or blocks will be provided by the tissue source site (TSS) contributing the sample with each labeled with the project-assigned ID (as specified in SOP #101D and 102).
3. Aperio Slide Scanner

### **II. PROCEDURE:**

#### **A. Preparation for review:**

1. All members of a centralized pathology board obtain their PathXchange credentials by going to the following website: <http://www.pathxchange.org/user/register>
2. Once the credentials are secured, they should be communicated to the appropriate OCG project manager.
3. Upon arrival at the pathology core, the pathology coordinator will verify that all slides and reports submitted are labeled with the same project-assigned ID.
  - The report will be scanned and uploaded to the PathXchange website (<http://www.pathxchange.org>).

4. Pathology coordinator will send the appropriate number of slides or blocks to the histology service to perform hematoxylin & eosin (H&E) as well as necessary immunohistochemical (IHC) protocols.
  - IHC to be performed is for **CDKN2A**
  - The processing should not take longer than 14 days.
5. After the H&E and IHC processing is completed, the pathology coordinator will scan the whole slide on the Aperio system and deposit them, together w/ a blank review form on the PathXchange website (<http://www.pathxchange.org>).
6. The coordinator will send an e-mail to the members of the pathology review core (PRC) (with copy to the OCG project manager) informing them that materials for review have been deposited in a folder. This communication must specify the number and name of files, as well as the project-assigned ID(s) for the case(s) under review.

**B. Review:**

1. Within ten days of receipt of the e-mail from the coordinator, all members of the PRC will return their pathology review form to the pathology coordinator via e-mail.
2. The tumors will be classified using the WHO classification
3. If a consensus is reached, 2 out of 3 reviewers agree, and the case passes the specified criteria the pathology coordinator will create a final pathology report and submit it to the Data Coordinating Center and the Genome Science Center at British Columbia (GSC-BC) no later than 4 days. Steps 1-3 will take 2 weeks total.
4. Cases considered inadequate for diagnosis due to low quality of FFPE sections will be labeled as such and taken out of the study.
5. Discrepant cases (cases in which the 2 out of 3 majority consensus is not reached) will be submitted for a web-based consensus review, to be convened by Dr. Teresa Darragh. The schedule of such consensus reviews will be dictated by the number of discordant cases accrued as follows:
  - When 6 or more discordant cases have been accrued, a consensus review panel must be convened.
  - If there are less than 6 discordant cases, but the oldest accrued case is more than six months old, a consensus review panel must be convened.

**CONTACTS:****Project Team Representative:**

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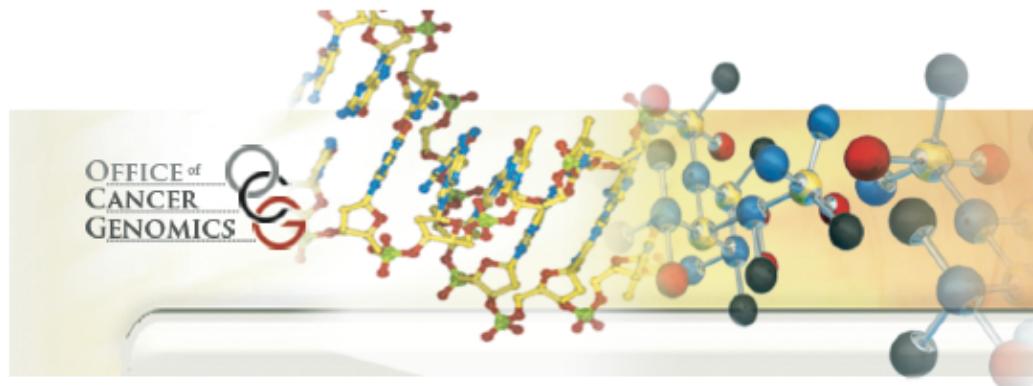
Thomas C. Wright  
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HTMCP SOP #107D

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# National Cancer Institute



## BLGSP PROTOCOLS

U.S. DEPARTMENT  
OF HEALTH AND  
HUMAN SERVICES

National Institutes  
of Health



Adopted: \_\_\_\_\_  
2<sup>nd</sup> Version: \_\_\_\_\_  
3<sup>rd</sup> Version: \_\_\_\_\_  
Reviewed: \_\_\_\_\_  
4<sup>th</sup> Version: \_\_\_\_\_

## **DOCUMENT REQUIREMENTS FOR SAMPLE SUBMISSION TO THE BURKITT LYMPHOMA GENOME SEQUENCING PROJECT**

### **I. INTRODUCTION:**

The National Cancer Institute's Office of Cancer Genomics (OCG) and the Foundation for Burkitt Lymphoma Research have developed an initiative to establish a genomic databank to explore potential genetic changes in patients with Burkitt Lymphoma.

**It is imperative that all personnel involved in the project read all the protocols and adhere to them at all times. It is your responsibility as a contributor to the BLGSP to familiarize yourself with all aspects of the procedures and assure their compliance.**

### **A. SCOPE AND PURPOSE:**

1. To list all the documents needed in order to start collection of samples for the Burkitt Lymphoma Genome Sequencing Project (BLGSP).
2. This protocol applies to all Tissue Source Sites (TSSs) providing tissues prospectively.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times and which samples were affected. This information should be given within 48 hours of the occurrence to the project team by sending an e-mail to Dr. Robin S. Broughton ([robin.broughton@nih.gov](mailto:robin.broughton@nih.gov)) with the details.

### **B. REQUIREMENTS:**

1. Every TSS must have an IRB-approved protocol in place that allows collection of tumor tissue, matched normal tissue (blood, whenever possible) and clinical data that can be used in a characterization project. The protocol must have explicit language permitting the molecular characterization of the samples by genomic-scale methodologies, and subsequent deposition of the data into a public, but protected database.

2. Every patient accrued to the project must be enrolled in the protocol and agree to participate by signing an informed consent.
3. If you require assistance drafting such protocol or informed consent form, please contact the Project Team (PT) representative (address below). OCG has templates that contain the appropriate language.
4. TSSs must have in place a materials transfer agreement (MTA) with The Research Institute at Nationwide Children's Hospital (NCH; see address below) to allow transfer of tissues and pathology reports. A sample MTA can be provided by the PT upon request.
5. OCG will store a copy of the IRB-approved protocol and a blank informed consent form. Additionally, certification that such protocol exists, and that patients have been consented, must be produced by the TSS Institution before the samples can be accepted and costs can be reimbursed. A template of such certification document can be found in Appendix A.
6. The completed Institutional certification must be sent to the PT before any sample can be shipped.

## **CONTACTS:**

### **Project Team (PT) Representative:**

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## BLGSP SOP #002

Adopted: \_\_\_\_\_  
 2<sup>nd</sup> Version: \_\_\_\_\_  
 3<sup>rd</sup> Version: \_\_\_\_\_  
 Reviewed: \_\_\_\_\_  
 4<sup>th</sup> Version: \_\_\_\_\_

## **PROCESSING TISSUE FOR MOLECULAR CHARACTERIZATION OF BURKITT LYMPHOMA TUMORS**

### **I. INTRODUCTION**

#### **A. SCOPE AND PURPOSE:**

1. To establish a procedure for tissue processing and storage by Tissue Source Sites (TSS).
2. This protocol applies to all TSS providing tissues prospectively.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times, and which samples were affected. This information should be given within 48 hours of occurrence to the project team by sending an e-mail to Dr. Robin S. Broughton ([robin.broughton@nih.gov](mailto:robin.broughton@nih.gov)) with the details.

#### **B. SAFETY PRECAUTIONS:**

1. Wear personal protective equipment (PPE) such as lab coats and gloves.
2. Liquid nitrogen is extremely cold and can cause ‘burns’. Wear gloves that are specially made to withstand liquid nitrogen, eye protection (preferably Face Shield) and a lab coat to protect skin from splashes and spills. Liquid nitrogen is an asphyxiant; be sure to use in a well-ventilated area.
3. Acute overexposure to formaldehyde solutions and/or vapors causes severe eye, skin, and respiratory tract irritation.

#### **C. EQUIPMENT AND MATERIALS:**

1. Personal protective equipment (PPE) to include heavy duty gloves, eye protection (preferably Face Shield), lab coat and closed-toe shoes
2. Plastic cassette mold(s) for Formalin fixation.
3. Cryovials (e.g. 2mL vials from ChartBiomed, Part Number 10778828)
4. Freezer resistant labels with project-assigned ID (obtained from Project Team, see BLGSP SOP #003)
5. Dewar thermo-flask
6. Isopentane
7. Liquid Nitrogen
8. Formalin (10% solution)
9. Fine point Cryomarker (e.g. Nalge Nunc Cryomarker Black #6313-0020)

**MARK ALL CONTAINERS WITH THE LABELS CARRYING PATIENT PROJECT-ASSIGNED ID OBTAINED PRIOR TO SURGERY.**

**II. PROCEDURE:**

- A. Tissue diagnosed as Burkitt Lymphoma should be processed as follows:
    - 1. Wearing sterile gloves cut the tissue into multiple 2 mm thin sections using a sterile scalpel.
    - 2. Place tissue into various containers as follows:
      - i. **24-HOUR FORMALIN FIXATION:** Submit two representative tissue pieces for diagnosis, including 1-2 blocks to your Histology Lab. Tissue in formalin should be no more than 2 mm in thickness for proper fixation.
      - ii. **FREEZING TISSUE:** Select two or three representative pieces of tissue measuring about 10 x 10 x 2 mm in dimension. Freeze as many pieces as possible. Do not freeze the tissue with Freon.
- Freeze the tissues as described below:
- (i) **Perform snap freezing of fresh tissue ASAP**
    - 1. It is generally accepted that for the best tissue preservation snap freezing should take place within 20 minutes after tissue is ablated from patient.
    - 2. Do not perform snap freezing with bare hands. Wear gloves at all times.
  - (ii) **Set Up Freezing Station**
    - 1. Fill a small 100 ml metal beaker about 1/4 full with isopentane (2-methylbutane, certified grade).
    - 2. Fill the Dewar thermo-flask about 1/3 full with liquid nitrogen.
    - 3. Use extreme caution when dispensing liquid nitrogen
  - (iii) **Label Cryovial As Needed**
    - 1. Use a cryomarker for labelling.
      - a. Use a cryovial for tissue snap freezing.
      - b. Label cryovial with freezer-resistant labels obtained prior to surgery (see BLGSP SOP #003).
  - (iv) **Freezing Tissue in Cryovial**
    - a. Cut a 1cm by 3 cm strip of histowrap
    - b. Put the tissue on the histowrap strip
    - c. Place the histowrap strip containing tissue into a labelled cryovial, using a pair of forceps.
    - d. Screw on the cap **tightly** or else isopentane will seep into the vial during freezing and create a liquid in the vial upon thawing.
    - e. Lower the 100 ml metal beaker containing isopentane half-way into the liquid nitrogen for cooling. The liquid nitrogen will boil as the beaker is lowered, when the isopentane is reaching its freezing

## BLGSP SOP #002

- point the tone of the boiling will increase for 2-3 seconds.
  - f. Lift the beaker out of the liquid nitrogen once you see beads of solid isopentane at the bottom of the beaker (about 2 minutes).
  - g. Use long forceps to hold the cryovial down into the cooled isopentane. Hold for at least 1 minute.
  - h. Use the long forceps to take out the cryovial/ frozen tissue.
  - i. Store Frozen tissue vial(s) in Liquid Nitrogen Storage Tanks.
- B. Make a gross report of the sample using the dictation template below.
- C. Any questions regarding this protocol should be directed to the BLGSP Pathology Coordinators at 402-559-7689 or 402-559-7526.

**THE FROZEN SPECIMENS SHOULD BE KEPT FROZEN ON DRY ICE AT ALL TIMES DURING TRANSPORT TO AND FROM STORAGE TANKS.**

**BLGSP STUDY GROSS DICTATION TEMPLATE**

**History:**

The patient is a...

**Source/Gross:**

The specimen is received (**fresh vs. fixed**) in (# containers), each labeled with the project-assigned ID “#” and designated “#.” The specimen consists of (**gross to include number of fragments, size, appearance, etc.**)

Specimens submitted are:

Fixed in formalin for 24 hours – (**size, # of pieces in each block, and cassette designation**)

Snap Frozen – (**size and # of blocks**)

**CONTACTS:****BLGSP Pathology Coordinators:**

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Amelia and Austin Vickery Professor of Pathology  
Co-Director, Center for Lymphoma and Leukemia Research  
University of Nebraska  
Department of Pathology  
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University of Nebraska Medical Center  
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Omaha, NE 68105  
Phone: 402-559-7689  
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OR

Cynthia M. Lachel  
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E-mail: cindy.lachel@unmc.edu

**Project Team (PT) Representative:**

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e-mail: [robin.broughton@nih.gov](mailto:robin.broughton@nih.gov)

Adopted: \_\_\_\_\_  
2<sup>nd</sup> Version: \_\_\_\_\_  
3<sup>rd</sup> Version: \_\_\_\_\_  
Reviewed: \_\_\_\_\_  
4<sup>th</sup> Version: \_\_\_\_\_

## **PROSPECTIVE SAMPLE SUBMISSION PROCEDURE FOR THE BURKITT LYMPHOMA GENOME SEQUENCING PROJECT**

### **I. INTRODUCTION:**

The National Cancer Institute's Office of Cancer Genomics (OCG) and the Foundation for Burkitt Lymphoma Research have developed an initiative to establish a genomic databank to explore potential genetic changes in patients with Burkitt Lymphoma.

#### **A. SCOPE AND PURPOSE:**

1. To establish a general procedure to inform personnel of all the steps necessary for a successful submission of a sample to the Burkitt Lymphoma Genome Sequencing Project (BLGSP).
2. This protocol applies to all Tissue Source Sites (TSSs) providing tissues prospectively.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times, and which samples were affected. This information should be given within 48 hours of the occurrence to the project team (PT) representative by sending an e-mail to Dr. Robin S. Broughton ([robin.broughton@nih.gov](mailto:robin.broughton@nih.gov)) with the details.

### **II. PROCEDURES:**

#### **A. BEFORE PATIENT ACCRUAL BEGINS:**

1. Make sure all the documents required for sample shipment as spelled out in BLGSP SOP# 001 are in place before you start case accruals.

#### **B. BEFORE PATIENT SURGERY:**

1. Create a TSS-assigned ID for your patient. Your institution will be the keeper of the key as described in your approved IRB protocol.
2. Contact the data coordinating center (DCC, see address below) with your TSS-assigned ID to obtain a project-assigned ID to use in all documents regarding the case

and all materials shipped. **The TSS is responsible for maintaining the link between project-assigned ID and TSS-assigned ID in order to retrieve clinical information when required.** It is the TSSs responsibility to be able to track the patient's records back in the event that the original researcher(s) at the institution lose their affiliation.

3. Contact the PT and obtain forty (40) freezer-resistant labels that you should use to mark all containers/slides carrying materials for the project.
4. Make sure to prepare the tissue freezing station and have ready all the materials needed for tissue processing (BLGSP SOP# 002).
5. Inform the research nurse that a 10ml peripheral blood sample must be obtained from the patient to use as a non-tumoral control (see Appendix B). Store the blood sample in the refrigerator until processing (see BLGSP SOP# 004). Time in storage must be decided at site and reported back to Project Team.

#### C. DURING PATIENT SURGERY:

1. Inform the surgical staff of the tissue requirements for the project (see Appendix B).
2. Have a person ready to transport the ablated tissue to the processing station. It is generally accepted that for the best tissue preservation snap freezing should take place within 20 minutes after tissue is obtained from the patient.

#### D. AFTER SURGERY:

1. Process solid tissue as described in the tissue processing protocol (BLGSP SOP #002). Timely processing is crucial, it is generally accepted that for the best tissue preservation snap freezing should take place within 20 minutes after tissue is obtained from the patient.
2. Process the blood sample according to protocol (see BLGSP SOP #004). Store isolated cells in liquid nitrogen storage until shipment.
3. Obtain eight to ten (8-10) unstained 4  $\mu\text{m}$  sections from the formalin-fixed block. Affix one of the provided freezer-resistant labels to each slide.

#### E. PREPARING SAMPLES AND SHIPMENT:

1. Contact The Research Institute at Nationwide Children's Hospital (NCH) coordinator to obtain a cryoport transport vessel to ship the cryovials containing frozen tumor sample sections and frozen blood cells.
2. Once the cryoport arrives follow the frozen sample shipment protocol (BLGSP SOP #006) and send the frozen samples to NCH. Upon shipping, provide both the GSC-BC and PT with tracking number.
3. Send the eight to ten (8-10) unstained 4  $\mu\text{m}$  sections obtained from the formalin fixed blocks to the pathology coordinator at the University of Nebraska (see address below). On shipment, provide both the pathology coordinator and PT with tracking

- number. For shipment use a closable box (such as Thermo Scientific\* Plastic Slide Box, capacity 25 slides, catalog# B1780).
4. Collect all the clinical data requested in the sample requirements (Appendix B) and send electronically to the DCC.

**NOTES:**

- A checklist is provided to help you track all the steps required by this process (Appendix C). Please use it!
- If any one of the required items (institutional certification, confirmation of informed consent, frozen tissue, frozen blood cells, unstained slides and clinical data) are not present, the submission is incomplete and reimbursement of costs cannot proceed.
- **At no point in the process can traditionally-used identifying information (such as the patient name, address, phone number, medical record number, or social security number) be used to label samples. Only use the project-assigned ID and labels provided by the Project Team.**

## **CONTACTS:**

### **Project Team Representative:**

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### **Data Coordinating Center:**

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E-mail: [gesuwanp@mail.nih.gov](mailto:gesuwanp@mail.nih.gov)

### **BLGSP Pathology Coordinators:**

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OR

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E-mail: cindy.lachel@unmc.edu

**NCH Coordinator:**

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Lead Clinical Outreach Coordinator & BCR Project Development Specialist  
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E-mail: [Hollie.Harper@nationwidechildrens.org](mailto:Hollie.Harper@nationwidechildrens.org)

## **APPENDIX A: Template of Institutional Certification**

**[This Institutional Certification should be submitted on the PI's institutional letterhead.]**

Date: Month Day, Year

To: Elizabeth Gillanders, Ph.D.  
Branch Chief and NCI Data Access Committee Chair  
National Institutes of Health  
MSC 7324  
6130 Executive Blvd  
Rockville, MD 20892

Re: Certification of [name of PI's institution] to Accompany Submission of the Dataset for the HMP Demonstration Project, [insert name of Project] to the NIH Database of Genotypes and Phenotypes (dbGaP).

Dear Dr. Gillanders:

[Name of PI's institution] adheres to the *Policy for Sharing of Data Obtained in NIH Supported or Conducted Genome-Wide Association Studies (GWAS)*, Notice Number: NOT-OD-07-088. [Name of PI's institution] provides the following Certification in regard to the Dataset for the [name of Project], which is being deposited into dbGaP.

- The data submission is consistent with all applicable laws and regulations, as well as institutional policies.
- All uses of these data that are deemed acceptable and approved per NIH policy and that follow the dbGaP procedures for access are allowable, with the following restriction:

These data may be used for general scientific research by qualified investigators that are performing research projects and that have likely relevance to developing more effective treatments, diagnostic tests, or prognostic markers, including cancer research.

- The identities of research participants will not be disclosed to dbGaP
- An Institutional Review Board has reviewed and verified that:
  - The submission of data to dbGaP and subsequent sharing for research purposes are consistent with the informed consent of the study participants from whom the data were obtained;

- The plan for de-identifying datasets is consistent with the standards outlined in NOT-OD-07-088;
- [Name of institution] has considered the risks to the individuals, their families, and groups or populations associated with data submitted to dbGaP and determined risks to be reasonable in relation to anticipated benefits to society; and
- The phenotype data to be submitted were collected in a manner consistent with 45 CFR Part 46. .

The suggested Acknowledgement Statement to accompany the data set is:

**Acknowledgement Statement**

This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under XXXXXXXXX.

If additional information is required, please do not hesitate to contact us.

Sincerely,

**[Need signatures from both the signing official at the PI's institution and the PI for the Project.]**

## APPENDIX B: Sample Requirements

### Burkitt Lymphoma Genome Sequencing Project Tissue Sample Requirements for Accrual

#### Tissue Requirements:

To be accepted to the project, the following conditions have to be met at the tissue level.

- Paired tumor and normal (non-involved tissue or blood) must be available in sufficient quantities (~100mg of frozen tumor tissue and 10ml of blood).
- Tissues (both normal and tumor) need to be snap frozen. Time between tissue extraction and freezing must be recorded.
- Optimal storage of the tissues is in N<sub>2</sub>(liq), but -80°C or lower is acceptable. The form of tissue storage must be recorded.
- There must be enough tissue of both to produce a 4µm thick section from the top for H&E staining, then 8-10 sections of 20µm thickness, followed by another 4µm section to stain by H&E. **The number of sections needed is based on a block with a surface area of about one square cm. If the area is smaller, proportionately more sections will be required.**
- Tumors need to have a minimum percent of tumor nuclei between 70-80% as assessed on an H&E section physically adjacent to the specimen that is candidate for generating the RNA and DNA.
- A paraffin embedded block for pathology consensus review must exist for the tumor.

## Burkitt Lymphoma Genome Sequencing Project

### Clinical Data Requirements for Accrual

#### **Clinical Data Requirements:**

To be accepted to the project, the following conditions must be met at the clinical data level. The samples must meet ALL the clinical data elements (CDEs) here listed.

These clinical data elements must be reported to the DCC as an initial report within two weeks of enrolling the patient. Updates must be sent to the DCC as the patient returns for periodic visits (ideally every 3-4 months).

- Patients need to be consented in such a way that allows for the use of their tissues for genomic-scale molecular characterization.

Data element	Data type	Example
Unique identifier	text	XX-XXXX
WHO diagnosis	text	BL
Date pathologic diagnosis	date (dd.mm.yyyy)	01.02.2003
Age at diagnosis	number	55
Gender	text	M
Race + Ethnicity (2 CDEs, CDC/Census std)		
Stage (Ann Arbor)	text	4A
Performance status (ECOG)	text	1
Lactate dehydrogenase level (LDH) (as ratio of measured value/upper limit of normal)	number	1.3
Sites of extranodal disease	text	bone marrow, lung
Maximum tumor bulk (cm)	number	8
Anatomic site of maximum tumor bulk	text	bone marrow, lung
Primary treatment	text	CHOP-R
Primary treatment start date		
Response to primary treatment	text	Complete
Date of assessment of response		
Date progressive disease, if occurred	date (dd.mm.yyyy)	11.11.2005

Primary site of progression	text	Lung
Secondary treatment	text	GDP
Secondary treatment start date		
Hematopoietic stem cell transplant (HSCT) (yes/no)	text	Yes
Date HSCT	date (dd.mm.yyyy)	03.01.2006
Malignant cells in bone marrow histology (yes/no)	text	Yes
Surface antigens, IHC, CD		
HIV status (pos/neg)	text	Negative
EBV status of malignant cells (EBER) (pos/neg)	text	Positive
Date last follow-up	date (dd.mm.yyyy)	11.11.2006
Status at last follow-up (alive/dead)	text	Alive
Number of months from diagnosis to last follow-up (living patients only)	number	
Cause of death	text	lymphoma
Date of death	date (dd.mm.yyyy)	05.24.2009

## **APPENDIX C: Checklist of Task Completion for Sample Submission**

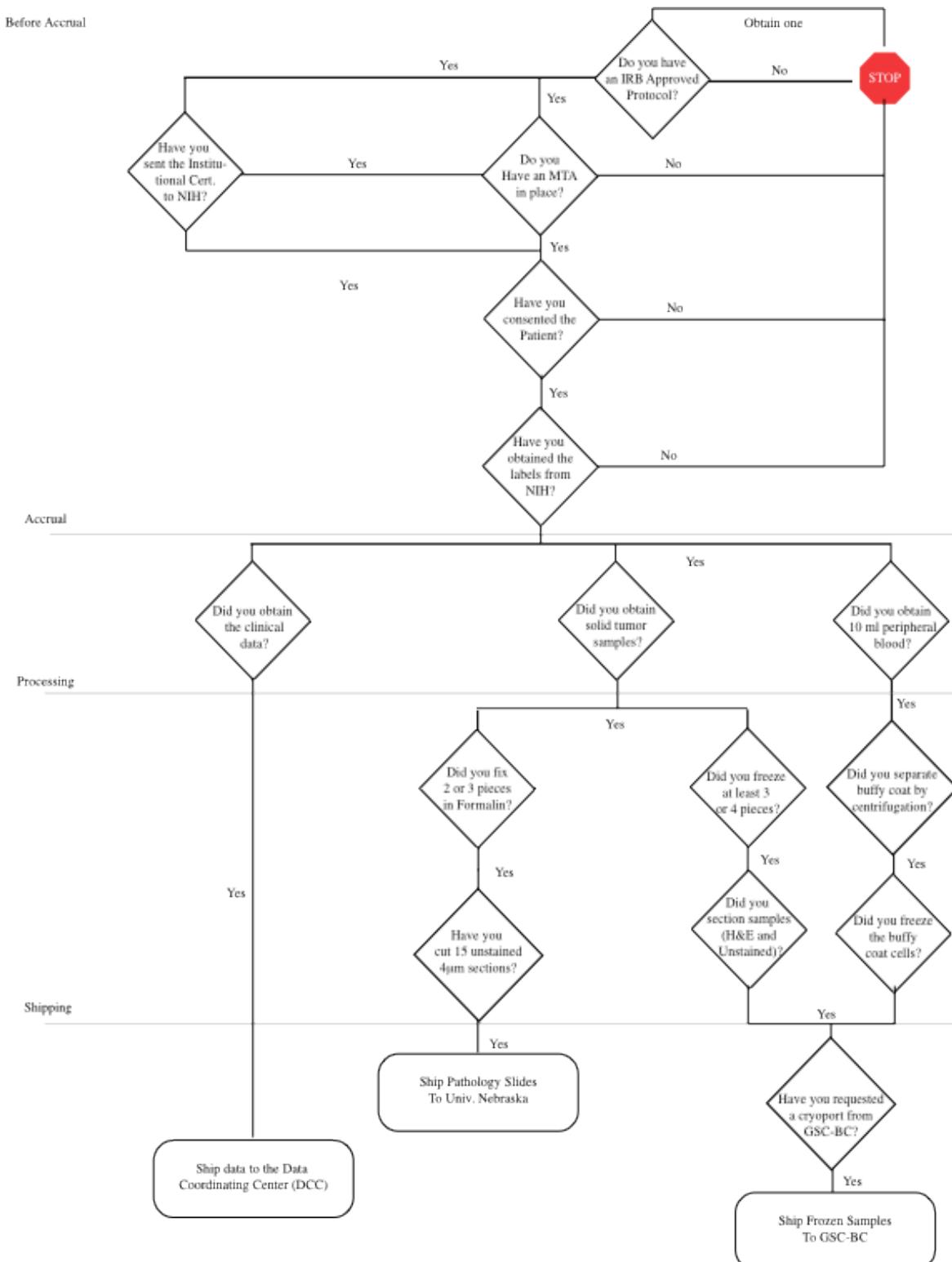
**Date:**

**Institution:**

**Operator:**

- ❖ Do you have an IRB approved protocol?
- ❖ Have you consented the patient?
- ❖ Have you sent your Institutional Certification to the Project Team?
- ❖ Have you obtained the project-assigned ID and labels from the Project Team?
- ❖ Do you have frozen sections in cryovial and H&E stained slides? Are they labeled?
- ❖ Do you have frozen blood cells? Are they correctly labeled?
- ❖ Have you secured a cryoport from the Genome Science Center at British Columbia?
- ❖ Do you have (15) unstained 4  $\mu\text{m}$  sections from the Formalin-fixed block? Are they labeled?
- ❖ Do you have the clinical data elements required by the Project? (Appendix B)

**ONLY if all the above items check out, you are ready to ship the samples.**



## BLGSP SOP#004

Adopted: \_\_\_\_\_  
 2<sup>nd</sup> Version: \_\_\_\_\_  
 3<sup>rd</sup> Version: \_\_\_\_\_  
 Reviewed: \_\_\_\_\_  
 4<sup>th</sup> Version: \_\_\_\_\_

## **PROCESSING BLOOD SAMPLES FOR THE BURKITT LYMPHOMA GENOME SEQUENCING PROJECT**

### **I. INTRODUCTION:**

The National Cancer Institute's Office of Cancer Genomics (OCG) and the Foundation for Burkitt Lymphoma Research have developed an initiative to establish a genomic databank to explore potential genetic changes in patients with Burkitt Lymphoma.

#### **A. SCOPE AND PURPOSE:**

1. To establish a common procedure for blood processing previous to shipment to the Genome Science Center at British Columbia (GSC-BC) by tissue source sites (TSS).
2. This protocol applies to all TSSs providing tissues prospectively.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times, and which samples were affected. This information should be given within 48 hours of occurrence to the project team by sending an e-mail to Dr. Robin S. Broughton ([robin.broughton@nih.gov](mailto:robin.broughton@nih.gov)) with the details.

#### **B. SAFETY PRECAUTIONS:**

1. Wear personal protective equipment (PPE) such as lab coats and gloves.

#### **C. EQUIPMENT AND MATERIALS:**

1. Personal protective equipment (PPE) to include heavy duty gloves, eye protection (preferably Face Shield), lab coat and closed-toe shoes
2. Clinical Centrifuge
3. Red Blood Cell (RBC) Lysis Buffer (0.15 M NH<sub>4</sub>Cl, 10 mM KHCO<sub>3</sub>, 0.1 mM EDTA in dH<sub>2</sub>O, 0.2 µm filtered)
4. Freezing Media (10% DMSO, 20% FCS, RPMI 1640)
5. Conical tubes (e.g. 15 and 50 ml polypropylene tubes from BD Biosciences, Part Numbers 352097 and 352098, respectively)
6. Cryovials (e.g. 2mL vials from ChartBiomed, Part Number 10778828)
7. Freezer resistant labels with project-assigned ID (obtained from Project Team, see BLGSP SOP #003)
8. Dewar thermo-flask
9. Liquid nitrogen
10. Isopentane (2 methyl butane)

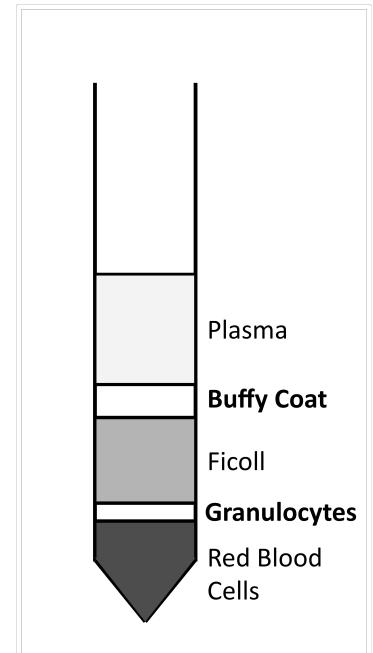
**MARK ALL CONTAINERS WITH THE LABELS CARRYING PATIENT PROJECT-ASSIGNED ID OBTAINED PRIOR TO SURGERY.**

**II. PROCEDURE:**

A. Collect 10 ml of blood in tubes containing anticoagulant (e.g. heparin (green top), EDTA (lavender top), or ACD (yellow top)). If there is indication that **tumor cells are present in blood**, fractionate the blood as soon as possible after collection (see section B). If fractionation is not needed, then red cell lysis with collection of all the nucleated calls is sufficient.

**B. Blood Separation:**

1. In a 50 ml conical tube, dilute 10 ml of the whole blood with 40 ml of buffer.
2. To a 50 ml conical tube, add 15 ml of Ficoll. Carefully layer 30 ml of the diluted blood over the Ficoll.
3. Fractionate the diluted blood by centrifuging at  $400 X g$  for 30 min at room temperature with the brake off. This will separate the blood into three prominent layers: an upper plasma layer, a middle Ficoll layer, and a lower red blood cell (RBC) layer. At the interface between the plasma layer and the Ficoll layer there will be a thin layer containing the white blood cells (WBCs) / buffy coat and between the Ficoll and RBC layer a thin interface containing the granulocytes (see Figure). **NOTE:** In a typical clinical centrifuge 1500-2000  $X g$  is  $\sim 800$  rpm. Check the appropriate settings for your centrifuge using the nomogram in your user's manual.
4. Use a disposable, plastic transfer pipet (e.g. Falcon Cat #357524) to aspirate off the plasma (upper layer) down to  $\sim 1$  mm from the buffy coat (see Figure). Discard the plasma into a container with bleach When removing the plasma do not disturb the WBC layer, also called the buffy coat, which forms a thin film between the upper plasma layer and the lower layer of packed RBCs. Samples with exceptionally high WBC counts will have a thicker buffy coat.
5. Recover the WBCs in  $\leq 0.5$  ml with a 1 ml pipetman. Do not take too much Ficoll (the layer below the buffy coat), as it is toxic to cells. Estimate the volume recovered and write the information into the datasheet.
6. Place the recovered buffy coat into a freezer-resistant labeled cryovial cooled on ice. Labels are obtained prior to surgery from the Project Team (BLGSP SOP #003). Screw on the cap **tightly** to prevent isopentane from seeping into the vial during freezing as it creates a liquid in the vial upon thawing.



## BLGSP SOP#004

7. Aspirate off the Ficoll layer carefully into an Erlenmeyer flask with bleach (see Figure) taking care not to disturb the granulocyte layer between the Ficoll layer and the pelleted RBCs. The granulocytes sit on the surface of the RBCs and are visible as a white haze. Discard the Ficoll.
8. Recover the granulocytes, usually the volume is  $\sim \leq 0.5$  ml, using a 1 ml pipetman. Place cells into a labeled 15 ml conical tube.
9. Add 1 ml of the RBC Lysis Buffer to the granulocytes and incubate at room temperature for 5-10 min.
10. Centrifuge the granulocytes at 300 X g for 10 min at room temperature with the brake on.
11. Aspirate and discard the supernatant ( $\sim 1.5$  ml) and re-suspend the granulocyte cell pellet in 0.5 ml of Freezing Media by gently pipetting up and down.
12. Place the recovered granulocytes into a cooled freezer-resistant labeled cryovial. Labels can be obtained from the Project Team (BLGSP SOP #003). Screw on the cap **tightly** to prevent isopentane from seeping into the vial during freezing.

**C. Set Up Freezing Station**

1. Fill a small 100 ml metal beaker about 1/4 full with isopentane (2-methylbutane, certified grade).
2. Fill the Dewar thermo-flask about 1/3 full with liquid nitrogen.
3. Use extreme caution when dispensing liquid nitrogen.

**D. Freezing Blood Cells in Cryovials**

1. Using long forceps lower the 100 ml metal beaker containing isopentane half-way into the liquid nitrogen for cooling. The liquid nitrogen will boil as the beaker is lowered, when the isopentane is reaching its freezing point the tone of the boiling will increase for 2-3 seconds.
  2. Lift the beaker out of the liquid nitrogen once you see beads of solid isopentane at the bottom of the beaker (about 2 minutes).
  3. Use long forceps to hold the cryovial down into the cooled isopentane. Submerge cryovial for at least 1 minute.
  4. Take out the cryovial containing frozen tissue.
  5. Store frozen tissue vial(s) in liquid nitrogen storage tanks or -80°C Freezers.
- E. Any questions regarding this protocol should be directed to the BL Pathology Coordinators at 402-559-7689 or 402-559-7526.

**THE FROZEN SPECIMENS SHOULD BE KEPT FROZEN ON DRY ICE AT ALL TIMES DURING TRANSPORT TO AND FROM STORAGE TANKS.**

**CONTACTS:****BLGSP Pathology Coordinators:**

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Adopted: \_\_\_\_\_  
2<sup>nd</sup> Version: \_\_\_\_\_  
3<sup>rd</sup> Version: \_\_\_\_\_  
Reviewed: \_\_\_\_\_  
4<sup>th</sup> Version: \_\_\_\_\_

## SAMPLE SHIPPING GUIDELINES FOR THE BURKITT LYMPHOMA GENOME SEQUENCING PROJECT

### I. INTRODUCTION:

Tumor samples from Burkitt Lymphoma patients are rare and they may be accrued at specific tumor source sites (TSS) at a rate of 3-5 per calendar year. Some tumor samples may also be HIV-infected. Shipping costs for infectious labeled material in vapor phase liquid nitrogen containers (cryoports) are expensive.

#### A. SCOPE AND PURPOSE:

1. To establish a sample shipping guideline standard to be applied to all samples contributed to the Burkitt Lymphoma Genome Sequencing Project (BLGSP) that balances the need for expeditious transport while maintaining cost efficiency.
2. This procedure applies to all TSSs.

### II. ADOPTED STANDARD:

- Immediate requests for a cryoport will be made to The Research Institute at Nationwide Children's Hospital (NCH) coordinator when the contributing TSS has in its possession 3 or more matched tumor-normal tissues.
- However, if less than three cases are accrued, and the date of oldest sample resection is more than 4 months, shipment of this/these sample(s) is warranted.

Any questions regarding this protocol should be directed to Dr. Robin S. Broughton at 301-451-3860.

## **CONTACTS:**

### **Project Team Representative:**

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Fax: 301-480-4368  
E-mail: [robin.broughton@nih.gov](mailto:robin.broughton@nih.gov)

### **NCH Coordinator:**

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E-mail: [Hollie.Harper@nationwidechildrens.org](mailto:Hollie.Harper@nationwidechildrens.org)

Adopted: \_\_\_\_\_  
2<sup>nd</sup> Version: \_\_\_\_\_  
3<sup>rd</sup> Version: \_\_\_\_\_  
Reviewed: \_\_\_\_\_  
4<sup>th</sup> Version: \_\_\_\_\_

## **SHIPPING CRYOPORTS CONTAINING FROZEN BIOSAMPLES FOR PROCESSING AND EXTRACTION OF NUCLEIC ACIDS**

### **I. INTRODUCTION:**

Cryoports are shipped from The Research Institute at Nationwide Children's Hospital (NCH) to the Tissue Source Site (TSS). TSSs are instructed to use this SOP when shipping samples to NCH.

#### **A. SCOPE AND PURPOSE:**

1. To establish a procedure for personnel in shipping the cryoports.
2. This procedure applies to all laboratory personnel.
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times and samples affected. This information should be given immediately to the project team by sending an e-mail to Dr. Robin S. Broughton (**robin.broughton@nih.gov**) with the details.

#### **B. SAFETY PRECAUTIONS:**

1. Wear personal protective equipment (PPE) such as lab coats and gloves.
2. Liquid nitrogen is extremely cold and can cause 'burns'. Wear gloves that are specially made to withstand liquid nitrogen, eye protection and a lab coat to protect skin from splashes and spills. Liquid nitrogen is an asphyxiant; be sure to use in a well-ventilated area.
3. Always keep the cryoport in the upright position.

#### **C. EQUIPMENT AND MATERIALS:**

1. Cryoport, obtained in 3 or 4 days in advance from the NCH Coordinator (see below).
2. Personal protective equipment (PPE) to include heavy duty gloves, eye protection (preferably Face Shield), lab coat and closed-toe shoes
3. Shipping documents

## II. PROCEDURE:

1. Request cryoport from NCH shipping coordinator 3-4 days in advance of sectioning samples.
2. Complete the appropriate shipping forms needed for the sample(s).
3. Complete the sample shipping document with the project-assigned ID obtained prior to surgery, the sample type information and any comments. Sign and date the form and have a second individual verify the contents of the shipment and sign and date the form.
4. Don personal protection equipment.
5. Open the cryoport shipping vessel and remove the temperature probe that has been wrapped in bubble wrap and placed between the cryoport and the outside shipping vessel. Lift the cryoport out of the shipping vessel to access the data logger which has also been wrapped in bubble wrap and placed between the cryoport and the shipping vessel.
6. Open cryoport lid carefully.
7. Take the temperature of the cryoport prior to placing the samples in the cryoport.
  - a. Turn the On/Off switch on the digital thermometer to the “On” position.
  - b. Press the Celsius/Fahrenheit to read “C” in the upper right corner of the temperature screen.
  - c. Place the temperature probe into the cryoport for a minimum of five minutes.
  - d. After five minutes, record the temperature of the cryoport on the Cryoport Temperature Log that is enclosed in the plastic tie envelope.
  - e. If the temperature is -170°C or colder, it can be used to ship the samples to NCH. Place your samples in the cryoport and place the lid on the shipper and use one of the plastic ties to secure the lid. **ALERT:** If the temperature is warmer than -170°C, please contact the NCH coordinator for instructions.
  - f. Wrap the data logger and temperature probe and return all items to the shipping vessel in reverse order as listed above.
8. Carefully close the lid. Affix a plastic zip tie through the loop of the lid and the loop on the cryoport.
9. Place all shipping documents, including the Sample Shipping Document and the Cryoport Temperature Log, into the plastic sleeve.
10. Notify the shipping carrier for pick-up. Under normal conditions, shipments should only be sent to NCH on Monday through Wednesday. If an exception is needed, NCH must be contacted at 614-355-2901 for further instructions and to alert the appropriate NCH personnel of any schedule changes.
11. Attach the enclosed Federal Express shipping label to the handle of the outside shipping vessel and use the other enclosed plastic tie to secure the outside lock before returning the cryoport to NCH.
12. TSS personnel will notify the coordinator by email stating the cryoport is being returned with tissue samples back to NCH.
13. The NCH Coordinator will track the cryoport in transit.

14. If there are any exceptions to the normal shipping schedule or in the event of an anticipated shipment delay, the Coordinator will notify the NCH on-call personnel of the potential arrival of samples after normal working hours or on the weekend.
15. Upon receiving the cryoport, the temperature will be recorded and quality control verified by a second individual.
16. Any questions regarding shipments to NCH should be directed to the NCH Coordinator at 614-355-2901.

## **CONTACTS:**

### **NCH Coordinator:**

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## BLGSP SOP #007

Adopted: \_\_\_\_\_  
2<sup>nd</sup> Version: \_\_\_\_\_  
3<sup>rd</sup> Version: \_\_\_\_\_  
Reviewed: \_\_\_\_\_  
4<sup>th</sup> Version: \_\_\_\_\_

## SAMPLE IDENTIFIER STANDARDS FOR THE BURKITT LYMPHOMA GENOME SEQUENCING PROJECT

### I. INTRODUCTION:

To assure the privacy of all human subjects that have consented to donate their tissues and clinical data to the Burkitt Lymphoma Genome Sequencing Project (BLGSP), all the materials given to the project must be de-identified prior to shipment and study. This project-assigned ID must have a rational structure that permits tracking of which subproject, tissue source site (TSS) and case is labeled.

#### A. SCOPE AND PURPOSE:

1. To establish a sample identifying standard to be applied to all samples and data contributed to the BLGSP.
2. This procedure applies to all laboratory personnel.

### II. ADOPTED STANDARD:

- Samples contributed to the BLGSP must be labeled with an ID obtained from the Data Coordinating Center (DCC) by the TSS previous to shipment.
- These code must have the following form:

BLGSP - 71 - ##### - ## - ##X

Where:

1. BLGSP stands for Burkitt Lymphoma Genome Sequencing Project
2. 71 is the tumor code for Non-Hodgkin's lymphoma, Burkitt lymphoma
3. The next five digits are the sample identifier
4. The next two digits specify the tissue code (see list on next page)
5. The final three characters denote the nucleic acid code (see list on next page)

Any questions regarding this protocol should be directed to the Project Team (PT) representative Dr. Robin S. Broughton at 301-451-3860.

## BLGSP SOP #007

**Tissue Codes:**

Sample Code	Description	Code
Primary Tumor	Primary Tumor	01
Recurrent Tumor	Recurrent Tumor	02
Primary Blood Cancer	Primary Blood Derived Cancer – Peripheral blood	03
Recurrent Blood Cancer	Blood derived Tumor Relapse - Bone Marrow	04
Addtl - New Primary	Additional - New Primary	05
Human Tumor Original	Human Tumor Original Cells	08
Primary Bld Cancer BM	Primary Blood Derived Cancer – Bone Marrow	09
Blood Derived Normal	Blood Derived Normal	10
Solid Tissue Normal	Solid Tissue Normal	11
Buccal Cell Normal	Buccal Cell Normal	12
EBV Normal	EBV Immortalized Normal	13
BM Normal	Bone Marrow Normal	14
Recurrent Blood Cancer	Blood derived Tumor Relapse – Peripheral blood	40

**Nuclei acid codes:**

- 01D=DNA, unamplified, from the first isolation of a tissue
- 01W=DNA, WGA'ed by Qiagen (1 of the 2 done)
- 01X=DNA, WGA'ed by Qiagen (2 of the 2 done)
- 01R=RNA

Note: If additional isolations are needed, the # would change to 02D, etc.

Adopted: \_\_\_\_\_  
 2° Version: \_\_\_\_\_  
 3° Version: \_\_\_\_\_  
 4° Version: \_\_\_\_\_  
 Under Revision: \_\_\_\_\_

**VERY USEFUL INSTRUCTIONS ON HOW TO COMPLETE A STUDY PROTOCOL  
REQUEST TO THE INSTITUTIONAL REVIEW BOARD (IRB)  
FOR THE BURKITT LYMPHOMA GENOME SEQUENCING PROJECT**

**INTRODUCTION:**

The Burkitt Lymphoma Genome Sequencing Project's (BLGSP) goal is to develop a genomic databank of the molecular changes in Burkitt Lymphoma that will be available to the research community world-wide. It will allow the comparison between the cancer-related alterations in patients with Burkitt Lymphoma and those without. The project aims to generate large scale, high-quality data on the cancers' genomes and transcriptomes using 2<sup>nd</sup> and 3<sup>rd</sup> generation sequencing technology which means that the changes identified will include large genomic rearrangements, expression profile changes and detection of mutations.

In order for cases to be included in the project, the patients must provide consent of participation in an approved IRB protocol specifying that the samples can be used for genomic characterization and that the data deposited in a publicly available, yet patient privacy designed database. The Office of Cancer Genomics (OCG) of the National Cancer Institute has created a generic template that contains the appropriate language to help the Tissue Source Site (TSS) in producing the IRB document. This template lacks details that are Institution-specific and should not be considered complete.

**SCOPE AND PURPOSE:**

- To establish a set of instructions allowing each TSS to create their own IRB protocol to contribute samples to the BLGSP.
- These instructions should be useful to every TSS contributing samples to the BLGSP.

**INSTRUCTIONS:**

1. Obtain the IRB protocol template from either the SOP package sent when you agreed to participate in the BLGSP or the SharePoint site ([https://ocg-sps.nci.nih.gov/Burkitt\\_Lymphoma/default.aspx](https://ocg-sps.nci.nih.gov/Burkitt_Lymphoma/default.aspx)). You may also request a copy from the project team (see address below).
2. Fill in your organization name, PI's name and other pertinent information in the form. The Project name should be "Burkitt Lymphoma Genome Sequencing Project" and its acronym is BLGSP.

## BLGSP SOP #008

3. The project rationale can be found in the introductory section above.
4. The total number of samples that will be analyzed is 120.
5. Details on amount of tissue requested is in BLGSP SOP #003 under the sample requirement section (page 8)
6. Details on the blood collection for germline DNA extraction can be found in BLGSP SOP #004.
7. All the operational details of the project are clearly specified in the SOPs sent to the TSSs. It is expected that all participating personnel will read the SOPs, be familiar with the project procedures and requirements and follow them in all instances.

**Should you have any question please contact Dr. Broughton at 301-451-3860**

**Project Team Representative:**

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## BLGSP SOP #009

Adopted: \_\_\_\_\_  
 2<sup>nd</sup> Version: \_\_\_\_\_  
 3<sup>rd</sup> Version: \_\_\_\_\_  
 Reviewed: \_\_\_\_\_  
 4<sup>th</sup> Version: \_\_\_\_\_

## **CENTRALIZED PATHOLOGY REVIEW PROCESS FOR THE BURKITT LYMPHOMA GENOME SEQUENCING PROJECT**

### INTRODUCTION:

Pathological diagnosis of tumor can be impacted by the subjective nature of the process as well as the subjective definition of the criteria used in the assessment. To ensure that samples meet the tissue requirements for the Burkitt Lymphoma Genome Sequencing Project (BLGSP) and are Burkitt Lymphoma, a Pathology Review Committee (PRC) of three board-certified pathologists is established. The review of tissues by a group minimizes the subjectivity that is unavoidable in pathology reviews and allows an efficient resolution of discrepancies.

#### **A. SCOPE AND PURPOSE:**

1. To establish a standard procedure for the centralized pathology review of tissue submitted to the BLGSP.

#### **B. EQUIPMENT AND MATERIALS:**

1. De-identified pathology report provided by the tissue source site (TSS) contributing the sample.
2. One hematoxylin & eosin (H&E) and a minimum of 15 unstained 4  $\mu\text{m}$  thick sections from the formalin-fixed paraffin-embedded (FFPE) diagnostic block OR a diagnostic block and a top and bottom section from the frozen tissue (for estimating % nuclei in the tissue processed for nucleic acid extraction to be done by the Pathology Coordinator). These sections will be provided by the tissue source site (TSS) contributing the case and should be labeled with the project-assigned ID (as specified in BLGSP SOP #007).
3. Bioimagine or Aperio Slide Scanner

## **II. PROCEDURE:**

#### **A. Preparation for review:**

1. Immediately upon arrival to the Pathology Review Lab (PRL), the Pathology Coordinator will verify that all slides and reports submitted are labeled with the same project-assigned ID for each case.
  - The report will be scanned and uploaded to the PathXchange website (<http://www.pathxchange.org>).

If only slides are received, the Pathology Coordinator will send the appropriate number of slides for H&E, immunohistochemical (IHC) studies, and fluorescence *in*

*situ* hybridization (FISH). If the paraffin block is received, an H&E stained section will be prepared to identify the distribution of the tumor in the block and slides will be prepared for IHC analysis. The Pathology Coordinator will select an appropriate area in the block for the tissue microarray (TMA), circle the relevant area on the H&E stained slide, and submit the block to the core laboratory for preparation of the TMA. A full TMA will be constructed once blocks from 30 cases have been received or every 3 months.

**NOTE:** Performing FISH analysis on slides is suboptimal, thus diagnostic blocks are highly preferred.

#### Immunohistochemical analysis

- IHC to be performed include:  
**CD20, CD3, CD10, BCL2, BCL6, Ki67 and MYC**

#### FISH analysis

- FISH analysis will be performed on individual slides and TMAs for all cases to determine the presence of **MYC** to immunoglobulin locus translocation.
- FISH analysis for BCL2 and BCL6 rearrangement will be performed on the subset of cases in which BCL2 is found to be expressed by IHC, or in which atypical features raise the option of differential diagnosis of diffuse large B-cell lymphoma.
- It is estimated that a complete FISH panel (MYC, BCL2, BCL6) will be performed on approximately 10% of submitted cases.

**Note:** Initial sample processing, H&E and IHC analysis should take no longer than 5 days, using either submitted unstained slides or a paraffin block (which will be cut by the reference laboratory). FISH analysis, when performed on individual slides or after a sufficient number of cases have accrued for TMA construction, will take approximately 7-14 days to complete.

2. Once all processing is completed, the Pathology Coordinator will:
  - scan the H&E and IHC slides on the Bioimagene system;
  - deposit images of the slides and a blank review form in the PathXchange website (<http://www.pathxchange.org>);
  - deposit an official report of the FISH result in the PathXchange website (<http://www.pathxchange.org>)
3. The Pathology Coordinator will send an e-mail to members of the PRC informing them that materials for review have been deposited in a folder. This communication must specify the number and name of files, as well as the project-assigned ID for the case(s) under review. This deposition and communication must occur within 48 hours of scanning the slides by the Pathology Coordinator.

#### B. Review:

1. Within three days of receipt of the e-mail from the Pathology Coordinator, all members of the PRC will return their pathology review form to the Pathology Coordinator via e-mail.

## BLGSP SOP #009

2. If consensus is reached and the case passes the specified criteria, the Pathology Coordinator will create a final pathology report and submit it to the Data Coordinating Center and the Genome Science Center at British Columbia so sequencing on those cases can begin.
3. Cases considered inadequate for diagnosis will be labeled as such and taken out of the study.
4. Discrepant cases will be submitted for web-based review, to be convened by Dr. John Chan. The schedule of such consensus reviews will be dictated by the discordant cases accrued as follows:
  - When 6 or more discordant cases have been accrued, a consensus review panel must be convened.
  - If there are less than 6 discordant cases, but the oldest accrued case is more than six months old, a consensus review panel must be convened.

Any questions regarding this protocol should be directed to BLGSP Pathology Coordinator at 402-559-7689 or 402-559-7526.

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BLGSP SOP #009

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## BLGSP SOP #010

Adopted: \_\_\_\_\_  
 2<sup>nd</sup> Version: \_\_\_\_\_  
 3<sup>rd</sup> Version: \_\_\_\_\_  
 Reviewed: \_\_\_\_\_  
 4<sup>th</sup> Version: \_\_\_\_\_

## **HEMATOXYLIN & EOSIN PROTOCOL TO DETERMINE THE DIAGNOSIS OF BURKITT LYMPHOMA FROM FORMALIN FIXED PARAFFIN EMBEDDED TISSUES**

### I. INTRODUCTION:

An essential component in the pathological diagnosis of Burkitt Lymphoma is the classical “starry sky” appearance of tumor tissue stained with hematoxylin & eosin (H&E) visualized under low-power microscopy. A hallmark of Burkitt Lymphoma is the increased mitotic rate of B cells accompanied by increased cell death. The dead cells are ingested by macrophages leaving an empty space in the tumor tissue, which appears as a white spot on a black background giving the starry sky pattern. To ensure that samples meet this basic tissue requirement for the Burkitt Lymphoma Genome Sequencing Project (BLGSP), a standard protocol for H&E staining has been established.

### A. SCOPE AND PURPOSE:

1. To establish a standard procedure for H&E staining of tissue submitted to the BLGSP in order to determine that samples meet basic tissue requirements for the BLGSP.

### B. SAFETY PRECAUTIONS:

1. Wear personal protective equipment (PPE) including chemical splash goggles, gloves and protective clothing (e.g. impervious lab coat, long sleeves, closed-toed footwear).
2. Always use the rack handles to submerge racks into the series of chambers containing xylene, ethanol, and aqueous solutions as gloves are not suitable for immersion protection, only splash protection.

### C. HAZARDOUS MATERIALS:

<b>Materials</b>	<b>Corrosive</b>	<b>Irritant</b>	<b>Flammable</b>	<b>Other Comments:</b>
Xylene		X	X	See comment 1, below.
Ethanol (100, 95, 80%)		X	X	See comment 2, below.
Hematoxylin	X	X		See comment 3, below.
Eosin		X		
Mounting Medium		X	X	See comment 4, below.

**Other comments:**

1. May affect central nervous system and may be narcotic at high concentration. Keep away from heat, sparks, and open flame.
2. May affect central nervous system.
3. May cause kidney damage. May cause central nervous system effects.
4. Possible risk of harm to unborn child. May cause central nervous system depression. Aspiration hazard if swallowed.

**D. EQUIPMENT AND REAGENTS:**

**PLEASE NOTE:** The vendors/part numbers listed below for each item are only suggestions and primarily intended to provide examples of the needed items. It is permissible to order from another vendor as long as the correct item is purchased.

1. 8 glass staining dishes (e.g. Fisherbrand Glass Staining Dish, Part Number 08-810)
2. 2 slide racks (e.g. Wheaton Science Products, Part Number 900204)
3. Laboratory wipes (e.g. Kimwipes, Part Number 34155)
4. Glass coverslips (e.g. Fisherbrand Cover Glasses, Part Number 12-544E)
5. Xylene (e.g. Sigma-Aldrich histological grade, Part Number 534056)
6. Ethanol
7. Hematoxylin, Mayer's (e.g. Sigma Hematoxylin Solution, Mayer's, Part Number MHS16)
8. Eosin (e.g. Sigma-Aldrich Eosin Y solution, aqueous, Part Number HT110216)
9. Histological mounting medium (e.g. Sigma-Aldrich, Canada balsam Mounting medium for microscopy, Part Number C1795)
10. Standard light microscope (e.g. Olympus IX71 Inverted Microscope)

**II. PROCEDURE:**

1. Place slide(s) containing paraffin sections into slide rack.
2. Deparaffinize and rehydrate sections according to the following:
  - Xylene                    3 minutes, 3 times      →      Submerge slide(s) (in slide rack) into a staining dish containing xylene **3 times for 3 minutes** each time
3. Use a laboratory wipe to gently blot excess xylene from slide rack before submerging slides (in slide rack) in ethanol according to the following:
  - 100% ethanol            3 minutes, 3 times
  - 95% ethanol             3 minutes, 1 time
  - 80% ethanol             3 minutes, 1 time
  - deionized H<sub>2</sub>O        5 minutes, 1 time
4. Blot excess water from the slide rack before staining with hematoxylin according to the following:

- Hematoxylin            1 minute, 1 time
  - deionized water      1 minute, 1 time
5. Blot excess water from the slide rack before staining with eosin according to the following:
- Eosin                30 - 45 seconds, 1 time
  - 95% ethanol          1 minute, 2 times
  - 100% ethanol        1 minute, 2 times
6. Blot excess ethanol before placing slide rack into a staining dish containing xylene.
- Xylene                2 minutes, 2 times
7. Remove slide(s) from slide rack, blot excess xylene from slide(s) using a laboratory wipe, and then overlay the tissue on the slide(s) with 2-3 drops of mounting media taking care to avoid bubbles.
8. Angle the coverslip and let it fall gently onto the slide. Allow the mounting media to spread beneath the coverslip, covering all of the tissue.  
*NOTE:* If air bubbles do occur, squeeze them out by applying light pressure with forceps to the coverslip and draw the bubbles to the end of the slide.
9. Dry in a fume hood overnight.
10. Visualize slides under low power (20x or 40x) using a standard light microscope.

## CONTACTS:

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