C-CFR Biospecimen Repository
Core Standards for the Processing and Storage of Samples
SOP Executive Summary

The Colon CFR (C-CFR) biospecimen repository is comprised of samples derived from blood, archival tissues, and fresh tissues that are stored at all six collaborative sites. The C-CFR established a minimum standard for the collection, processing, storage and dispatch of these specimens in 1998 when recruitment first began. Over time this standard has been refined to accommodate different funding levels, changes to recruitment focus, sample demands on the biorepository, technology improvements, and to capitalize on site-specific strengths.

The core standard operating procedures for blood processing and tissue handling are summarized below. Procedural differences between sites are identified, and addressed individually.

Detailed site-specific SOPs for sample collection, processing, and storage are available for download from the NCI website.

1. Blood Processing

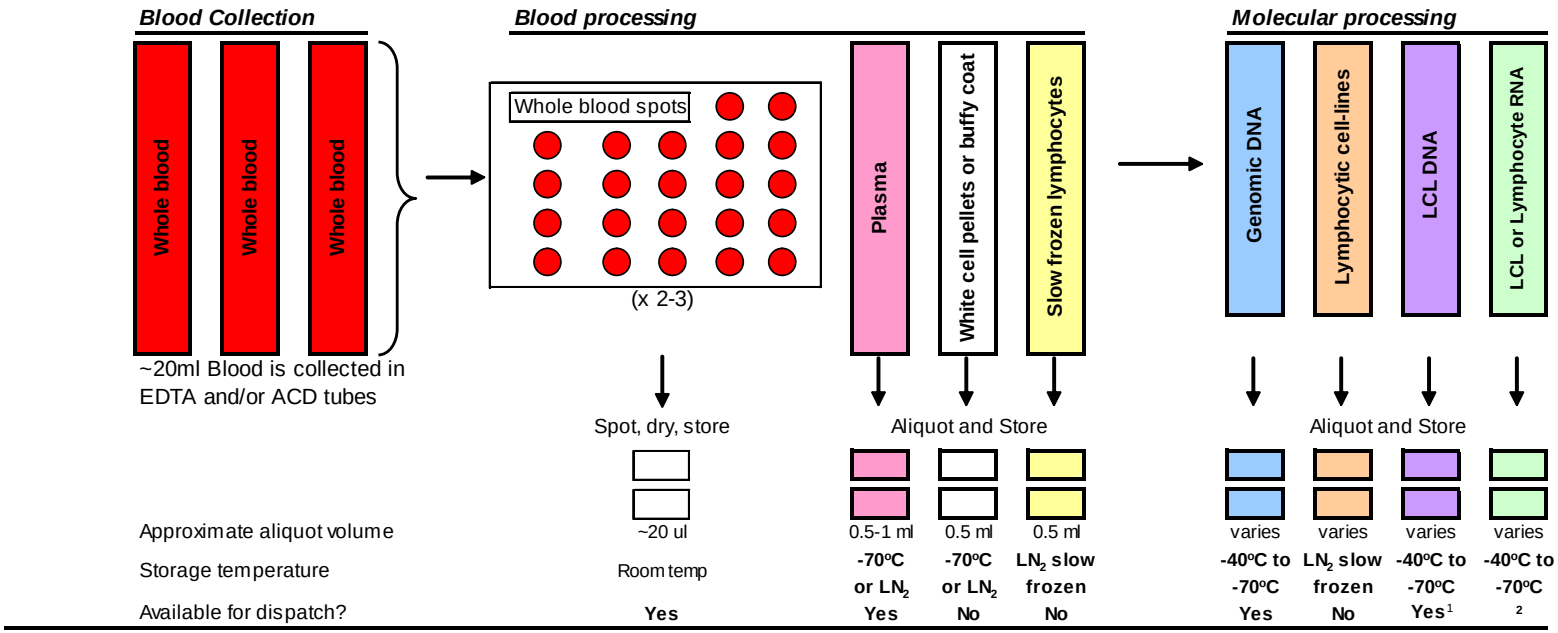
The core SOP for blood processing and storage of blood products is given in Figure 1. All sites are in compliance with minimum core standards.

Notes:

- **Blood tube collection.**
  - EBV transformation was not supported in the second phase of funding, thus the number of ACD tubes required was reduced from 2 in Phase I, to a single ACD tube in Phase II. Australia and Hawaii elected to continue collecting 2 ACD tubes during Phase II, while Ontario chose to collect 40mls of ACD and eliminate EDTA collection. These deviations from core SOP exceed minimum requirements, and provide all required blood products.
  - FHCRC did not collect ACD tubes from Phase I controls until 2002 in order to obtain blood samples on more controls than originally funded (800 vs. 250). At the time, the CFR had excluded controls from plans for transformation.
- **Blood Spots.**
  - Two sites, Australia and Hawaii exceed the minimum requirement of 1ml in 44 spots (1.5mls in 66spots, and 1.4ml in 45 spots respectively).
  - Ontario has changed protocol to spotting 0.480ml in 4 large spots in response to changes in the availability and format of Guthrie cards. To date there has been very little demand for this product outside internal QC procedures; therefore, this new format will likely continue to exceed demand.

- **Plasma.**
    - The total amount of plasma collected varies from site to site in accordance with the amount of EDTA blood that is drawn, however all sites store at least 3mls of plasma.
    - Minimum volume for each aliquot is 0.5ml, however the number and volume of aliquots stored varies depending upon storage space available at each site.
- **White Cell Pellets**
    - Phase I funding supported the extraction of DNA from white cell pellets, thus the DNA product forms the standard requirement. However, in addition to the DNA product, many sites also store extra white cell pellets as a backup for future DNA extraction.
    - Phase II funding does not provide for DNA extraction. Despite this many sites continue to extract DNA, so the core requirement allows for storage of white cell pellets, of extracted DNA, or a combination of both.
- **Slow Frozen Lymphocytes**
    - The amount of slow frozen lymphocytes varies dependent upon the amount of ACD blood that is processed at each site, however at least 2 aliquots of viable cells are stored for most collections.
    - As described above, FHCRC did not collect ACD tubes from Phase I controls until 2002 in order to bleed more controls than funded (800 vs. 250). At the time, the CFR had excluded controls from plans for transformation.
    - The volume of each aliquot varies dependent on available storage space.
- **Genomic DNA**
    - Extraction method and amount of starting material extracted varies between sites. However all resulting DNA meets QC standards.
    - See section 4. 'Quality of C-CFR DNA' for details on the performance of the C-CFR DNA samples.
- **Lymphoblastic Cell-lines**
    - Cell-lines were established for Phase I probands with positive first degree relative family history, and some selected controls. Phase II does not support EBV transformations.
    - Coriel has established, expanded and stored cell-lines for Seattle, Hawaii and USC; while Mayo, Ontario and Australia utilized local facilities for the establishment and storage of their LCLs. Mayo has subsequently transferred their cell-line repository to Coriel as a move towards centralization of this resource.
    - All cell-lines, regardless of where they were established, meet QC standards. QC checks include testing for mycoplasma, microbial contamination, and viability. Coriell has performed QC testing on a random sample of cell-lines from across sites to ensure consistency.
- **Lymphoblastic Cell-line DNA**
    - Amount of DNA available and number of aliquots stored varies dependent on the amount of starting material extracted. No minimum requirement is set as this is a renewable resource which can be regenerated at any time.
- **RNA**
    - RNA is not a requirement of the core repository, and is not routinely extracted. However, it can be isolated from either slow-frozen lymphocytes or LCLs.

Figure 1. Colorectal Cancer Family Registry (C-CFR) Blood Collection, Processing, and Storage Flow Diagram



Site-specific collection, processing, aliquotting:		Total blood; # spots	Number x volume of aliquots stored			Number x volume of aliquots stored			
CFR min. std.	15 ml EDTA; 14 Ph-I/7 Ph-II ml ACD	1 ml; 44 spots	7ml	WBC/DNA[3]	4x0.5ml	--	varies	varies	n/a
Hawaii	20 ml EDTA; 16 ml ACD	1.4 ml; 45 spots	16x0.5ml	4x1.0ml[3]	4x1.0ml	varies	varies	varies	n/a
Mayo	15 ml EDTA; 8.5 ml ACD or heparin	1 ml; 44 spots	6x1.0ml	DNA[3]	3x0.5ml	1xvaries	varies	ND	n/a
FHCRC	20 ml EDTA; 8.5 ml ACD[4]	1 ml; 44 spots	14x0.5ml	4x0.5ml[3]	4x0.5ml	varies	varies	varies	n/a
USC	30 ml EDTA; 8.5 ml ACD	1ml; 44 spots	24x0.5ml	3x1.5ml[3]	8x0.5ml	varies	varies	varies	n/a
Australia	18 ml EDTA; 18 ml ACD	1.5 ml; 66 spots	4 x 1.0ml	1x0.5ml[5]	4x0.5ml	[6]	4x1ml	[6]	n/a
Ontario	0 ml EDTA; 40 ml ACD	0.480 ml, 4 large spots	3 x 1.0ml	4 pellets[3]	2x1.0ml	varies	4X1ml	varies	n/a

Note:
ND - not done to date; n/a - not applicable (not core); LN_2 - liquid nitrogen/cryofreezer

[1] If selected for EBV transformation (Phase I probands with positive FDR family history, possibly others)

[2] RNA is not routinely isolated, but could possibly be provided upon special request.

[3] The C-CFR standard was to obtain genomic DNA. Sites extracted DNA from whole blood or isolated buffy coat, white cell pellets, or granulocytes for DNA. All resulting DNA meets QC standards.

[4] FHCRC did not collect ACD tubes from Phase I controls until 2002 in order to bleed more controls than funded (800 vs. 250). At the time, the CFR had excluded controls from plans for transformation.

[5] Australia stores one 0.5 ml aliquot of granulocytes for future DNA extraction and uses the entire buffy coat for DNA at processing.

[6] 2 concentrations are stored: 1x0.5ml stock DNA and 1x0.5 dilute (50ng/ul)

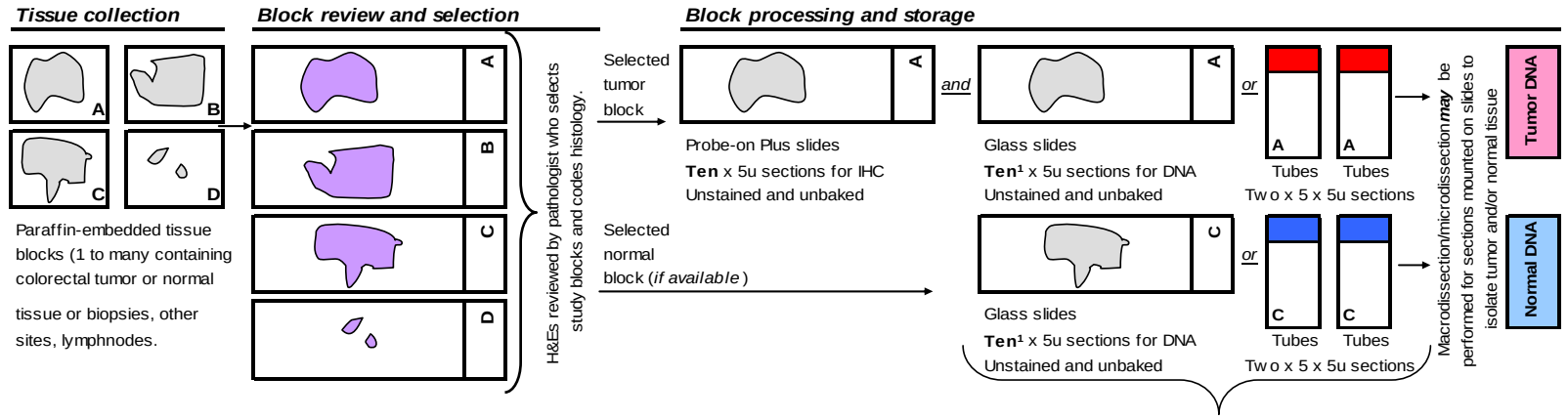
2. Archival Tissue Processing

The C-CFR accessions blocks from a wide variety f tissues, including tumors, normal mucosa, lymph nodes, biopsies, adenomas and polyps.
The core SOP for processing and storage of archival tissue products is given in Figure 2.

Notes:

- **Archival Blocks**
  - The core SOP does not require storage of blocks. Many blocks are accessioned for sectioning from pathology services, and must be returned to the source lab.
  - Some sites are able to retain blocks, such as Hawaii, while others are able to generate a research block from the accessioned material. These blocks are a resource for generating more sectioned material, but are not available for dispatch.
- **H&E slides**
  - One H&E slide is required per block; many sites exceed this. The H&E slide is currently available to researchers only on the basis that it is returned. The C-CFR is currently planning to scan these slides as part of a virtual tissue repository whereby researchers will have remote access to the scanned H&E image.
- **Charged Slides**
  - Core SOP minimum initially required three charged slides. These slides were not available for dispatch, but used for MLH1, MSH2 and MSH6 immunohistochemistry (IHC). A decision was later made to include PMS2 in the IHC panel, thus the minimum requirement was raised to 4.
  - Most sites exceed SOP requirements, and have charged slides available for dispatch.
- **Plain glass slides**
  - Initial core requirements were for 10 micron sections (n=10) and 4-5 micron sections (n=10) to be collected.
  - As demand for tissue increased, the amount of material available for DNA extraction was increased. This extra material was able to be stored as glass slides, or as sections in tubes at the discretion of the site as space and resources allowed.
- **Tissue micro-array**
  - USC is now producing tissue microarrays for archival blocks in addition to core SOP requirements for tissue storage.

Figure 2. Colorectal Cancer Family Registry (C-CFR) Tissue Collection, Processing, and Storage Flow Diagram



Purpose	Haematoxylin and eosin (H&E) stain	Immunohistochemistry	DNA extraction	DNA
Available for dispatch?	Yes, for borrow	Limited source	Yes	Yes

Site-specific collection, processing, aliquotting (when adequate tissue is available):

	Haematoxylin and eosin (H&E) stain	Immunohistochemistry	DNA extraction	DNA?
CFR min. std.	1 H&E per block	Ten 5u sections on Probe-on+ slides	Twenty (total) 5u sections on slides *or four x 5 5u sections in tubes*	Yes
Hawaii	2 H&Es per block	Ten 5u sections on Probe-on+ slides	20x5u on slides and 10x10x4u sections in tubes (50/50 tumor/normal)	Yes
Mayo	Phase I: 2 H&Es per block	Ten 5u sections on Probe-on+ slides	Ten (total) 10u sections on slides	Yes
FHCRC	Phase I: 1 H&E per block Phase II: 3 H&Es[2] per block	Ten 5u sections on Probe-on+ slides	Phase I: Four tubes with 5x5u sections in tubes (2 tumor; 2 normal) Phase II: Twenty 5u sections on slides (10 tumor; 10 normal)	Yes
USC	2 H&Es per block	Ten 5u sections on Probe-on+ slides	Phase I: 26x5u on slides and 4x5u sections in tubes Phase II: Five 5u sections on slides	Yes
Australia	2 H&Es per block	Ten 5u sections on Probe-on+ slides	Twenty (total) 4u sections on slides (10 tumor; 10 normal)	Yes
Ontario	3 H&Es[2] per block	Six 5u sections on Probe-on+ slides	Ten 10u sections on slides	Yes

Note:
Ontario: When pemitted, Ontario stores a representative block from tumour and normal which can be used for sections for future IHC.
USC: When possible, USC also prepares 2 tumor/normal arrays, 2 tumor-only arrays, and prepares 4 cores per block, which are stored in tubes.

[1] The C-CFR standard for obtaining tissue for DNA was either 20 sections when tumor and normal could be isolated from the same section or 10 tumor block sections plus 10 normal block sections.

[2] An initial set of H&E slides are prepared. At time to sectioning for IHC and DNA sections a beginning and end H&E slide are prepared.

3. Fresh Tissue Collection

In 2002, a pilot study was initiated for the collection of 270fresh colorectal cancers at the Mayo Clinic, Ontario, USC Consortium (USC and Cleveland Clinic-CCF) and Hawaii sites.

Tissue is collected as soon as possible after resection to avoid degradation of the sample. Wherever possible, the following samples are prepared

- Cancer tissue, frozen in LN_2
- Adjacent normal mucosa, frozen in LN_2
- Cancer tissue preserved in RNA stabilizing solution
- Adjacent normal preserved in RNA stabilizing solution

To date, 272 samples have been collected, exceeding our target. The number of cancers collected at each site is:

Hawaii	4
USC/CCF	58
Ontario	171
Mayo	83

4. Quality of C-CFR DNA

The C-CFR has dispatched ~50,000 aliquots of genomic DNA to external and internal investigators for a vast variety of analyses and methodologies. Technologies used to analyze these samples include microsatellites, sequencing, SNP analysis (Illumina and Affymetrix platforms), dHPLC, methylation analysis (MehtyLight, bisulfite sequencing), MLPA, Southern blot, RT-PCR, TaqMan and more. Fewer than 1% of these samples needed to be resent due to performance related to the quality of the DNA, demonstrating that the C-CFR is consistently generating high quality DNA.

For one particular study, Genomic DNA was shipped to TGen from all 6 CFR sites.
The DNA had been extracted at each CFR using a variety of methods. The blood had been collected up to 9 years prior to extraction and stored on-site as summarized. To date, 4,507 of these samples have been processed using Illumina GoldenGate Assay at TGen. Ninty-nine (99%) of these samples passed TGen's Sample QC/QA and the overall call rate was >85%.

DNA received by TGen is routinely purified upon receipt to ensure DNA is of high enough quality to be analyzed on the Illumina platform. In a pilot test to determine whether the DNAs needed to be re-purified, 46 samples (23 from Mayo and 23 from USC) tested for call rate and concordance with the Illumina GoldenGate assay.

I. Test: Compatibility of Illumina's GoldenGate Assay with non-repurified and re-purified DNA samples.

II. Approach: Illumina's DNA Test Panel (OPA)
    - Off the shelf product containing 360 GoldenGate validated SNPs that test the quality of samples before they are run on a custom Oligo Pool All (OPA).
    - They ran a randomly selected set of 23 samples from each site. Each sample was represented twice per run; once with the samples as they were received and following purification using a Millipore DNA purification kit.
    - The samples that were run un-purified were also normalized using the concentrations calculated by the site source. For the re-purified samples, following purification, the

samples were quantified at TGEN and normalized to 50 ng/µl via Picogreen ® (Molecular Probes).

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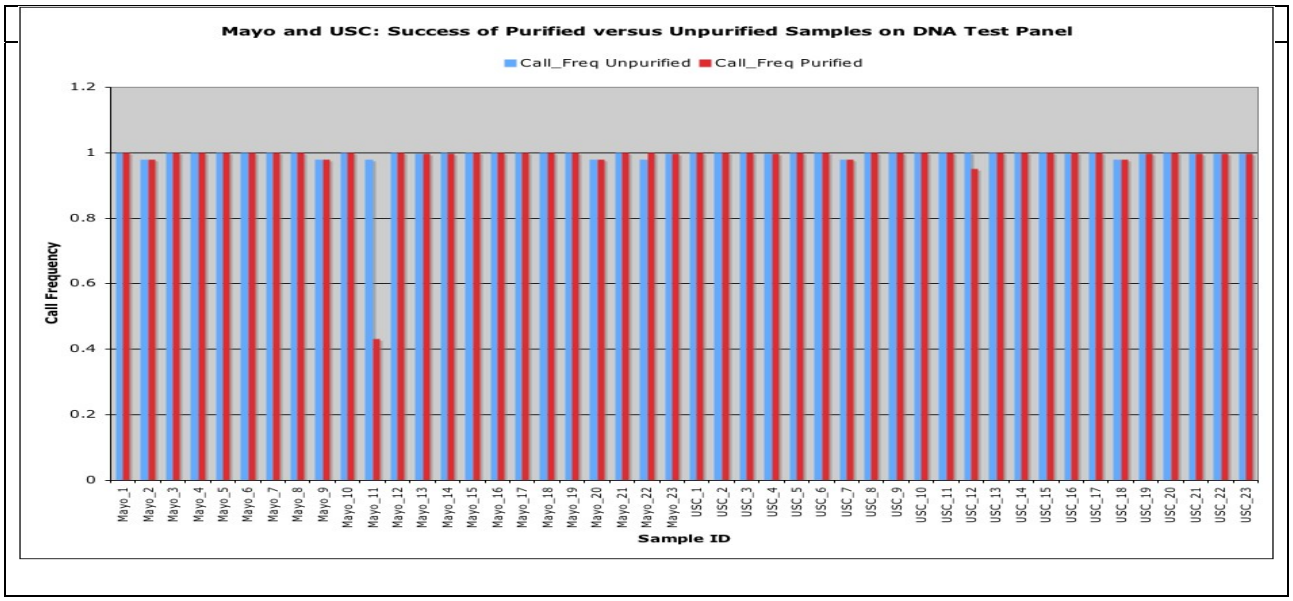
III. Results: As shown below, they found negligible difference in genotype calls between re-purified and non-purified sample call rates. Sample Mayo_11 was assumed to be lost during the purification process.



5. CFR Ethics and Access Policy

Researchers seeking to obtain and use C-CFR biospecimens must submit all proposals for approval by the Steering Committee, and Advisory Committee, and comply with all IRB, data use, material transfer agreement (MTA) and confidentiality requirements. The procedure for this process is shown below in Figure 3 and described in more detail below.

A. Informed Consent

Each Colon CFR recruitment center obtains informed consent for each biospecimen collection event. These consents are reviewed and approved by the centers' Institutional review boards (IRB). The consent addresses the use of biospecimens by private entitites, the possible future development of commercial products through research and disclosure of individual results. All biospecimens transferred to the central repository will be stripped of any personal identifiers.

B. Access to Biospecimens and Data

The Colon CFR has developed clear guidelines for reviewing applications requesting use of Colon CFR biospecimens that are consistent with ethical principles, prevailing laws and regulations. These are guided by:

- Scientific validity of the research project
- The applicant investigator's agreement covering confidentiality, use, disposal and security of the biospecimens and associated data
- The applicant investigator's written agreement in an MTA to comply with site-specific policies as well as the NIH Research Tools Policy
- Ethical oversight
- Adequate funding for the biospecimen request

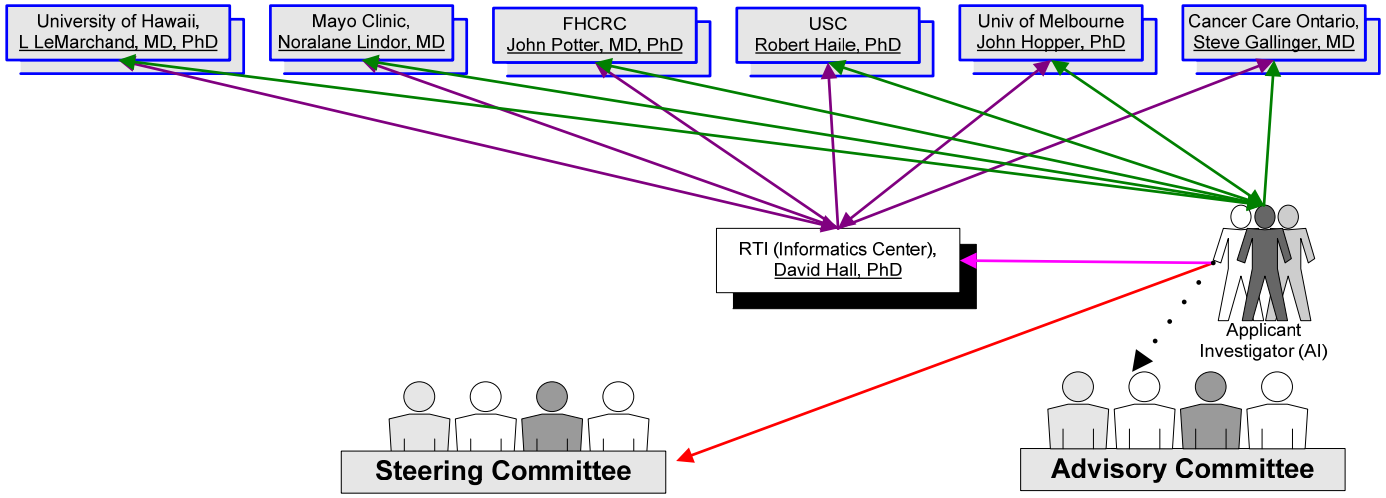
C. Privacy Protection

Each Colon CFR recruitment center institutes a level of security that is appropriate to their repository and to protect study participant privacy. All biospecimens are stripped of any personal identifiers and are labelled with a unique identifier.

D. Intellectual Property

All transfers of materials to applicant investigators are done under the terms of a Material Transfer Agreement with terms consistent with the NIH Research Tools Policy and the NIH Data Sharing Policy. It is recognized that biospecimen repository staff member act as custodians of biospecimens and that biorepositories have no inherent rights to future Intellectual Property in inventions made by investigators using samples obtained from the biorepository.

Figure 3. Colorectal Cancer Family Registry (C-CFR) Ethical, Legal and Access Policy Flow Diagram



I. Recruitment Centers (RC):
(1) Has *IRB approval*.
(2) Obtain Informed Consent for all Biospecimens
(3) Has been issued a *Certificate of Confidentiality*
(4) Acts as Gatekeeper to their biospecimens and institutes security to protect privacy

II. RC signs 1-time *Data Use Agreement* with RTI

III. AI submits application to CFR SC

IV. If applicable (requests biospecimens or participant recontact), AI submits application to the CFR AC

V. If approved:,
(1) Each collaborating RC signs *Collaboration Agreement* w/ AI
(2) AI obtains IRB approval
(3) CFR site obtains IRB approval/exemption
(4) CFR site initiates *MTA* (if applicable)
(5) AI signs:
  a. *Confidentiality Pledge* (if needed)
  b. *Biospecimen Request Form* and invoice
  c. *MTA*
(6) AI sends payment for biospecimens
(7) CFR site dispatches biospecimens

VI. For data requests:
(1) RTI initiates *Data Use Agreement* w/ AI
(2) AI signs and returns *DUA* to RTI
(3) RTI releases data to AI
(4) RTI notifies CFR PIs of data release via e-mail.

IRB Approval: Type of approval required will depend on proposal. Key issues include: 1) will the data released by identifiable or stricly anonymized (constitute a "limited data set")? 2) does the proposal involve a one-way exchange of data or a 2-way exchange (will CFR get data back?); 3) does the proposal involved the collection of study of data or the study of existing data?

Certificate of Confidentiality: A *Certificate of Confidentiality* is issued on behalf of the Secretary of the Department of Health and Human Services (DHHS) and protects Investigators against the involuntary release of information about participants collected during the course of study.

Data Use Agreement: This agreement must be executed with a covered entity in connection with obtaining a "limited data set". The covered entity may disclose a limited data set only if it also obtains specified assurances from the data recipient in the form of a DUA. RC each signed a one-time DUA with RTI. AI signs a project-specific DUA to obtain data files.

Collaboration Agreement: AI must obtain copies of this simple agreement showing that the RC has agreed to collaborate on approved project.

Material Transfer Agreement: Governs the transfer of one institution's proprietary materials to another organization for research purposes. MTAs are necessary when the site receives a request for research materials from an investigator outside the custodial institution. MTAs protect the site's ownership interest in the material that is being distributed to the other party by containing provisions regarding the use of the material.

Confidentiality Pledge serves to document: (1) IRB approval of the research involving access to repository data and/or specimens and (2) Confidentiality protections to be honored by the researcher and any study staff utilizing stored specimens and/or data. It is completed by each researcher and any study staff whose new research activity involves access to the repository's stored specimens and/or data.

Biospecimen Request Form: Confirms criteria, clarifies/tracks steps in collaboration, calculates biospecimen costs, serves an invoice.

NOTE: AI – Applicant Investigator; RC – Recruitment Center; SC – C-CFR Steering Committee; AC – C-CFR Advisory Committee; RTI – Research Triangle Institute; MTA – Material Transfer Agreement; DUA – Data Use Agreement