Recommendations for Collection and Handling of Specimens from Group Breast Cancer Clinical Trials, from Onsite Collection through Shipping to the Central Bank

An international collaboration among the Breast International Group, the NCI Group Banking Committee, and the North American breast cancer Cooperative Groups
Goals:

1. To **promote and ensure proper collection of high-quality research specimens** such that each patient diagnosed with breast cancer can have a **reliable, interpretable molecular diagnosis**.

2. To provide a **known baseline of standardization of specimen collection and handling procedures**, to the extent possible, such that **more global biomarker analysis across studies is possible**.

3. To promote specimen collection that would allow for **future technologies**, particularly in the molecular arena, to be applied to these specimens for research.

4. To provide **guidelines that Group trial leadership can incorporate into clinical trial protocols**.
Quick questionnaire sent to Groups on specimen collection, handling, processing, storage practices

Sent to GBC Best Practices & Ops Subcommittee. Covered:

- Procurement methods for **fresh, frozen, and fixed solid tissue**
- Procurement methods for **blood components** (for serum, for plasma, for PBMC, for DNA, for RNA)
- Level of **detail of annotation kept on how a sample was actually handled** – esp. time before freezing, reagents & fixatives, duration of fixation, temperature, etc.
- **Actions required at local site** (e.g., spinning, aliquoting)
- **Processing** methodology – TMA, nucleic acid extraction from solid tissue and blood, cell sorting from blood, Quality Assurance
- **Storage of nucleic acid**, blocks, unstained slides, shavings, frozen tissue, whole blood, serum, plasma, PBMC, DNA, RNA.
- **Data linkages** - Procedures for linking information on specimens to the clinical trial database
- Specimen-related SOP’s were collected from NCI and BIG Groups.
Quick questionnaire on Group specimen collection, handling, & processing practices

A. TISSUE

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<thead>
<tr>
<th>Type</th>
<th>Collection devices (e.g., punches, capsules, molds)</th>
<th>Collection buffer (e.g., RPMI, RNA later, formalin)</th>
<th>Variety (e.g., slides, cores, block, shaving)</th>
<th>Shipping kit &amp; method of shipment (e.g., IATA completed shipper)</th>
<th>Time before freezing</th>
<th>Tissue annotation</th>
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<td>Fresh</td>
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<td>Frozen</td>
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<td>Fixed</td>
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Additional Useful Info: Please include below any additional useful information you would like to provide on your Group’s solid tissue procurement and handling that you did not address above:
Quick questionnaire on Group practices, continued

**Tissue Processing Methods**

**TMA**

Do you re-embed multi-site donor blocks? Yes _____ No _____
Do you include cell-line/control tissue in final TMA? Yes ___ No ___
How many cores do you include on a single TMA block? _____
Do you include replicate cores on the TMA? ___ If so, on same block or different blocks and how many?: _____
What size core do you include on the TMA? _____
What density of cores do you use on the TMA? _____
Do you take an extra core for nucleic acid extraction? _____ Do you take cores for TMA’s before processing of FFPE tissue for RNA extraction?
Any addition information on your TMA methodology? __________________________

**Nucleic Acid Extraction**

Please list methods used for nucleic acid extraction (including kit names): __________________________
Do you extract nucleic acid upfront, or do you store tissue for later extraction? ________
Any addition information on your nucleic acid extraction methodology? ________

**Quality Assurance**

What are your QA procedures for ensuring the quality of...
FFPE tissue: __________________________
Fresh/frozen tissue: __________________________
Nucleic acid? (including quantification) __________________________

**Storage**

Please describe your methods of storage and general conditions for...
Nucleic Acid __________________________
Blocks __________________________
Unstained Slides __________________________
Shavings __________________________
Frozen Tissue __________________________
Group SOP’s are being compiled via the Group Banking Committee and posted to a secure Web site.

Web page for Working Groups on common specimen collection/processing procedures for Group breast cancer trials

Some of the documents posted here are still in draft stages. Therefore, please keep confidential the information found on this site, unless it is obviously of a public nature.

Permissions
- List of those with access to this site
- Add new link

Weblinks
- Harris et al. abstract re: Agilent, Affymetrix profiing of FFPE
- Affymetrix Best Practices Paraffin Embedded Protocol Working Group
- Abstract: 2006 Laboratory Investigation article on stability of RNA in fresh tissue
- Add new link

Questionnaires on Group banking practices
- Completed questionnaires from Groups
- Add new link

SOP’s, meeting reports, comments
- MEETING REPORTS of working groups
- GOG and COG SOP’s
- MIND ACT specimen SOP’s
- New South Wales Breast Cancer Tissue Bank SOP’s (some draft only)
- Northwestern U./ECOG documents from Raji (including PACCT-1)
- Other SOP’s, comments
- Oxford specimen collection SOP’s
- P53 (EORTC study) fresh frozen procedure
- RTOG’s SOP’s for specimen collection
- SWOG’s SOP’s
- Add new link
Blood, Serum, and Plasma Working Group
Chaired by Brian Leyland-Jones, M.D., Ph.D.

FFPE Working Group
Chaired by Adekunle Raji

Fresh Frozen Tissue Working Group
Chaired by Matthew Ellis, M.B., Ph.D.
OBJECTIVES: TO RECOMMEND:

- Minimum facility requirements
- Central banking of and access to research specimens
- Specimen collection kits
- Labeling
- Database documentation/annotation
- Storage before and after shipping
- Shipping
- Notes on downstream processing
OBJECTIVES: TO RECOMMEND:

- Some guidance to central banks – e.g., TMA design, QA of FFPE blocks, notes on downstream processing

- However, mainly addresses collection, handling, and shipment by procurement sites.
BLOOD, SERUM, AND PLASMA WORKING GROUP
of the BIG/GBC/North American breast cancer Cooperative Groups

Chair: Brian Leyland-Jones, M.D., Ph.D.
TransHERA, McGill University

Mark Abramovitz, Ph.D.
McGill University

Christine Ambrosone, Ph.D.
Roswell Park Cancer Institute/SWOG

Fatima Cardoso, M.D.
TRANS BIG, Jules Bordet Institute

Daniel Hayes, M.D.
The University of Michigan/SWOG

Klaus Pantel, M.D., Ph.D.
Universitätsklinikum Hamburg, Germany

Adekunle Raji, Michael Pins, M.D.
ECOG Pathology Coordinating Office

Federico Rojo, M.D.
Vall d'Hebron Hospital, Spain

Geraldine Thomas, Ph.D.
TRANS BIG, South West Wales Cancer Institute

JoAnne Zujewski, M.D.,
Cancer Therapy Evaluation Program, NCI

Coordinator: Rebecca Enos, MPH
NCI Contractor

Comments also provided by Jacqueline Lafky of NCCTG and
Sandra Brewer-Stakely, and Jackie Smith of COG/GOG
GLOBAL MINIMAL STANDARD
FOR BLOOD COLLECTION

1. **1 tube whole blood** (either EDTA/purple top or PAXgene DNA) for germline DNA

2. **20 mL for serum at each collection timepoint** (see below). Either red top or SST could be used. SST is acceptable minimum & easier to use.

3. **Storage temperature:** $-80^\circ\text{C}$

**NO FREEZE-THAWING. SAMPLES SHOULD BE FROZEN ONCE, AND THEN THAWED ONLY ONCE, BY THE RESEARCHER.**

*Notes:* The collection volumes recommended above should serve to ensure adequacy of specimen both for studies embedded within the trial protocol as well as for future research.

With current technologies, a few ng of DNA can support analyses of multiple SNPs. Moreover, SNP multiplexing is possible. Therefore, one EDTA or PAXgene tube should yield sufficient DNA – a couple hundred micrograms of DNA – for future studies.

Onsite processing of plasma was considered too complicated to ensure consistent, good-quality specimen & compliance, if plasma collection were expected across trials.
SERUM COLLECTION TIME POINTS

**NEOADJUVANT STUDIES**
- 20 ml for serum at baseline
- 20 ml for serum at 2-4 weeks (usually 2 wks)
- 20 ml for serum at 12-16 weeks

**ADJUVANT & METASTATIC STUDIES**
- 20 ml for serum at baseline
- 20 ml for serum at first time point during study (e.g., end of Cycle 1)
- 20 ml for serum at a second time point during the study (e.g., end of Cycle 4)
- Additional time points if deemed appropriate

**ALIQUOTING**
Aliquot serum for freezing into as small amounts as possible to avoid freeze-thawing:
- Either 0.5 straws as used by Dr. Ambrosone, or
- Cryovials (less expensive) as used by Dr. Thomas in the UK
1. **Pre-made kits sent by the central bank sites**, containing the necessary tubes, bar code, tracking sheet, etc., can vastly expedite collection of good-quality specimen and reduce variability in collection procedure.

2. When **kits aren’t available** and a blood draw is imminent, **drawing into an EDTA tube with immediate (same day) shipment** to site is recommended.
1. If circulating tumor cells are to be collected, the **ECOG CPT tube protocol** can serve as the recommended standard.

2. CPT tubes already contain the gradient solution. Site spins it down, and the central bank isolates the PBMC’s.

3. **ABSOLUTELY REGARDED AS RESEARCH: FOLLOW PROTOCOL**
FFPE WORKING GROUP
of the BIG/GBC/North American breast cancer Cooperative Groups
Chair: Adekunle Raji
ECOG Pathology Coordinating Office

Mark Abramovitz, Ph.D.
McGill University

John Bartlett, Ph.D.
TRANS BIG, University of Edinburgh

Sofia Braga, M.D.
BIG/TRANS BIG Secretariat

Carsten Denkert, M.D.
BIG, German Breast Group

Wedad Hanna, M.D.
TransHERA, Sunnybrook & Women’s, Toronto

Daniel Hayes, M.D.
The University of Michigan/SWOG

Stephen Hewitt, M.D., Ph.D.
TARP Lab, NCI

Scott Jewell, Ph.D.
CALGB PCO, Group Banking Committee

Denis Larsimont, M.D.
Jules Bordet Institute

Brian Leyland-Jones, M.D., Ph.D.
McGill University

Rudolf Maibach, Ph.D.
ICSBG

Soonmyung Paik, M.D.
NSABP pathology & specimen bank

Michael Pins, M.D.
ECOG Pathology Coordinating Office

Federico Rojo, M.D.
Vall d’Hebron Hospital, Spain

Sheila Taube, Ph.D.
Cancer Diagnosis Program, NCI

Marc van de Vijver, M.D., Ph.D.
Nederlands Kanker Instituut

Giuseppe Viale, M.D.
BIG, University of Milan

JoAnne Zujewski, M.D., Ph.D.
Cancer Therapy Evaluation Program, NCI

Coordinator: Rebecca Enos, MPH
NCI Contractor
FFPE

Recommended mandatory tissue submission for research:

1. Block of primary tumor and block of adjacent non-tumor.
2. If block submission is not possible, then diagnostic block submitted to central bank for TMA coring.
3. If neither of the above is possible: A written, clear explanation as to why.
4. If available, block from local/distant recurrence resection (or TMA coring if no block).
5. If available, axillary node block (or TMA coring if no block).
6. Specimen submission form as per protocol.

Under consideration: Non-compliant sites should be excluded from Group membership, support, and access to Group specimens by the site’s researchers.
Pathologist support for submitting research specimens:

- Increase pathologist involvement in, and awareness of, clinical research. **Involve pathologist on clinical trial steering committee.**

- Ensure that **money allotted to pathology gets to pathologists.**

- Consider **re-apportioning Group resources to allow increased reimbursement of pathologists. $50-$250.**

- Promise **timely return of block** if needed for patient care.

- List reasons why research block submission is essential.

  **Self-explanatory kits for specimen submission.**

  **Increase pathologist awareness of studies of specimen handling’s impact on specimen quality.**
FFPE (continued)

Fixative and buffer type: Recommend use ONE TYPE: **10% neutral phosphate-buffered formalin**

**Time to fixation, duration of fixation, FFPE “Don’ts”**.

**Paraffin infiltration**
FRESH/FROZEN WORKING GROUP
of the BIG/GBC/North American breast cancer Cooperative Groups

Chair: Matthew Ellis, M.B., Ph.D.
Washington University, St. Louis, Missouri
CALGB/ACOSOG

Mark Abramovitz, Ph.D.
McGill University

Sofia Braga, M.D.
BIG/TRANS BIG Secretariat

John Forbes, FRACS, M.S., FRCS, MBBS
Australian New Zealand Breast Cancer Trials Group

Nadia Harbeck, M.D.
TRANS BIG, Technical University of Munich

Stephen Hewitt, M.D., Ph.D.
TARP Lab, NCI

Brian Leyland-Jones, M.D., Ph.D.
McGill University

John Olson, M.D., Ph.D.
Duke University, ACOSOG

Federico Rojo, M.D.
Vall d’Hebron Hospital, Spain

Arun Seth, Ph.D.
Sunnybrook and Women’s, Toronto, Canada

Baljit Singh, M.D.
New York University/CALGB, I-SPY

Laura van ‘t Veer, Ph.D.
Stella Mook, M.D.
TRANS BIG, Nederlands Kanker Instituut

Mark Watson, M.D., Ph.D.
Washington University, St. Louis/ACOSOG

JoAnne Zujewski, M.D.
Cancer Therapy Evaluation Program, NCI

Coordinator: Rebecca Enos, MPH
NCI Contractor
FRESH/FROZEN TISSUE

Rationale for fresh/frozen tissue collection

“Do’s and Don’t’s”

Recommended SOP’s:

1. Brochure used by BIG 00-01 / EORTC 10994 p53 study
2. SOP for TuBaFrost (European Human Frozen Tumour Tissue Bank)
3. MINDACT SOP’s
4. ACOSOG-Z1031 SOP

Settings for specimen acquisition:

Diagnostic setting
Post-diagnostic preoperative setting
Surgical setting
Obtain specimen, ideally by in vivo guided biopsy (ultrasound) when most convenient for patient

Initial presentation

Initial biopsy

No banking incorporated

Obtain specimen, ideally by in vivo guided biopsy (ultrasound) when most convenient for patient

Initial biopsy

Banking Incorporated

Obtain specimen in OR through ex vivo guided biopsy device

Diagnostic Specimens Formalin

Potential Research Specimens Frozen

Frozen specimens initially held in abeyance in case they are needed for diagnosis (will always have to be sectioned in the case of a negative biopsy so suspicion for cancer should be high)
<table>
<thead>
<tr>
<th>PROS AND CONS</th>
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<tr>
<td>Post-diagnosis banking</td>
<td><strong>Advantages:</strong> Most patients going to the OR already have a tissue diagnosis, so this is routine for the primary surgery setting. In the setting of primary systemic therapy, most patients require a post-diagnosis procedure (sentinel nodes, tumor clip, etc.) that can be coordinated with the tissue procurement team so that additional samples can be obtained. <strong>A definitive banking event allows time for the informed consent process to be incorporated into the therapeutic discussions.</strong> <strong>Disadvantages:</strong> A second biopsy event is necessary and patient may refuse if experience was uncomfortable (unless patient sedated or anesthetized).</td>
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<tr>
<td>Initial biopsy, banking incorporated</td>
<td><strong>Advantages:</strong> Research biopsies and clinical biopsies are taken at the same time. <strong>Disadvantages:</strong> Requires a system for holding research biopsies in abeyance. Could result in a delay in reading pathology, to take frozen tissue into account. Tissue procurement team must be on hand when diagnostic clinics are ongoing, and team must show up promptly to avoid delays. Reduced time for adequate consent process and discussions complicated by lack of a certain breast cancer diagnosis.</td>
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ACOSOG Kit for frozen tissue acquisition

1) OCT
2) Tissue cassettes
3) Ex Vivo guided biopsy device
4) Specimen tubes
5) Biopsy gun
6) Forceps for tissue manipulation
7) Dry ice shipment
8) Package reusable
Other elements of guideline:

Preparation and storage of fresh tissue specimens
  Time to freezing and freezing temperature
  Options for snap-freezing
  Use of RNAlater® as alternative to snap-freezing
  Sample annotation
  Specimen shipping

Quality assurance

Team approach and training

Notes on “downstream” processing
Current plans

- Submitted to journal

- If accepted, citation will be posted at ctep.cancer.gov/guidelines/spec_bc_grptrials.html

- 6-month to yearly follow-up/ updating of recommendations