

RAFT STUDY

RENAL ANHYDRAMNIOS FETAL THERAPY TRIAL



JOHNS HOPKINS
M E D I C I N E

JOHNS HOPKINS
ALL CHILDREN'S HOSPITAL



Lab Manual

Version: 7/28/2020

JHAC Pediatric Biorepository
501 6th Avenue South
St. Petersburg, FL 33701
<http://www.hopkinsallchildrens.org>
P: 727-767-2450 F: 727-767-2905

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1. Contacts

Please direct specimen processing questions to the relevant staff listed below – questions relating to study issues may be sent to the study coordinator and lab manual questions to the JHAC Pediatric Biorepository.

1.1. Study Contacts

1.1.1 Principal Investigator:

Eric Jelin, MD
Assistant Professor of Surgery
Director, Fetal Program, Johns Hopkins Children's Center
The Johns Hopkins Hospital
1800 Orleans Street
The Charlotte R. Bloomberg Children's Center Building, Suite 7323
Baltimore, MD 21287
P 443-997-5437
ejelin1@jhmi.edu

1.1.2 Primary Study Coordinator:

Denise Wolfson, BSN, RN
Fetal Therapy Program
P 443-287-9549
dwolfso3@jhmi.edu

1.1.3 Site Manager:

Carolyn Koenig, RN, MS, CRA
JHU/Tufts Innovation Center
P 513-233-1416
carolynhkoenig@gmail.com

1.2. JHAC Pediatric Biorepository Contacts

1.2.1. Primary Contact(s):

JHAC Pediatric Biorepository
Johns Hopkins All Children's Hospital
501 6th Avenue South
St. Petersburg, FL 33701
P 727-767-2450 | F 727-767-2905
ACHBiorepository@jhmi.edu

1.2.2. Secondary Contact:

Billy Schleif, MS, MT
Manager, JHAC Pediatric Biorepository
Johns Hopkins All Children's Hospital
501 6th Avenue South
St. Petersburg, FL 33701
P 727-767-4267 | F 727-767-8398
Billy.Schleif@jhmi.edu

2. Specimen Collection Kit and Procedure

RAFT uses pre-labeled study kits containing only the specific components required for each cohort's sample collection requirements. Any kit may be used for any research participant at any visit, however, individual components inside the kit should never be substituted between different kits. Kits are assigned to a specific research participant using the tracking form at collection. Please verify the research participant's consent matches the RAFT RESEARCH Specimen Tracking Form consent check boxes and only consented biospecimens are collected. Any biospecimen-related consent discrepancies noted after collection should immediately be reported directly to the JHAC Biorepository and the study team so records are updated appropriately and biospecimens can be disposed if needed.

2.1. Specimen Collection Kit Contents

- 2.1.1. Amniotic Fluid Specimen Collection Kit
 - 2.1.1.1. Biospecimen Transport Bag
 - 2.1.1.2. RAFT Research Specimen Tracking Form
 - 2.1.1.3. One 15 mL Conical Tube
 - 2.1.1.4. Processing Supply Bag:
 - 2.1.1.4.1. One 4.5 mL polypropylene NUNC tube
 - 2.1.1.4.2. One Transfer pipette
 - 2.1.1.4.3. Eight 1.4 mL aliquot tubes with light blue caps
 - 2.1.1.4.4. One 2.0 mL Cryovial (with multi-colored lid)
Note: This cryovial contains 0.2 mL of phosphate buffered saline (PBS)

2.2. Equipment & Supplies Required but Not Included:

- 2.2.1. All materials required for amniotic fluid collection.
- 2.2.2. Intra-Facility Transport Cooler (or equivalent) to hold wet ice
- 2.2.3. Centrifuge, refrigerated preferred, but not essential if temperature readings available throughout sample processing.
- 2.2.4. Minus 20 °C Freezer - short-term storage (if needed)
- 2.2.5. Minus 80 °C Freezer - long-term storage
- 2.2.6. Biohazardous waste containers (following local and institutional regulations).
- 2.2.7. Dry ice for shipping.

3. Specimen Tracking Form Instructions

3.1. How to Fill Out the RAFT RESEARCH Specimen Tracking Form:

- 3.1.1. **Collection Staff:** please fill out top portion of the tracking form. All fields in the white sections are mandatory. The shaded areas are reserved for processing use only.
- 3.1.1.1. Study participant ID must be written into the tracking form to associate the kit components to the correct research ID. Do not use protected health information (PHI) or other non-approved identifiers.
 - 3.1.1.2. Kit Number: Ensure the collection kit inventory number matches the number on the associated specimen bag.
 - 3.1.1.3. Consent Checkboxes: Ensure each biospecimen collected is properly consented.
 - 3.1.1.4. Specimen Collection Table: Enter the collection date and time, and the collector's initials for each specimen collection. If no specimen is collected, enter NA in the collection method field.
- 3.1.2. **Processing Staff:** please fill out shaded areas of the tracking form
- 3.1.2.1. Processing Date, Start Time, and initials of processor in the specimen collection table. Note processing discrepancies in the comments section (i.e. sample was processed at the wrong centrifuge temperature).
 - 3.1.2.2. Enter the quality control metrics during specimen processing:
 - 3.1.2.2.1. Specimens are hemolyzed or other quality issues are present.
 - 3.1.2.3. Note the number of aliquots created next to the specimen type in the specimen collection table under comments. If electronic data entry reception is delayed or not used, enter the storage date and time, storage location, and volume in the comments section or on the back of the tracking form if space is limited.

4. Processing and Storage Instructions

4.1. Amniotic Fluid Processing

4.1.1. 15 mL Conical Tube

- 4.1.1.1. After collection, samples should be transported on wet ice. Sample processing must begin within one hour of collection.
- 4.1.1.2. Check the 15 mL conical tube to verify at least a 10 mL fill. Note discrepancies in the comments section of the research tracking form. Incompletely filled tubes should still be processed, however the 4.5 mL Nunc tube will not be used and can be discarded.
- 4.1.1.3. Spin the conical tube for 15 minutes at 2000g at 4°C. See Appendix A for determining RCF (relative centrifugal force). If a refrigerated centrifuge is not available for use, a room temperature centrifuge can be used, but the temperature must not exceed 26°C (79°F) during the spin. Be sure to properly balance the centrifuge before starting the process.
- 4.1.2.4 After centrifugation is complete, use the provided transfer pipette to remove the amniotic fluid supernatant without disturbing the cell pellet at the bottom of the tube. Starting at the top, transfer approximately 1 mL to each of the smaller 1.4 mL tubes. The transfer pipette has graduations on the side to help measure the required volume. Do not exceed 1 mL in the aliquot tubes, or it may rupture the cap during freezing.
- 4.1.2.4 Use the transfer pipette to remove the remaining volume to the larger 4.5 mL Nunc tube, again being careful not to disturb the cell pellet at the bottom of the conical tube. Leave approximately 1-3 mL of fluid covering the cell pellet.
- 4.1.2.4 Use the transfer pipette to gently resuspend the cell pellet at the bottom of the tube three times. Transfer 1.5 mL of the re-suspended cell pellet to the provided 2.0 mL cryovial.
- 4.1.2.5. Tighten lids securely on all cryovials and freeze **upright** at -80°C as soon as possible. If a -80°C freeze is not available, a -20°C freezer may be used for up to 24 hours. Place samples together into the appropriate racks described here:
 - 4.1.2.5.1. 1.4 mL cryovials should be stored in the 96-position white rack provided.
 - 4.1.2.5.2. The 2.0 mL cryovial and 4.5 mL cryovial should be stored in the 49-position box provided.
- 4.1.2.6. Discard the 15 mL conical tube and transfer pipette into an appropriate biohazardous waste container.

5. Packaging and Specimen Transport

5.1. Shipping Date Considerations and Laboratory Holidays (Non-local)

5.1.1. The JHAC Pediatric Biorepository is open Monday through Friday from 7:00am to 5:30 pm.

5.1.2. **Coordinate all batch shipments with the JHAC Biorepository prior to shipping. The Biorepository will provide the FedEx air bill once shipping dates are confirmed. Plan specimen shipments Monday through Thursday. Please do not ship specimens when delivery date falls on a hospital holiday (next bullet).**

Email: ACHBiorepository@jhmi.edu

Phone: **727-767-2450**

5.1.3. **Hospital Holidays**- the JHAC Biorepository will be closed and will not be available to receive specimens on the following holidays unless special arrangements are made:

5.1.3.1. Thanksgiving

5.1.3.2. Christmas Day

5.1.3.3. New Year's Day

5.1.3.4. Martin Luther King, Jr. Day

5.1.3.5. Memorial Day

5.1.3.6. 4th of July

5.1.3.7. Labor Day

5.2. Batch Shipping to the JHAC Pediatric Biorepository

5.2.1. Frozen Shipment on dry ice:

5.2.1.1. Place storage racks inside a sealable bag. Absorbent material sufficient to absorb all liquid contents in case of a spill must also be included.

5.2.1.2. Place sample bag within the provided Styrofoam shipping box with at least 2.5 kg of Dry Ice.

5.2.1.3. Label Styrofoam shipping box with UN1845 dry ice and UN3373 shipping labels (provided)

5.2.1.4. Place original RAFT Research Specimen Tracking Form inside box on top of Styrofoam lid. Be sure to make a copy for your records.

5.2.1.5. Affix FedEx air bill to box and ship specimen as priority overnight.

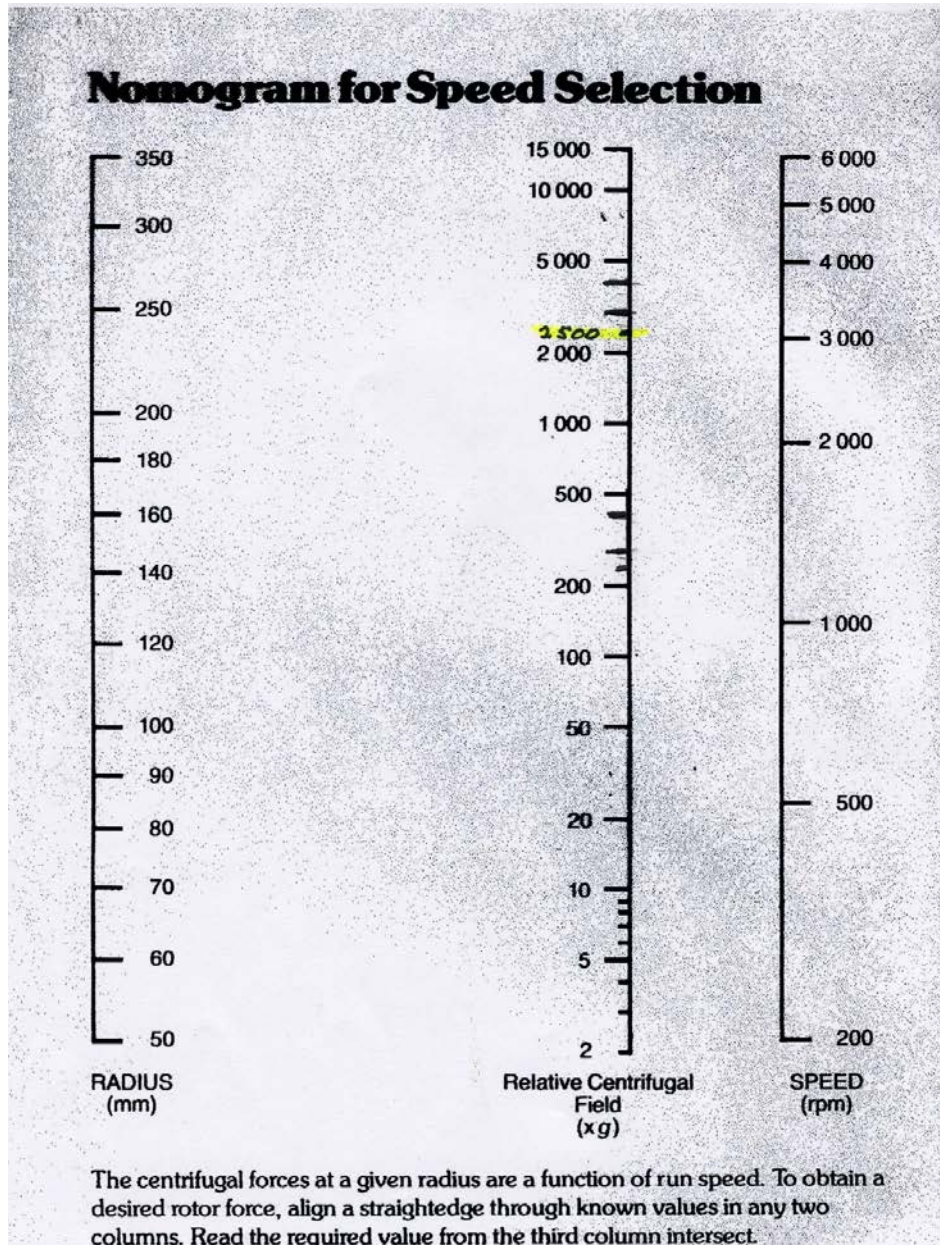
5.2.1.6. Notify ACHBiorepository@jhmi.edu samples are shipped.

6. Kit Resupply

6.1. New Kits are provided by the JHAC Biorepository.

6.1.1. Request new or replacement kits by contacting the Biorepository via email: ACHBiorepository@jhmi.edu.

Appendix A



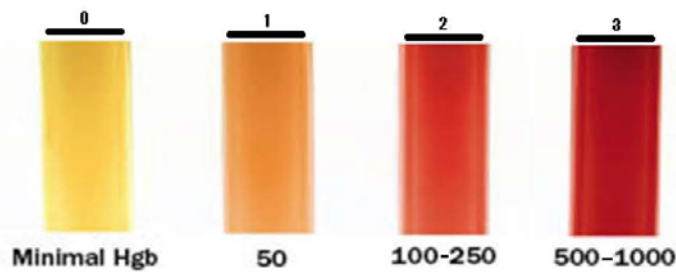
Appendix B



SPECIMEN EVALUATION

Type of specimen	Definition
Acceptable plasma or serum sample	No known interfering substances.
Hemolyzed sample	Visible hemolysis following centrifugation is defined as the presence of free hemoglobin in serum or plasma >100 mg/L. See Hemolysis chart below.
Lipemic sample	Visible lipemia, turbidity due to elevated concentrations of lipids, usually translates to a triglyceride level >1000 mg/dL (whole blood) or >300 mg/dL (serum or plasma).
Icteric sample	Visible detection of icterus is variable and unreliable.
Clotted sample	Those specimens that present with visible clots—either as a red cell clot in whole blood or a fibrin clot in plasma.

HEMOLYSIS CHART FOR SERUM OR PLASMA



Approximate Hemoglobin Concentration (mg/dL)

General guidance for acceptance
 <50 mg/dL – not hemolyzed
 >100 mg/dL – hemolyzed

Source: Mayo Clinic, Mayo Clinical Laboratories