I-SPOT Laboratory Manual Log of Changes from Version 3 to Version 4

Throughout document changed Footer from “July 2013 Version 3” to November 2013 Version 4”

Throughout document changed “aliquot” to “cryovial”

Page 2 – Updated Page numbers on the Table of Contents

Page 3 – Inserted: “tissue factor procoagulant activity (TF-PCA)” replacing TF-PCA alone

Page 3 – Inserted: “At the 48 hour draw, blood should be collected at the time of the scheduled finger stick check closest to 48 hours post randomization. If the time of the scheduled finger check coincides with a meal, the meal should be held until after the scheduled finger stick check and blood draw.”

Page 4 – Deleted: “After removal of aliquots of” and Inserted: “For blood processing first remove”

Page 5 – Removed: “two” from the phrase “with the plasma from the blue top tubes”

Page 11 – Replaced: “Exempt Human Specimen” with “UN 3373 Biological Substance Category B”

Page 12 – Replaced: “Under IATA, clinical or diagnostic specimens are classified under Division 6.2 (infectious substances) as “exempt” as there is minimal likelihood of an infectious agent or pathogen being present” with “*Biological Substance, Category B* means an infectious substance that is not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs. This includes Category B infectious substances transported for diagnostic or investigational purposes.”

Page 15 – Replaced: “Exempt Human Specimen” with “UN 3373 Biological Substance Category B diamond shaped”

Page 16 – Replaced: “Exempt Human Specimen” with “UN 3373 Category B label”

Laboratory Manual

Insights on Selected Procoagulation markers and Outcomes in stroke Trial

(*Response to Insulin Administration and Blood Glucose Control*)

Table of Contents

1. OVERVIEW

Goal: To assess the effects of strict hyperglycemia control and standard treatment of hyperglycemia on membrane-bound tissue factor procoagulant activity (TF-PCA) and markers of blood coagulation in Type 2 Diabetes Mellitus (T2DM) patients after acute ischemic stroke (AIS), and the relationships between markers of blood coagulation and clinical outcome and to determine if hyperglycemia control modulates the relationship between these biomarkers and clinical outcome in patients with T2DM with blood glucose >110 mg/dL or blood glucose ≥150 mg/dL after acute ischemic stroke (AIS).

Primary Aim: Compare the effects of strict hyperglycemia control with standard treatment of hyperglycemia on membrane-bound TF-PCA and markers of blood coagulation in T2DM patients after AIS.
Hypothesis: **The decrease in levels of markers of blood coagulation will be greater in patients treated with IV insulin to reduce BG than in patients treated with SQ Insulin as the standard fashion**.

Secondary Aim: Determine the relationship between levels of markers of blood coagulation and functional neurological outcome in SHINE treatment and control patients.
Hypothesis 1: The **decrease in levels of markers of blood coagulation will be greater in patients with than without favorable outcome** (defined as the baseline stroke severity adjusted measure of functional ability at 90 days after AIS).

Patient selection: At I-SPOT participating sites all patients entering the SHINE trial will be assessed for inclusion in the ancillary I-SPOT study. Patients must meet the following inclusion/exclusion criteria.

Inclusion

a. Able to provide a valid informed consent to be in the study (self or their legally accepted representative).

Exclusion

a. Current or anticipated use of systemic anticoagulants
b. Known moderate or severe hepatic insufficiency (as defined by INR>1.5 if known or history of variceal bleeding or hepatic encephalopathy)
c. Prior or concurrent thrombotic or hypercoagulable condition (Antiphospholipid antibody syndrome; Antithrombin III, Protein C or S deficiencies; Congenital or Inherited Factor deficiencies; Sickle cell disease)

Eligible patients for I-SPOT will have samples drawn before study drug start and again at 48 hours (between 46 and 54 hours). At the 48 hour draw, blood should be collected at the time of the scheduled finger stick check closest to 48 hours post randomization. If the

time of the scheduled finger check coincides with a meal, the meal should be held until after the scheduled finger stick check and blood draw.

For blood processing first~~After removal~~ remove ~~ of aliquots of~~ whole blood for TF-PCA, remaining blood must be spun within 60 minutes to harvest the plasma. Once aliquoted and frozen, patient samples can be stored in a -80° freezer for up to one month. Specimens for up to three subjects can be shipped together in the same shipping container, but a separate cardboard cryovial box must be used for each subject**.**
You must abide by your own institutions policies regarding certification for the collection/processing/shipping of blood products (contact your Environmental Health and Safety Office).

For each subject, I-SPOT Form "Blood Sample Collection Form" should be printed from WebDCU and used as a worksheet for data collection at each time point (baseline, 48 hours). Worksheet should be kept in the subject research file as a source document.

2. LAB KITS

Sites will be supplied with the following Shipping, Transport and Storage and Collection kits by the I-SPOT coordinating center.

Blood Collection Kits (2 per subject)
- 4.5 ml Blue top sodium citrate tube x 3
- Aliquot tubes x 10

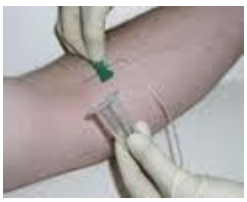
Specimen Transport and Storage (1 per subject)
- Cardboard cryovial box x 1

Specimen Shipping Kit (1 per subject)
- Insulated Shipping Container x 1
- Biohazard bags x 1
- Absorbent Strips x 3
- Zip Tie x 1
- Zip Top Bag for Sample Documents x 1
- UPS Airbill (supplied separately by Coordinating Center)
- "Exempt Human Specimen" label
- IATA dry ice label
- "This End Up" label
- Outer Cardboard Shipping Box

3. INSTRUCTIONS FOR COLLECTING AND PROCESSING BLOOD

DRAWING BLOOD

- Draw blood samples from a fresh venipuncture.



- If it is not possible to draw blood from a fresh venipuncture and is drawn from an existing line, at least 5 ml of blood must be discarded before obtaining samples.

- Fill all 3 tubes completely to ensure consistent ratio of blood to tube additive.

- Blood should be collected without use of tourniquet if possible. A tourniquet may be placed to identify the vein but should be removed while tubes are filling. Prolonged application of a tourniquet while tubes are filling may affect some laboratory measurements. Please mark on the CRF if a tourniquet was in place while tubes are filling.

- Collect blood through a needle no smaller than 21 gauge.

- Use Vacutainer® system to collect blood. **DO NOT** drip blood or use syringe to put blood directly into tubes.

- After tubes fill **gently** invert tubes 4 times to mix sodium citrate and blood.

PROCESSING BLOOD

- Blood may be processed immediately after it is drawn and centrifuge start time must be within one hour after blood is drawn.

- **BEFORE CENTRIFUGING TUBES** remove 2 ml of whole blood and divide between 2 cryovials. Blood can be taken from any of the three blue top tubes.

- Centrifuge remaining blood at 1500 x g for 20 min. at room temperature.

- Label each ~~aliquot~~ cryovial with the SHINE subject ID and either 'Baseline' or '48 hour'.

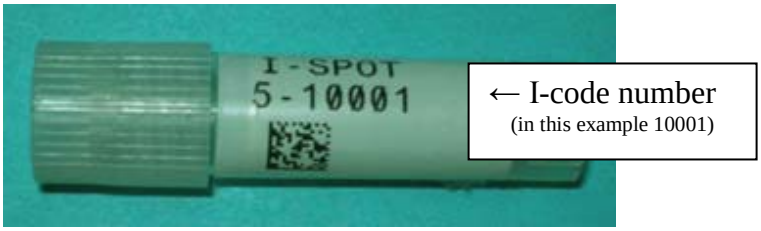
- Fill the ~~aliquot~~ cryovial tubes with approximately 0.3 ml of plasma, filling up to 8 ~~aliquot~~ cyrovial tubes with the plasma from the ~~two~~ blue top tubes after centrifuge is completed

- Place caps on the ~~aliquot~~ cryovial tubes tightly. Record the number of ~~aliquot~~ cryovial tubes with plasma and whole blood on Blood Sample Collection Form, for data entry into WebDCU.

- Care should be taken not to disturb the red cell layer during harvesting of the plasma.

- Place the ~~aliquot~~ cryovial tubes into the cardboard cryovial box and, using a permanent marker, label the box with the following information:

  - I-SPOT Study
  - Subject ID#
  - I-code number (Five-digit number)
  - Date and time of blood draw



Place the cardboard cryovial box **containing the ~~aliquot~~ cryovial tubes into the freezer immediately at - 80° (-70° is acceptable) C** until ready to ship. Make sure the samples are frozen so that the aliquot tubes are upright.
Do NOT lay the aliquot tubes on their side.

- Data Enter Form: Blood Sample Collection Form into WebDCU™.
- Repeat these steps for the 48 hour blood draw.

IMPORTANT:

BOTH blood draws for a subject must be placed into a single cryovial box.

DO NOT put more than one subject's specimens in a single cryovial box.

DO NOT take the cardboard cryovial box out of the freezer for the 48 hour samples until you are ready to place the ~~aliquot~~ cryovial tubes inside to prevent thawing of the frozen samples.

4. INSTRUCTIONS FOR SHIPPING BLOOD SAMPLES

You will receive an email from the I-SPOT Coordinating Center indicating when the I-SPOT samples should be shipped (within 30 days after an enrollment). This email will also contain the UPS airbill that will be printed and placed on the outside of the shipping box.

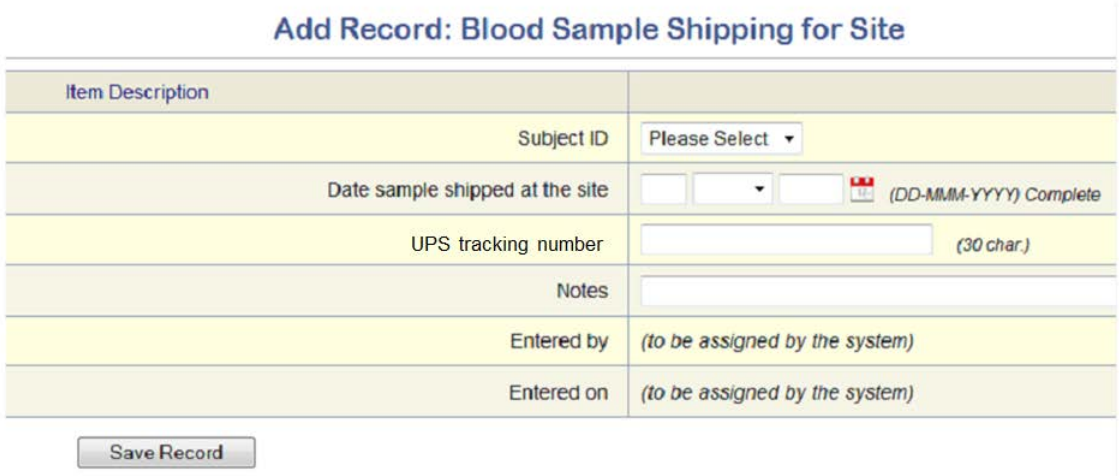
Make sure that the Baseline and 48hr are collected and placed in a single cryovial box for each subject before shipping.

Data enter the Sample Shipping information into WebDCU:

Please note that Blood Sample Collection Form must be completed, for all time points collected, **prior** to data entering ~~-~~the Sample Shipping information, because the

information that is populated on the shipping invoice is pulled from both Blood Sample Collection form and from the Sample Shipping information that is entered into WebDCU.

- Go to the 'project management' tab and select [Blood Sample Shipping for Site]. Click on the 'add new' button in the upper right hand corner. Select the subject, add the date of shipment, the UPS tracking number, and select 'save record'. After saving the record, the shipping invoice will be generated. Click on the green arrow next to the invoice number. View and print the shipping invoice and include the invoice in the shipment box.



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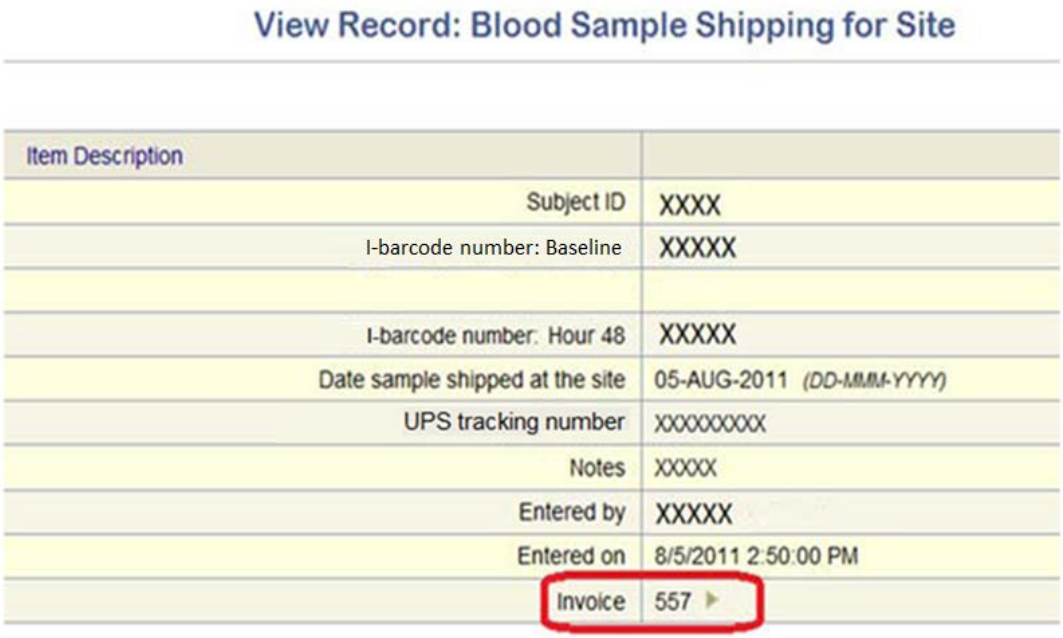
From WebDCU, print the shipping invoice for the subject/cryovial box being shipped.



To prepare the samples for shipment:

- **Check cardboard cryovial box to make sure ~~aliquot~~ cryovial tubes from both time points are in the box.** Make sure all specimens are thoroughly frozen. **Samples should be placed on dry ice in the shipping container immediately after removal from freezer to prevent thawing.**

- Add a layer of dry ice to the bottom of the shipping container. (*Please use proper precautions when handling the dry ice to prevent burns.)*

- Secure the lid on the cardboard cryovial box with tape or rubber band. Each cardboard cryovial box should contain samples for only one subject (Baseline and 48 hour). **DO NOT** mix multiple subjects' specimens within one cryovial box.

- Place the provided absorbent strips (3 total) in the bottom of the biohazard bag prior to adding cryoboxes.

- Place cardboard cryovial box, labeled as stated in section 2.0, into the absorbent strip-lined biohazard bag and secure the bag tightly to prevent samples from falling out of the cryoboxes.

- Place the biohazard bag on its side into the insulated Styrofoam shipping container with dry ice as shown below.

- Add sufficient dry ice to the shipping container and record the approximate weight of the dry ice (usually about 5 kgs) on the IATA dry ice label. Place the Styrofoam lid on the shipper.

- Seal the WebDCU™ shipping invoice in the Ziploc document bag and place on top of the Styrofoam shipper lid (i.e., between the Styrofoam shipping container

and outer cardboard shipping box).

- Close the outer cardboard shipping box so that the flaps that say "RUSH - PERISHABLE" are showing and seal with suitable packing tape.

- Label the outer cardboard shipping box with the following labels
    - "~~Exempt Human Specimen~~UN 3373 Biological Substance Category B" label
    - IATA dry ice label
    - "This End Up" label
- Securely attach the UPS airbill to the outside of the cardboard UPS shipping box.

- Ship the package via UPS Priority Overnight (Monday through Wednesday only in week with all 5 working days, to prevent arrival of the samples on a holiday or weekend).

5. DETAILED INSTRUCTIONS FOR PACKAGING AND SHIPPING SAMPLES

Preliminary Preparations

The PI and Primary SHINE Coordinator will receive a Sample Shipment Notification by email when it is time for the I-SPOT samples to be shipped to the I-SPOT Coordinating Center, Temple University Hospital, Philadelphia, (within 30 days after a single enrollment).
The email will contain a UPS airbill to be used for shipping. The site will be expected to accomplish shipment as quickly as possible after receipt of the email (Monday through Wednesday only). Temple Thrombosis Research Center Laboratory, the I-SPOT Coordinating Center and the NETT Coordinating Center will receive email notification once a site tenders their shipment to UPS, so that they can anticipate delivery the following morning.

Shipment Packaging Requirement

- Sample shipments are regulated by the International Air Transportation Association (IATA / http://www.iata.org/whatwedo/dangerous_goods) and the US Department of Transportation (DOT / http://hazmat.dot.gov).

- *Biological Substance, Category B* means an infectious substance that is not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs. This includes Category B infectious substances transported for diagnostic or

investigational purposes. ~~Under IATA, clinical or diagnostic specimens are classified under Division 6.2 (infectious substances) as "exempt" as there is minimal likelihood of an infectious agent or pathogen being present.~~ Packing must comply with the three-layer packaging system as regulated under the IATA packaging instructions 650.

- Waterproof Primary Container – plastic, glass, or metal container with positive closures (snap on, screw on, or push on lids) that are leak proof when the internal to external pressure gradient is at least 95 kPa at – 40 °C to +55 °C. Primary containers cannot contain more than 1,000 ml. The cryovials supplied for the study meet this requirement.

- Waterproof Secondary Container – With sufficient absorbing material to absorb the entire contents of one of the primary containers, and with adequate cushioning material to ensure that the primary containers do not break. The secondary container must be labeled with the international biohazard symbol. The cardboard cyrovial boxes supplied for the study meet this requirement if they are placed within a leak-proof sealable plastic biohazard bag.

- Styrofoam Container – Sturdy and rigid outer packaging of corrugated cardboard, wood, metal, plastic, or Styrofoam that is appropriately sized for intended use. Package must permit release of carbon dioxide from dry ice. For shipments of samples the shipping container used as the outer container must be a manufacturer-qualified low temperature shipping container. The Sytrofoam shipping container supplied for the study meets these requirements.

6. SHIPMENT DOCUMENTATION AND LABELING REQUIREMENTS

Shipping Invoices

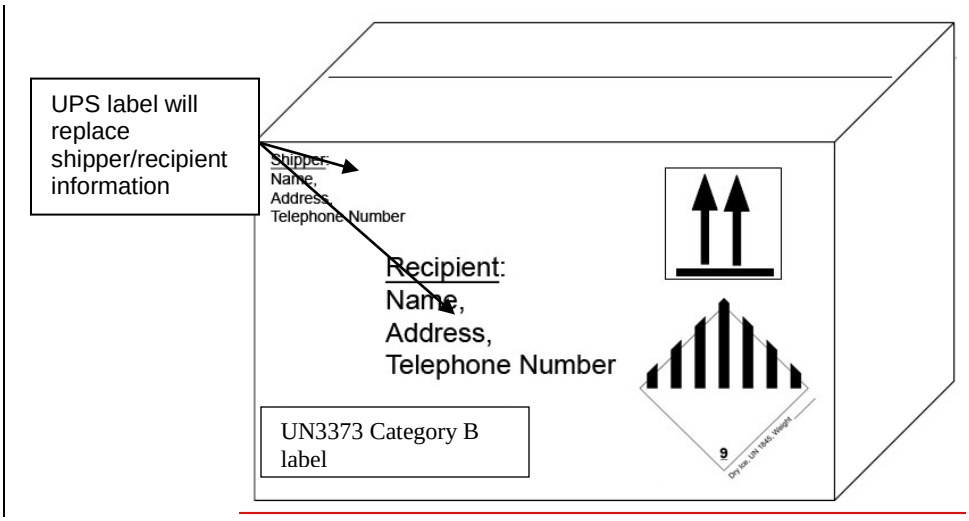
Shipping invoices, printed from WebDCU, should be put inside a Ziploc bag and placed on top of the Styrofoam shipping container (i.e., between the Styrofoam shipping container and outer cardboard shipping box) for each subjects/cryovial box being sent. Up to 3 shipping invoices (1 shipping invoice per subject) can be sent per shipment.

Shipping invoices include:

- Shipping site
- Subject ID
- Date that shipment leaves the research site
- Identity of the research site team member responsible for the shipment
- Shipment tracking number
- I-barcode numbers for each time point (baseline and 48 hours)
- Total count of the aliquot tubes contained for each time point

UPS Airbill and Labels

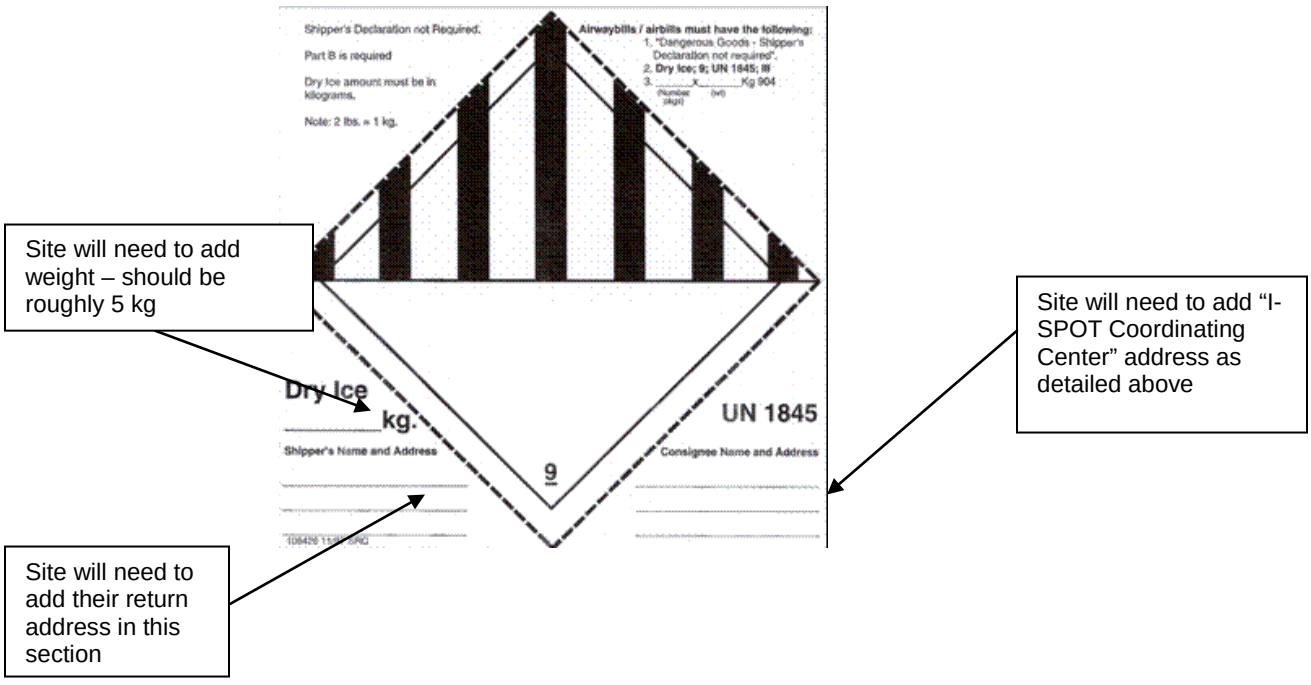
Affix the UPS airbill (which will be emailed when it's time to ship), the "~~Exempt Human Specimen~~UN3373 Biological Substance Category B" diamond-shaped label, the IATA dry ice label and the "This End Up" label (all found in the provided Ziploc document bag) to the outside of the cardboard shipping box as shown in the picture below.



- UPS shipping labels will be addressed as follows:

Temple University Hospital
I-SPOT Coordinating Center
Attn: Hannah Reimer, Emergency Medicine
3401 N. Broad St.
Philadelphia, PA 19140

- The IATA dry ice label should include the approximate weight of dry ice enclosed. A shipping container filled with dry ice will usually weigh approximately 5 kg. Make sure the weight is legible. (Do not use a gel pen or dry erase marker)



7. SHIPMENT RECEIPT

- The UPS tracking system will generate an automated email notification to Temple Coagulation Laboratory, the NETT Coordinating Center, and the I-SPOT Coordinating Center when the package is shipped, so the expected date of arrival will be known.

- I-SPOT Coordinating Center will document shipment receipt in WebDCU™.

- I-SPOT Coordinating Center will verify that the packaging conditions and paperwork are suitable, and will document any deviations in WebDCU™.

- I-SPOT Coordinating Center will verify that the aliquots shipped match what is recorded on the shipping invoice when ready to process specimens and will notify the I-SPOT Coordinating Center of any discrepancies.

8. LAB KIT RE-SUPPLY

Supplies will automatically be replenished based upon enrollment activities. If you are concerned about your inventory, please send an e-mail request to hreimer@temple.edu**. Please make sure to keep your shipping address up to date in the WebDCU Spoke Table**.

9. REPORTING OF RESULTS

Lab results are for research purposes only and will not be reported back to sites.

10. PUBLICATIONS AND PRESENTATIONS POLICIES AND PROCEDURES

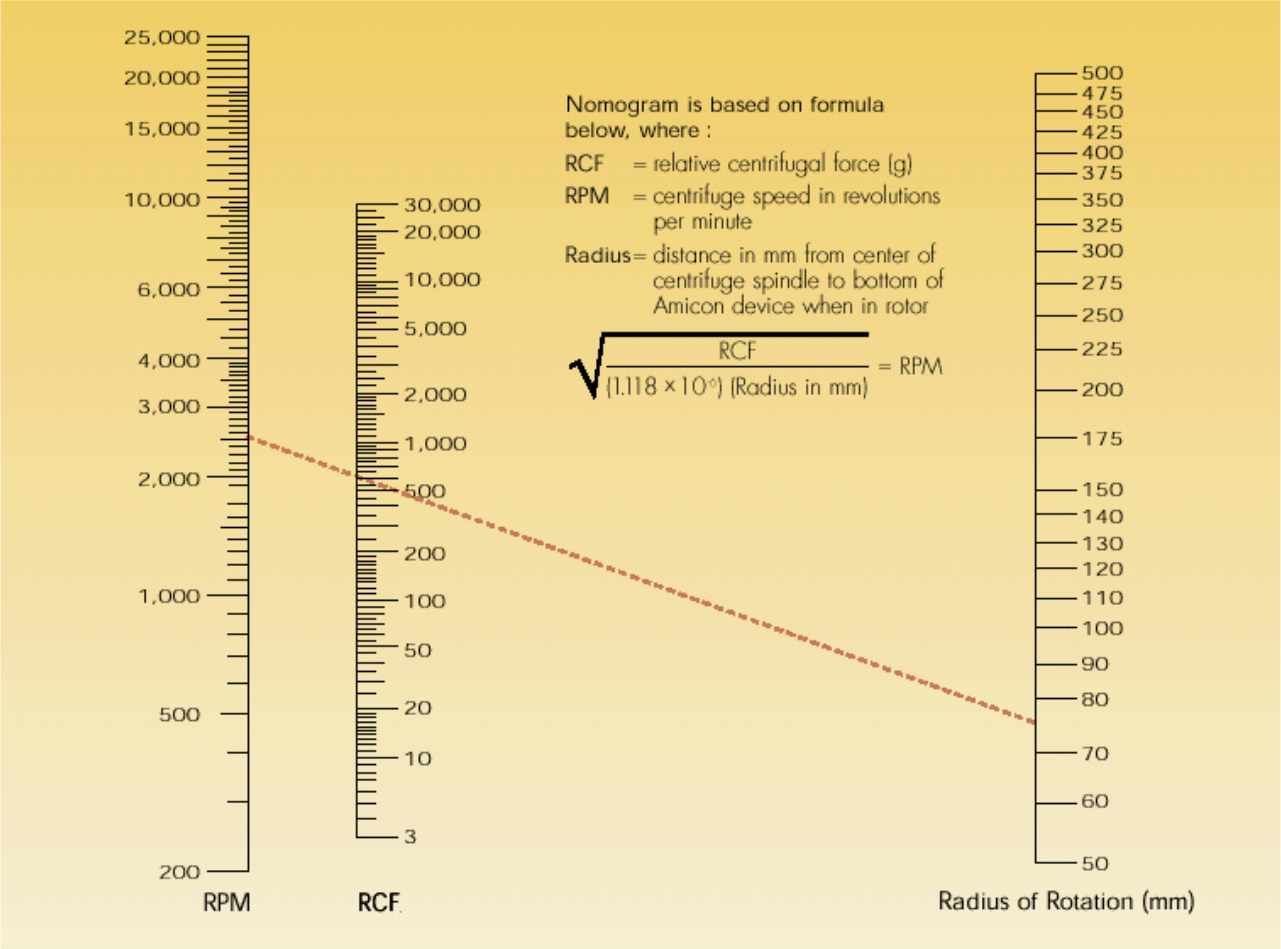
The I-SPOT Trial will adhere to the SHINE publications and presentations policies and procedures outlined in the SHINE Manual of Procedures (MOP).

11. CONTACT FOR PROBLEMS

Please contact the I-SPOT hotline 774-23I-SPOT (774-234-7768) for urgent issues or the Project Manager, Hannah Reimer at 215-707-5483 (office), 215-485-8183 (cell) or by email at hreimer@temple.edu.

The I-SPOT Coordinating Center would like to extend special thanks to the BioProTECT team for their hard work in creating the shipping procedures used in this study and their assistance and guidance in creating this manual.

Appendix A **Nomogram**



To convert RPM to relative centrifugal force (RCF): Determine centrifuge's radius of rotation (in mm) by measuring distance from center of centrifuge spindle to bottom of device when inserted into rotor. Lay a ruler and draw a line from radius value in right-hand column to 1500 RCF in the middle column and continue the line to the RPM column on the left. Then read the RPM value from column at left. This is the value you will enter into your centrifuge to equal 1500 RCF (G-force).

In the example above (red dotted line) the centrifuge has a radius of rotation of 75mm, the RCF of 600 which corresponds to RPM of 2500.