Heart Failure Network

Blood Collection Manual of Procedures

Version: April 14, 2016

Contact Information for questions related to this Manual:

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SUMMARY OF CHANGES FROM VERSION 11/19/14 TO VERSION 04/14/16

In addition to typographical and spelling errors, the following substantive changes were made to version 11/19/14 to the create version 04/14/16.

Updates related to completed and new trials

- ATHENA, IRONOUT, FIGHT, and NEAT study information removed from all applicable sections of the MOP.

- Addition of information related to new trials, SUBQ and INDIE

SUMMARY OF CHANGES FROM VERSION 10/14/14 TO VERSION 12/1/14

In addition to typographical and spelling errors, the following substantive changes were made to version 101414 to the create version 111914.

Shipping Address change for the Biomarker Core Lab

- 911 address change implemented at the HFN Biomarker Core Lab. References to the Core Lab address have been updated throughout the MOP to:

  Laboratory for Clinical Biochemistry Research
  360 South Park Drive
  Colchester, VT 05446

Additional information added to section 5, Shipping of Blood Samples to VT Core Lab

- Shipping to occur the first week of each month. Mondays or Tuesdays preferably, but absolutely no later than Wednesdays.
- Fed Ex Priority overnight service mandatory for sample shipments on dry ice to the core lab. Use of pre-printed Fed Ex airbills from the Core Lab highly encouraged.
- Current IATA 650 Shipping Instructions added.

General Table of Contents

1. Overview and Background

The Heart Failure Network is an NHLBI-funded multi-center, cooperative group, collaborating on randomized, controlled trials (RTC) to study treatments for and prevention of heart failure. The Laboratory for Clinical Biochemistry Research (LCBR) at the University of Vermont is responsible for laboratory protocol development, performing assays and reporting results for the following studies (as of the initiation of the network, Jan. 28, 2008):

HFN_SubQ
HFN_INDIE

The blood samples collected and processed by the HF Network technicians are the foundation for the tests performed by the Laboratory. The most important step (and potentially the most variable) in these tests is the collection and processing of blood samples.
If the blood sample itself is not correctly drawn and processed, the laboratory results will not be precise and may not be valid.

Additionally, the Laboratory will create and maintain the study's biomarker storage and repository. The types of specimens that will be collected and stored are serum and EDTA plasma.

If the participant consents to participate in the Genomic and Pharmacogenomic Substudy, EDTA whole blood will also be drawn **ONCE** at the time of enrollment (randomization). If for any reason the sample is not taken, it should be drawn at the next study visit.

This manual has been prepared to standardize methods for blood sample collection and handling throughout the study. Some of the tools used to assist in standardization include:

- Kits, which will contain the specific collection tubes and cryovials for each blood collection visit. The cryovials and transfer tubes will be frozen and shipped monthly to the LCBR for assay work and repository.

- Forms, which will capture information on sample collection, handling, and quality assurance parameters. This information is critical to the interpretation of assay results and maintenance of the samples.

- Labels, which will be used on forms, draw tubes and cryovials as necessary. Care must be taken to correctly identify the HF Network ID number. These unique labels will allow for each sample to be tracked individually throughout the study.

2. Blood Collection

2A. General Overview

As noted above, the LCBR will provide the necessary kits for each blood collection. Here we list some general safety issues.

General Safety Issues and Precautions for Handling Blood Specimens

In accordance with the Occupational Safety and Health Administration (OSHA) regulations on blood borne pathogens, the LCBR recommends the following general laboratory safety protocol for the field center laboratories:

- Use non-permeable lab coats, latex (or nitrile) gloves, and face shields when handling any blood in any situation in which splashes, spray, splatter, or droplets of blood may be generated and eye, nose, or mouth contamination can be reasonable anticipated.

- Disposable latex gloves and lab coats are worn when collecting and processing specimens. Hands are washed thoroughly with disinfectant soap prior to leaving the work area. Skin cuts or abrasions should be covered.

- Use of aerosol containers in all centrifuges is recommended.

- Follow "Standard Precautions" when handling any blood, urine or tissue products. All specimens must be handled as potentially infectious for laboratory workers.

- Contaminated needles and sharps shall be immediately placed in a puncture-resistant, leak proof biohazard container. Never recap or break needles.

- The Hepatitis B vaccine is offered to all unvaccinated technicians handling blood, and documentation of vaccination, or technician's declining to be vaccinated, should be kept on file at each Clinical Center.

- A solution of 0.1% sodium hypochlorite (household bleach) is used to clean up any spills of blood, plasma, or serum; and all laboratory work surfaces at the completion of work activities.

2B. Necessary Equipment & Supplies

1. Refrigerated centrifuge with swinging bucket rotor (capable of achieving 30,000 g-minute spin)

2. -80^{o}C (or colder) Freezer

3. Butterfly blood collection apparatus, labmat, biohazard disposal, pipets and tips, tube racks, cryovials, gloves, tube mixer.

2C. Collection by Venipuncture

General:

Blood drawing is standardized for the sitting position. Optimally, venipuncture is performed with a 21-gauge butterfly needle with 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. Other gauges may be used if necessary. The butterfly has a short, thin-walled needle, which minimizes trauma to the skin and vein. The use of 12 inches of tubing allows tubes to be changed without any movement of the needle in the vein.

Procedure:

1. Arrange draw tubes within easy reach and in order of draw. Assemble butterfly apparatus and vacutainer holder, gauze, and alcohol prep prior to tourniquet application.

2. Apply tourniquet.

3. Examine arm for the best site for venipuncture. Release tourniquet.

4. Cleanse venipuncture site. Prepare area by wiping with alcohol swab in a circular motion from center to periphery. Allow area to dry.

5. Reapply tourniquet

6. Grasp the donor's arm firmly, using your thumb to draw the skin taut. This anchors the vein. The thumb should be 1 or 2 inches below the venipuncture site.

7. With the needle bevel upward, enter the vein in a smooth continuous motion.

8. Make sure the donor's arm is in a flat or downward position while maintaining the tube below the site when the needle is in the vein. It may be helpful to have the donor make a fist with the opposite hand and place it under the elbow for support.

9. Grasp the flange of the needle holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.

10. Note the blood flow into the first collection tube. If the flow rate is very slow, the needle may not be positioned correctly.

11. Keep a constant, slight forward pressure (in the direction of the needle) on the end of the

tube. This prevents release of the shutoff valve and stopping of blood flow. Do not vary pressure nor reintroduce pressure after completion of the draw.

12. Fill each vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and blood flow ceases. Then remove the tube from the holder. The shutoff valve re-covers the point, stopping blood flow until the next tube is inserted (if necessary).

13. To remove the needle, lightly place clean gauze over the venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle into a puncture-proof container. Have the donor hold the gauze pad firmly for one to two minutes to prevent a hematoma.

14. Apply an adhesive or gauze bandage over the venipuncture site after making sure that the blood flow has stopped.

15. After each tube is filled, mix by gentle inversion for approximately 30 seconds then put tubes in crushed ice. Do not place the red-topped serum tubes on ice.

NOTE 1: If possible, it is best to release the tourniquet as soon as possible after flow has been established. In our experience, however, especially with sick and/or elderly subjects, this may result in flow stopping, and the trauma of a second venipuncture. Therefore, this is a judgment call, based upon the phlebotomist's experience and skill.

NOTE 2: Attention should be paid to minimizing turbulence whenever possible. Small steps, such as slanting the needle in the vacutainer to have the blood run down the side of the tube instead of shooting all the way to the bottom, may result in significant improvement.

Procedures for Difficult Draw:

1. If there is a sucking sound, turn needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.

2. If no blood appears, move the needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.

3. Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a Velcro type, quickly release and press back together.

3. Blood Drawing Master Schedule

Heart Failure Network Blood Collection Master Table

Study Name	Collection	Components	Volume	Time point	Time point	Time point
INDIE-HF				***Baseline/Visit 1***	***Visit 2***	***Visit 3***
	Biomarkers	EDTA, serum	8mL	x	x	x
	Biorepository	EDTA, serum	8mL	x	x	x
	DNA	EDTA	10mL	x		
	Total Volume			***26mL***	***16mL***	***16mL***
SubQ-HF				***Baseline***	***Day 7***	***Day 30***
	Biomarkers	EDTA, serum	8mL	x	x	x
	Biorepository	EDTA, serum	8mL	x	x	x
	DNA	EDTA	10mL	x		
	Total Volume			***26mL***	***16mL***	***16mL***

The Blood Drawing Master Schedule lists the tubes to be drawn at each time point of each protocol. The number of protocols will change with time, and this Master Schedule will be updated as necessary.

4. Processing & Local Storage of Biomarker Blood Samples

Centrifugation:
NOTE: EDTA whole blood for Genomic and Pharmacogenomic Substudy (DNA) is **NOT CENTRIFUGED.** Whole bloods from these tubes are transferred to 10mL transport tubes and frozen at -80◦C (or colder) immediately

1. After venipuncture is completed, EDTA tubes must be stored on crushed ice immediately. The red-topped serum tubes must stand at room temperature for at least 40 minutes but less than 90 minutes to allow clotting before centrifugation.

2. Once serum in completely clotted, centrifuge Serum, and EDTA tubes (4^{o}C) at least at 2,000 x g x 15 minutes or 3,000 x g x 10 minutes for a total of 30,000 g-minutes.

3. Once centrifugation is complete, tubes are carefully placed on ice and are ready to aliquot

General Notes on Aliquoting:

1. Aliquoting consists of removing the plasma in small amounts (for example 1.0mL) by pipet and placing it into the appropriate color-coded cryovials with O-rings for optimal seal. The LCBR will provide these vials in the collection kits. Color-coding is predetermined and used to identify sample type such as serum vs. EDTA plasma. If for any reason color- coded vials are not available, the sample type must be clearly identified on the label. The LCBR will provide the labels that will withstand moisture and ultra-cold temperatures.

2. This process must be done while the tubes and cryovials are on ice (unless otherwise noted).

3. Be careful not to disturb the top of the cell pellet with the pipet tip, as this will result in platelet, white cell and red cell contamination.

4. Use a new pipet tip or transfer pipet for each draw tube.

5. Once the sample is aliquotted, cryovials should be snap-frozen in an upright position immediately at -80^{o}C (or colder) or in a methanol- or ethanol-dry, ice bath or if the above are unavailable, simply on a block of dry ice or pressed into some crushed dry ice.

Note: Proper labeling is *critical* on these cryovials. The LCBR will supply labels guaranteed to withstand freezing and thawing. Most standard office printers or typewriters are not capable of printing in a permanent ink. Writing directly on the cryovials (i.e., not using a label) is not recommended**.**

Specific Aliquoting Schemes for Blood Collection

The blood draw tubes in the HF Network Biomarker and Biorepository blood collections are numbered as follows:

No.	Type	Volume	Cap Color	Purpose
1	Serum	4 ml	Red	Biomarkers
2	EDTA	4 ml	Purple	Biomarkers
3	Serum	4 ml	Red	Biorepository
4	EDTA	4ml	Purple	Biorepository

Each time point for each protocol will use a specific set of tubes.

An additional 10mL EDTA draw tube will be collected at 1 time point if the participant consents to the Genomic and Pharmacogenomic Substudy. EDTA whole blood for the DNA substudy will only be drawn at one time point. The timing of this blood collection is dependent on when consent is granted by the participant.

No.	Type	Volume	Cap Color	Purpose
1	EDTA	10 ml	white	Whole Blood for DNA

HFN_SubQ In HFN_SubQ bloods will be drawn at 3 time points;

The HFN_SubQ Baseline blood draw consists of 4 tubes: If the participant consents to DNA blood collection, EDTA Tube #5 will be drawn in addition to these 4 tubes. Tube #5 is only drawn at 1 visit (may occur at another visit if consent occurs after baseline visit).

Collection Tube	Min Volume Needed after Centrifugation	Number of Aliquots	Color Code	Volume per Aliquot
#1: 4 ml Serum(for biomarkers)	2.0 mL	2	red	1.0mL
#2: 4 ml EDTA(for biomarkers)	2.0 mL	2	purple	1.0mL
#3: 4 ml Serum(for repository)	2.0mL	2	red	1.0mL
#4: 4 ml EDTA(for repository)	2.0mL	2	purple	1.0mL
#5: 10mL EDTA **WHOLE BLOOD**	**NOT CENTRIFUGED**	1	White	10.0mL

The HFN SubQ Day 7 (for those randomized to device) and Day 30 blood draws consists of 4 tubes:

Collection Tubes	Min Volume Needed after Centrifugation	Number of Aliquots	Color Code	Volume per aliquot
#1:4 ml Serum (for biomarkers)	2.0 mL	2	red	1.0mL
#2:4 ml EDTA (for biomarkers)	2.0 mL	2	purple	1.0mL
#3:4 ml Serum (for repository)	2.0mL	2	red	1.0mL
#4:4 ml EDTA (for repository)	2.0mL	2	purple	1.0mL

HFN_INDIE In HFN_INDIE, bloods will be drawn at 3 time points.

The HFN_INDIE Baseline/Visit 1 blood draw consists of 4 tubes: If the participant consents to DNA blood collection, EDTA Tube #5 will be drawn in addition to these 4 tubes. Tube #5 is only drawn at 1 visit (may occur at another visit if consent occurs after baseline visit).

Collection Tube	Min Volume Needed after Centrifugation	Number of Aliquots	Color Code	Volume per Aliquot
#1: 4 ml Serum(for biomarkers)	2.0 mL	2	red	1.0mL
#2: 4 ml EDTA(for biomarkers)	2.0 mL	2	purple	1.0mL
#3: 4 ml Serum(for repository)	2.0mL	2	red	1.0mL
#4: 4 ml EDTA(for repository)	2.0mL	2	purple	1.0mL
#5: 10mL EDTA **WHOLE BLOOD**	**NOT CENTRIFUGED**	1	White	10.0mL

The HFN INDIE Visit 2 blood draw consists of 4 tubes:

Collection Tubes	Min Volume Needed after Centrifugation	Number of Aliquots	Color Code	Volume per aliquot
#1:4 ml Serum (for biomarkers)	2.0 mL	2	red	1.0mL
#2:4 ml EDTA (for biomarkers)	2.0 mL	2	purple	1.0mL
#3:4 ml Serum (for repository)	2.0mL	2	red	1.0mL
#4:4 ml EDTA (for repository)	2.0mL	2	purple	1.0mL

The HFN INDIE Visit 3 blood draw consists of 4 tubes:

Collection Tubes	Min Volume Needed after Centrifugation	Number of Aliquots	Color Code	Volume per aliquot
#1:4 ml Serum (for biomarkers)	2.0 mL	2	red	1.0mL
#2:4 ml EDTA (for biomarkers)	2.0 mL	2	purple	1.0mL
#3:4 ml Serum (for repository)	2.0mL	2	red	1.0mL
#4:4 ml EDTA (for repository)	2.0mL	2	purple	1.0mL

5. Shipping of Blood Samples to VT Core Lab

These instructions pertain to DNA, Biomarker, and Biorepository frozen blood samples shipments to the Vermont Core Lab. Shipping to the Core Lab should occur **the FIRST week of each month only**. If you cannot ship this week, please contact the Core Lab to arrange shipping for another time. Shipments should be made on either Monday, Tuesday preferably, but absolutely no later than Wednesday to allow time for shipping delays. All frozen sample shipments are to be made via **FedEx Priority Overnight** service on dry ice. Use the pre-printed Fed Ex airbills provided by the Core Lab to ensure the safe receipt of study samples to the Core Lab,

1. Line a shipping container with absorbent material (i.e. lab mat, or paper toweling).

2. Place approximately 5 to 10 lbs of dry ice on the bottom of the shipping container. Amount of dry ice will vary depending on the amount of samples being shipped. **Always err on the side of too much dry ice.**

3. Place another layer of absorbent material (i.e. lab mat) on top of the dry ice – so it will be between the dry ice and the freezer boxes.

4. Collect the freezer boxes containing samples to be shipped, and check the sample ID numbers against the Shipping Form for that shipment. See Appendix for box diagrams on freezer box organization.

5. Wrap absorbent material around the box and secure with a rubber band around the box.

6. Place each freezer box in a zip lock plastic bag and seal tightly.

7. Place zip locked freezer boxes in the shipping container. Note: the zip lock bags should NOT be in direct contact with the dry ice.

8. Add another layer of absorbent material on top of the freezer boxes in the shipping container.

9. Add remaining 5-10 lbs of dry ice to the shipping container. Close and tape down the Styrofoam lid in a few spots.

10. Seal Phlebotomy/Processing Forms in a zip lock bag and place on top of the Styrofoam lid. Include a cover sheet with recipient address and contact information.

11. Close the top of the outer cardboard sleeve of the shipping container with packing tape.

12. Affix shipping labels (pre-printed Fed Ex air bill, Biological Specimen Category B UN3373 label, and Dry Ice Class 9 UN1845 labels) to outside of shipping container (all labels are provided by the Core Lab. It is especially important to use the pre-

printed Fed ex air bills provided by the core lab)

13. Add extra shipping tape over the labels to ensure they will not fall off in transit.

Fill out the Shipping Log including the FedEx air bill #s and fax to the University of Vermont at (802) 656-8965.

NOTE: This shipping protocol follows the procedures mandated by the International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions 650 and 904. Copies of these regulations are included with this MOP.

Shipping Address:

Rebekah Boyle (802) 656-8938
University of Vermont – Pathology
360 South Park Drive
Colchester, VT 05446
Rebekah.Boyle@uvm.edu

The frozen samples will be cataloged and stored at -80^{o}C at the Vermont Core Lab either until they are analyzed or until the end of the study. Frozen DNA samples will be stored at -80C at the Vermont Core Lab until shipped to Montreal Heart Institute.

6. Forms

6a. VT Core Lab Blood Shipment Form

6b. SubQ Phlebotomy and Processing Form

6c. SubQ Blood Collection Processing Guide

6d. INDIE Phlebotomy and Processing Form

6e. INDIE Blood Collection and Processing Guide

6f. HFN Biomarker Core Lab Supply Request Form

HEART FAILURE NETWORK

Fax to: (802) 656-8965
ATTN: Rebekah Boyle

Heart Failure Network-VT Core Lab
SHIPPING FORM

Date of shipment: _____
Center: _____ Prepared by: _____

FedEx Air Bill#: _____

HFN-Protocol	Samples Included	BOX #
Example: SubQ	*SQ-015 Baseline*	*1*

For LCBR use only:
Date Received at LCBR:_____ Frozen: Y / N

Affix SubQ ID label here

HFN_SubQ
Blood Collection
PHLEBOTOMY / PROCESSING FORM

CHECK VISIT: ☐ **Baseline** ☐ **Day 7** ☐ **Day 30**

Blood Collection Date: ☐☐/☐☐☐/☐☐☐☐ (D D M M M Y Y Y Y) Phlebotomist Tech Initials: ☐☐☐ (First M Last)

BLOOD COLLECTION

1. Method: ☐ Venipuncture ☐ Other (specify): _____ Needle size: ☐☐ gauge

2. Record Start Time of Serum Tube# 01 Collection: ☐☐ (Hours) : ☐☐ (Minutes) AM or PM

3. Blood Volume per Tube:

	Yes	No	Filled Partial	Other (specify volume)	Tube Handling
*Bloods for **Biomarkers**:*					
Tube# 01 Serum 4.0 mL				_____	Room Temp 40 mins
Tube# 02 EDTA 4.0 mL				_____	Mix 30 sec; then on ice
*Bloods for **Biorepository***:					
Tube #03 Serum 4.0 mL				_____	Room Temp 40 mins
Tube #04 EDTA 4.0 mL					Mix 30 sec; then on ice
***Only collected at 1 time point**:*					
DNA EDTA 10mL	___	___	____	_____	Invert 5X; **DO NOT CENTRIFUGE**

PROCESSING

Centrifuge Start Time: ☐☐ (Hours) ☐☐ (Minutes) AM or PM Processor Tech Initials: ☐☐☐ (First M Last)

#	Draw Tube	Purpose	Cryo size (mL)	Cryo #	Color Code	Sample Vol. mL	If P*	If H*	If Done
01	Serum	Serum for Biomarkers	1.5mL	**01**	red	1.0			
		Serum for Biomarkers	1.5mL	**02**	red	1.0			
		EDTA plasma for Biomarkers	1.5mL	**03**	purple	1.0			
02	EDTA	EDTA plasma for Biomarkers	1.5mL	**04**	purple	1.0			
03	Serum	Serum for Biorepository	1.5mL	**05**	red	1.0			
		Serum for Biorepository	1.5mL	**06**	red	1.0			
04	EDTA	EDTA plasma for Biorepository	1.5mL	**07**	purple	1.0			
		EDTA plasma for Biorepository	1.5mL	**08**	purple	1.0			
05	EDTA	DNA-1 visit only-**do not centrifuge**	10mL	**DNA**	white	10.0			

** P = partially filled cryovial; H = hemolysis*

Comments:

_____

_____

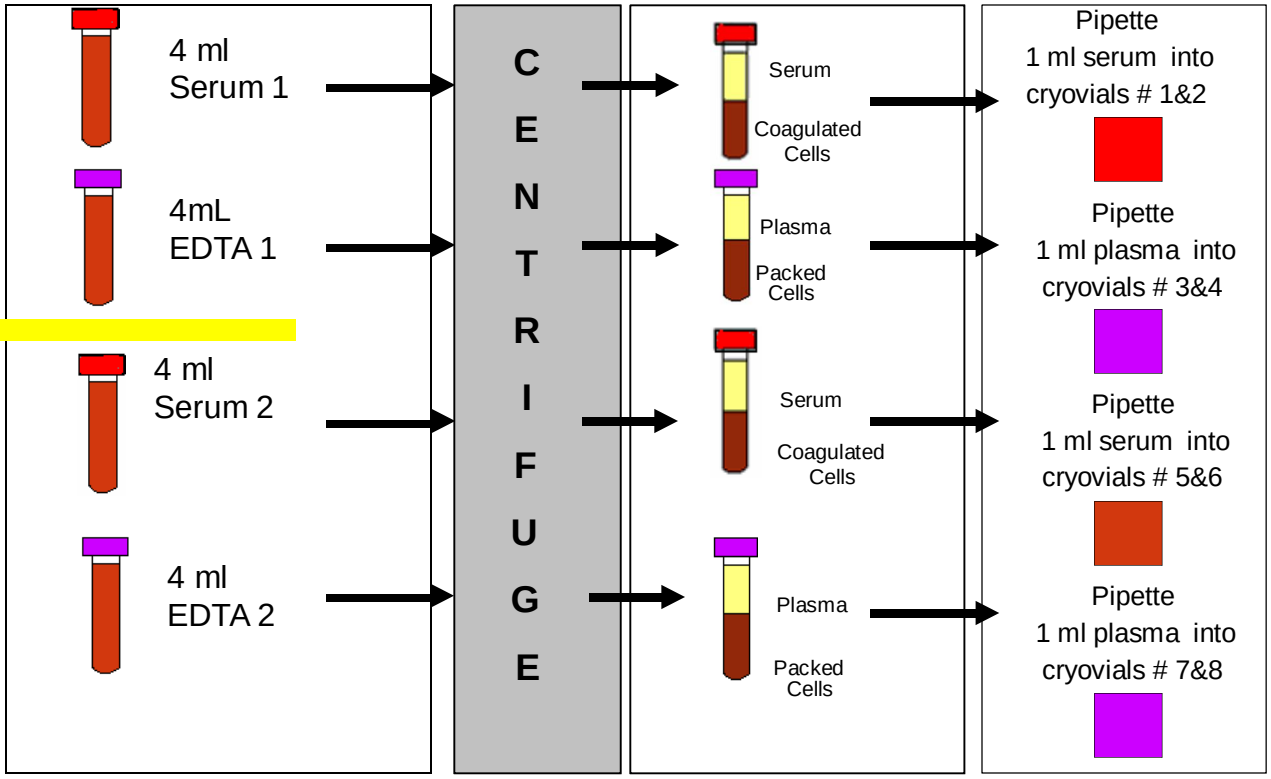
For LCBR use only:

Date Samples Received: ____/____/_____ Samples Received Frozen:__Yes ____No

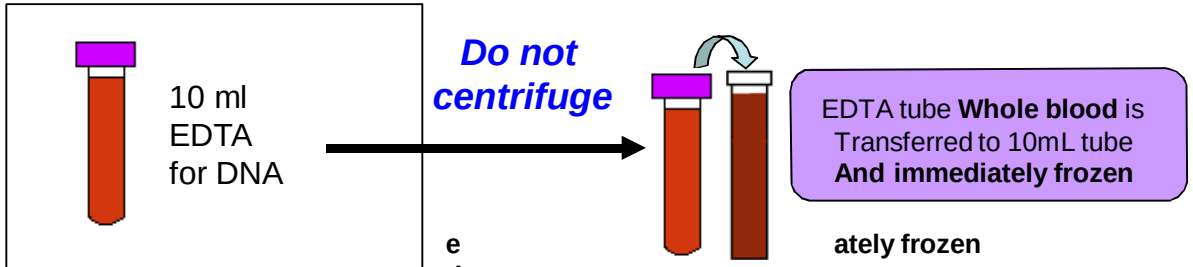
HFN-SubQ Protocol

Biomarker and DNA Blood Processing Guide

After the Baseline, Day 7, and Day 30 blood collections for *biomarkers* you should have:



If participant consents, EDTA whole blood will be collected at 1 time point for DNA:

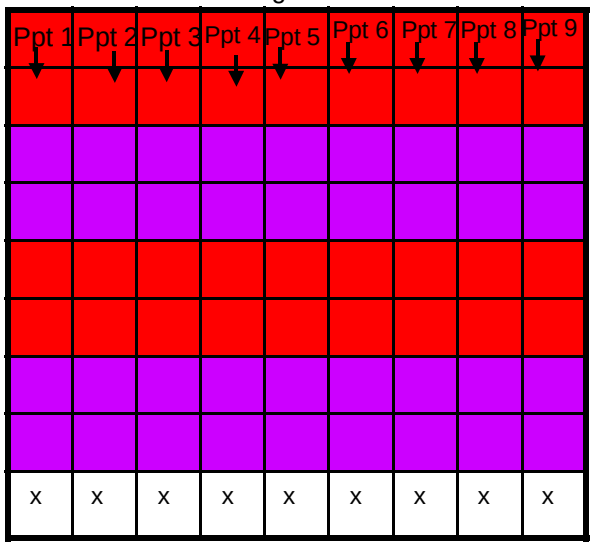


Freezer Boxes

Send to Vermont

Freezer box for Biomarker Cryovials

9x9 grid



Freezer box for Whole blood (DNA tubes)

7x7 grid

Pt 1	Pt 8	Pt 15	Pt 22	Pt 29	Pt 36	Pt 43
Pt 2	Pt 9	Pt 16	Pt 23	Pt 30	Pt 37	Pt 44
Pt 3	Pt 10	Pt 17	Pt 24	Pt 31	Pt 38	Pt 45
Pt 4	Pt 11	Pt 18	Pt 25	Pt 32	Pt 39	Pt 46
Pt 5	Pt 12	Pt 19	Pt 26	Pt 33	Pt 40	Pt 47
Pt 6	Pt 13	Pt 20	Pt 27	Pt 34	Pt 41	Pt 48
Pt 7	Pt 14	Pt 21	Pt 28	Pt 35	Pt 42	Pt 49

HEART FAILURE NETWORK

HFN_INDIE
Blood Collection
PHLEBOTOMY / PROCESSING FORM

Affix INDIE ID label here

CHECK VISIT: ☐ **Baseline/V1** ☐ **Visit 2** ☐ **Visit 3**

Blood Collection Date: ☐☐/☐☐☐/☐☐☐☐ (D D / M M M / Y Y Y Y) Phlebotomist Tech Initials: (First M Last)

BLOOD COLLECTION

1. Method: ☐ Venipuncture ☐ Other (specify): _____ Needle size: ☐☐ gauge

2. Record Start Time of Serum Tube# 01 Collection: ☐☐ (Hours) : ☐☐ (Minutes) AM or PM

3. Blood Volume per Tube:

	Yes	No	Filled Partial	Other (specify volume)	Tube Handling
*Bloods for **Biomarkers**:*					
Tube# 01 Serum 4.0 mL				_____	Room Temp 40 mins
Tube# 02 EDTA 4.0 mL				_____	Mix 30 sec; then on ice
*Bloods for **Biorepository**:*					
Tube #03 Serum 4.0 mL				_____	Room Temp 40 mins
Tube #04 EDTA 4.0 mL					Mix 30 sec; then on ice
***Only collected at 1 time point**:*					
DNA EDTA 10mL	___	___	_____	_____	Invert 5X; **DO NOT CENTRIFUGE**

PROCESSING

Centrifuge Start Time: ☐☐ (Hours) ☐☐ (Minutes) AM or PM Processor Tech Initials: ☐☐☐ (First M Last)

#	Draw Tube	Purpose	Cryo size (mL)	Cryo #	Color Code	Sample Vol. mL	If P*	If H*	If Done
01	Serum	Serum for Biomarkers	1.5mL	**01**	red	1.0			
		Serum for Biomarkers	1.5mL	**02**	red	1.0			
02	EDTA	EDTA plasma for Biomarkers	1.5mL	**03**	purple	1.0			
		EDTA plasma for Biomarkers	1.5mL	**04**	purple	1.0			
03	Serum	Serum for Biorepository	1.5mL	**05**	red	1.0			
		Serum for Biorepository	1.5mL	**06**	red	1.0			
04	EDTA	EDTA plasma for Biorepository	1.5mL	**07**	purple	1.0			
		EDTA plasma for Biorepository	1.5mL	**08**	purple	1.0			
05	EDTA	DNA-1 visit only-**do NOT centrifuge**	10mL	**DNA**	white	10.0			

P = partially filled cryovial; H = hemolysis

Comments:

_____

_____

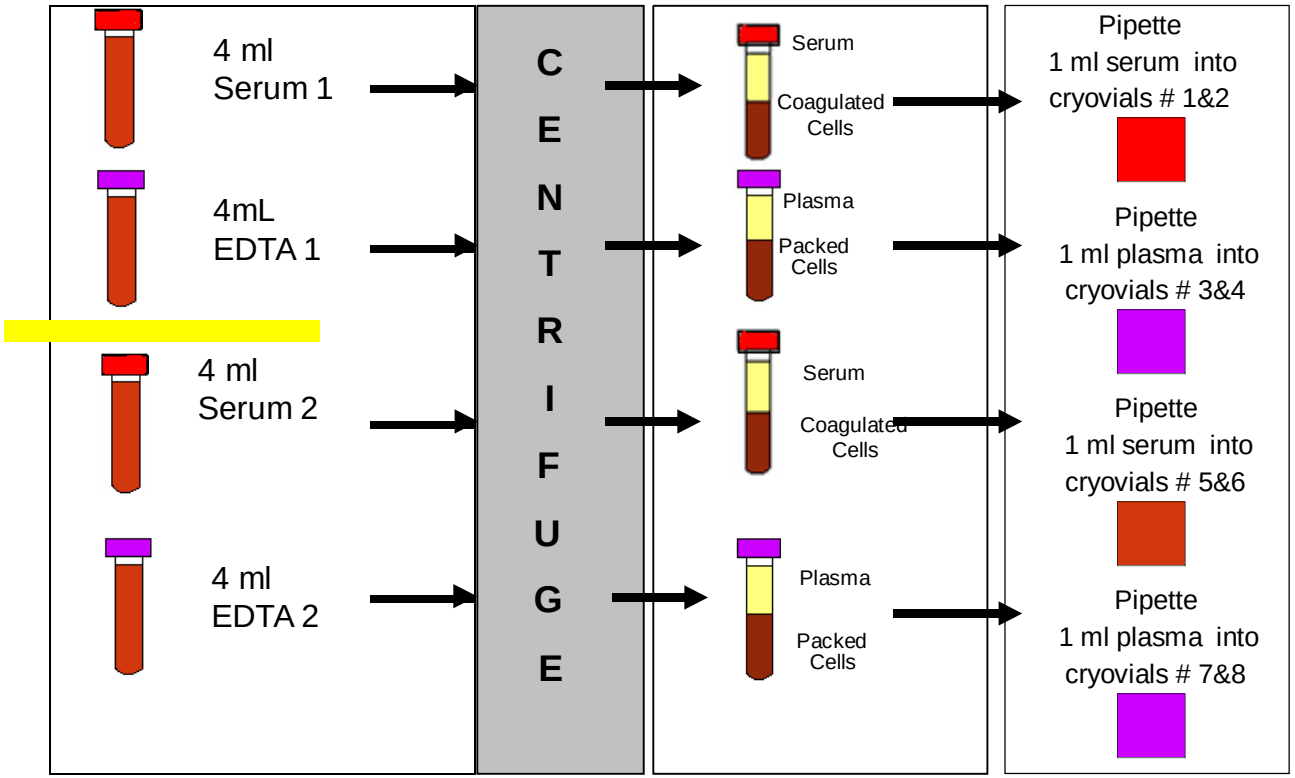
For LCBR use only:

Date Samples Received: ____/____/_____ Samples Received Frozen:__Yes ____No

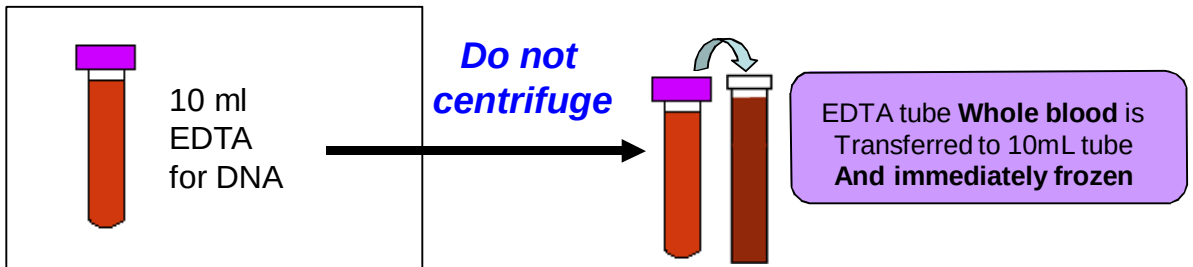
HFN-INDIE Protocol

Biomarker and DNA Blood Processing Guide

After the Baseline/Visit 1, Visit 2 and Visit 3 blood collections for biomarkers, you should have:



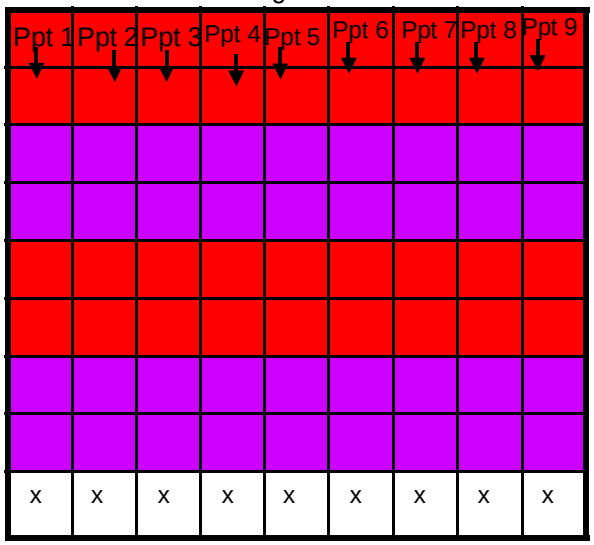
If participant consents, EDTA whole blood will be collected at 1 time point for DNA:



Freezer Boxes

Send to Vermont

Freezer box for Biomarker Cryovials
9x9 grid



Freezer box for Whole blood (DNA tubes)
7x7 grid

Pt 1	Pt 8	Pt 15	Pt 22	Pt 29	Pt 36	Pt 43
Pt 2	Pt 9	Pt 16	Pt 23	Pt 30	Pt 37	Pt 44
Pt 3	Pt 10	Pt 17	Pt 24	Pt 31	Pt 38	Pt 45
Pt 4	Pt 11	Pt 18	Pt 25	Pt 32	Pt 39	Pt 46
Pt 5	Pt 12	Pt 19	Pt 26	Pt 33	Pt 40	Pt 47
Pt 6	Pt 13	Pt 20	Pt 27	Pt 34	Pt 41	Pt 48
Pt 7	Pt 14	Pt 21	Pt 28	Pt 35	Pt 42	Pt 49

Heart Failure Network Core Lab Supply Request Form

FAX to: (802) 656-8965
Attn: R. BOYLE

Ordered by:
Site Name:
Site #:

LCBR:
DATE: / /
Needed By: / /

Comments:

General HFN Supplies

Fisher Sci. Catalog #	Item	Manufacturer	qty /unit	Unit (CIRCLE)	Qty Requested	Comment
not applicable	pre-printed Fed Ex air bills	Fed Ex	1 airbill	airbill		
05-680-00	Tube, Transport; Simport	Simport	25/pk *	pk		
02-681-338	1.5ml Cryos (500/pk)	Fisher Scientific	500/pk	pk		
02-681-361	RED caps (500/pk)	Fisher Scientific	500/pk	pk		
02-681-366	PURPLE caps (500/pk)	Fisher Scientific	500/pk	pk		
03-530-17	medium Insulated Shippers (14L x 14W x 14.63in.H)	SCA Thermosafe	1 box* or 4/cs	cs or box		
02-657-32	10mL K2 EDTA vacutainer	BD 366643	1 tube*	tube		
02-689-4	4mL K2 EDTA Vacutainer	BD 367844	1 tube*	tube		
02-685-111	4mL Serum Vacutainer (RED)	BD 367812	1 tube*	tube		
13-711-24	1.0mL transfer pipets	Samco	500/pk	pk		
06-670-50	4" x 4" Absorbent pads	Fisher Scientific	200/pk	pk		
22-130-067	IATA UN3373 Labels	Therapak	10/pk*	pk		
22-130-065	IATA UN1845 labels (dry ice)	Therapak	10/pk*	pk		
11-678-24B	2" Freezer Fiberboard Storage	Revco	1 box* or	pk or box		
13-989-218	81 cell grid for 2" freezer storage boxes	Revco	12/pk	pk		
11-678-24A	3" Freezer Fiberboard Storage	Revco	1 box* or	pk or box		
11-678-26D	49 cell grid for 3" freezer storage boxes	Revco	12/pk	pk		

	HFN Protocol	Unit	Qty	Comment
Blood Collection Kits and Labels	SubQ	1 kit		
	SubQ Labels	specify id range		
	INDIE	1 kit		
	INDIE Labels	specify id range		
	DNA	1 kit		

** items that will be ordered in bulk by Core Lab and shipped in smaller quantities to sites*

7. Freezer Box Diagrams

7a. Biomarker and Biorepository Samples Freezer Box Diagram

Freezer Box Diagram for Shipments of Frozen Blood Samples the Vermont HFN Core Lab

Box holds frozen EDTA and Serum cryovials for up to 9 participants/timepoints.
8 Cryovials per participant/timepoint: FOUR (4) purple-capped/EDTA (#3,4,7, and 8) and FOUR (4) red- capped/Sera (# 1,2, 5, and 6) 2" Revco Freezer box with a 9x9 grid

Ppt 1 Cryo 1	Ppt 2 Cryo 1Ppt	Ppt 3 Cryo 1	Ppt 4 Cryo 1	Ppt 5 Cryo 1	Ppt 6 Cryo 1	Ppt 7 Cryo 1	Ppt 8 Cryo 1	Ppt 9 Cryo 1
Ppt 1 Cryo 2	Ppt 2 Cryo 2Ppt	Ppt 3 Cryo 2	Ppt 4 Cryo 2	Ppt 5 Cryo 2	Ppt 6 Cryo 2	Ppt 7 Cryo 2	Ppt 8 Cryo 2	Ppt 9 Cryo 2
Ppt 1 Cryo 3	Ppt 2 Cryo 3Ppt 2	Ppt 3 Cryo 3	Ppt 4 Cryo 3	Ppt 5 Cryo 3	Ppt 6 Cryo 3	Ppt 7 Cryo 3	Ppt 8 Cryo 3	Ppt 9 Cryo 3
Ppt 1 Cryo 4	Ppt 2 Cryo 4	Ppt 3 Cryo 4	Ppt 4 Cryo 4	Ppt 5 Cryo 4	Ppt 6 Cryo 4	Ppt 7 Cryo 4	Ppt 8 Cryo 4	Ppt 9 Cryo 4
Ppt 1 Cryo 5	Ppt 2 Cryo 5	Ppt 3 Cryo 5	Ppt 4 Cryo 5	Ppt 5 Cryo 5	Ppt 6 Cryo 5	Ppt 7 Cryo 5	Ppt 8 Cryo 5	Ppt 9 Cryo 5
Ppt 1 Cryo 6	Ppt 2 Cryo 6	Ppt 3 Cryo 6	Ppt 4 Cryo 6	Ppt 5 Cryo 6	Ppt 6 Cryo 6	Ppt 7 Cryo 6	Ppt 8 Cryo 6	Ppt 9 Cryo 6
Ppt 1 Cryo 7	Ppt 2 Cryo 7	Ppt 3 Cryo 7	Ppt 4 Cryo 7	Ppt 5 Cryo 7	Ppt 6 Cryo 7	Ppt 7 Cryo 7	Ppt 8 Cryo 7	Ppt 9 Cryo 7
Ppt 1 Cryo 8	Ppt 2 Cryo 8	Ppt 3 Cryo 8	Ppt 4 Cryo 8	Ppt 5 Cryo 8	Ppt 6 Cryo 8	Ppt 7 Cryo 8	Ppt 8 Cryo 8	Ppt 9 Cryo 8
X	X	X	X	X	X	X	X	X

7b. DNA Sample Freezer Box Diagram

Freezer Box Diagram for Shipment of
Frozen EDTA Whole Blood (DNA Blood Sample) to the Vermont Core Lab

EDTA Whole Blood – White-capped 10mL Tube

10 ml sample volume. 1 tube per participant.

Box holds frozen EDTA Whole blood tubes for up to 49 participants.

3" Revco Freezer box with a 7x7 grid

PT 1	PT 8	PT 15	PT 22	PT 29	PT 36	PT 43
PT 2	PT 9	PT 16	PT 23	PT 30	PT 37	PT 44
PT 3	PT 10	PT 17	PT 24	PT 31	PT 38	PT 45
PT 4	PT 11	PT 18	PT 25	PT 32	PT 39	PT 46
PT 5	PT 12	PT 19	PT 26	PT 33	PT 40	PT 47
PT 6	PT 13	PT 20	PT 27	PT 34	PT 41	PT 48
PT 7	PT 14	PT 21	PT 28	PT 35	PT 42	PT 49

PACKING INSTRUCTION 650

STATE VARIATIONS: BHG-02, CAG-05, DQG-03, FRG-05, GBG-05, VCG-04

OPERATOR VARIATIONS: AF-02, AM-06/10, AR-02, AS-08, BR-14, BZ-07, CI-01, CO-07, CS-07, FX-09, IJ-06/10, JJ-06, JK-03, KC-08, KE-06, LA-07, LH-05, MN-03, MS-06, MX-06/11, OO-01, OU-12/16, PX-08, SQ-10, SV-12, TN-05, TY-03, UA-14, UU-05

This instruction applies to UN 3373 on passenger and cargo aircraft and Cargo Aircraft Only.

General Requirements

The packagings must be of good quality, strong enough to withstand the shocks and loadings normally encountered during transport, including trans-shipment between transport units and between transport units and warehouses as well as any removal from a pallet or overpack for subsequent manual or mechanical handling. Packagings must be constructed and closed so as to prevent any loss of contents that might be caused under normal conditions of transport, by vibration, or by changes in temperature, humidity or pressure.

The packaging must consist of three components:

(a) a primary receptacle(s);

(b) a secondary packaging; and

(c) a rigid outer packaging.

Primary receptacles must be packed in secondary packagings in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packagings must be secured in outer packagings with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

Packages must be prepared as follows:

(a) For liquid substances:

- The primary receptacle(s) must be leakproof and must not contain more than 1 L;
- The secondary packaging must be leakproof;
- If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them;
- Absorbent material must be placed between the primary receptacle and the secondary packaging. The absorbent material, such as cotton wool, must be in sufficient quantity to absorb the entire contents of the primary receptacle(s) so that any release of the liquid substance will not compromise the integrity of the cushioning material or of the outer packaging;
- The primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure of 95 kPa in the range of -40°C to 55°C (-40°F to 130°F).

Note:
The capability of a packaging to withstand an internal pressure without leakage that produces the specified pressure differential should be determined by testing samples of primary receptacles or secondary packagings. Pressure differential is the difference between the pressure exerted on the inside of the receptacle or packaging and the pressure on the outside. The appropriate test method should be selected based on receptacle or packaging type. Acceptable test methods include any method that produces the required pressure differential between the inside and outside of a primary receptacle or a secondary packaging. The test may be conducted using internal hydraulic or pneumatic pressure (gauge) or external vacuum test methods. Internal hydraulic or pneumatic pressure can be applied in most cases as the required pressure differential can be achieved under most circumstances. An external vacuum test is not acceptable if the specified pressure differential is not achieved and maintained. The external vacuum test is a generally acceptable method for rigid receptacles and packagings but is not normally acceptable for:

- flexible receptacles and flexible packagings;
- receptacles and packagings filled and closed under a absolute atmospheric pressure lower than 95 kPa.
- The outer packaging must not contain more than 4 L. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold.

(b) For solid substances:

- The primary receptacle(s) must be siftproof and must not exceed the outer packaging weight limit;
- The secondary packaging must be siftproof;
- If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them;
- Except for packages containing body parts, organs or whole bodies, the outer packaging must not contain more than 4 kg. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold;
- If there is any doubt as to whether or not residual liquid may be present in the primary receptacle during transport then a packaging suitable for liquids, including absorbent materials, must be used.

An itemized list of contents must be enclosed between the secondary packaging and the outer packaging.

At least one surface of the outer packaging must have a minimum dimension of 100 mm × 100 mm (4 in × 4 in).

The completed package must be capable of successfully passing the drop test described in 6.5.1.1 except that the height of the drop must not be less than 1.2 m. Following the appropriate drop sequence, there must be no leakage from the primary receptacle(s) which must remain protected by absorbent material, when required, in the secondary packaging.

For transport, the mark illustrated below must be displayed on the external surface of the outer packaging on a background of a contrasting colour and must be clearly visible and legible. The mark must be in the form of a square set at an angle of 45° (diamond-shaped) with each side having a length of at least 50 mm (2 in), the width of the line must be at least 2 mm and the letters and numbers must be at least 6 mm high. The proper shipping name "Biological Substance, Category B" in letters at least 6 mm high must be marked on the outer packaging adjacent to the diamond-shaped mark.

Unless all package markings are clearly visible, the following conditions apply when packages are placed in an overpack:

- the overpack must be marked with the word "Overpack"; and
- the package markings must be reproduced on the outside of the overpack.

A Shipper's Declaration for Dangerous Goods is not required.

Alternative packagings for the transport of animal material may be authorized by the competent authority in accordance with the provisions in 5.0.6.7.

Specific Requirements

Refrigerated or frozen specimens: Ice, dry ice and liquid nitrogen:

- When dry ice or liquid nitrogen is used to keep specimens cold, all applicable requirements of these Regulations must be met. When used, ice or dry ice must be placed outside the secondary packagings or in the outer packaging or an overpack. Interior supports must be provided to secure the secondary packagings in the original position after the ice or dry ice has dissipated. If ice is used, the outside packaging or overpack must be leakproof. If dry ice is used, the packaging must be designed and constructed to permit the release of carbon dioxide gas to prevent a build-up of pressure that could rupture the packagings.
- The primary receptacle and the secondary packaging must maintain their integrity at the temperature of the refrigerant used as well as the temperatures and the pressures, which could result if refrigeration were to be lost.

Infectious substances assigned to UN 3373 which are packed and marked in accordance with this packing instruction are not subject to any other requirement of these Regulations except for the following:

(a) the name and address of the shipper and of the consignee must be provided on each package;

(b) the name and telephone number of a person responsible must be provided on the air waybill or on the package;

(c) the classification must be in accordance to 3.6.2;

(d) the incident reporting requirements in 9.6.1 must be met; and

(e) the inspection for damage or leakage requirements in 9.4.1 and 9.4.2.

Note:
When the shipper or consignee is also the 'person responsible' as referred to in b) above, the name and address need be marked only once in order to satisfy the name and address marking provisions in both a) and b), above.

Passengers and crew members are prohibited from transporting infectious substances as or in carry-on baggage, checked baggage or on their person.

If an Air Waybill is used, the "Nature and Quantity of Goods" box must show "UN 3373", the text "BIOLOGICAL SUBSTANCE, CATEGORY B" and the number of packages.

Clear instructions on filling and closing such packages must be provided by packaging manufacturers and subsequent distributors to the shipper or to the person who prepares the package (e.g. patient) to enable the package to be correctly

Other dangerous goods must not be packed in the same packaging as Division 6.2 Infectious Substances unless they are necessary for maintaining the viability, stabilizing or preventing degradation or neutralizing the hazards of the infectious substances. A quantity of 30 mL or less of dangerous goods included in Classes 3, 8 or 9 may be packed in each primary receptacle containing infectious substances provided these substances meet the requirements of 2.6. When these small quantities of dangerous goods are packed with infectious substances in accordance with this packing instruction, no other requirements in these Regulations need be met.

9. Commercial Invoice Template (for Canadian shipments of bloods to Vermont):

Proforma Invoice

DATE _____

Sender:

Insert your site return address

Recipient:

Rebekah Boyle, Lab Manager
Tel (802) 656-8938
Laboratory for Clinical Biochemistry Research
UVM – Pathology
360 South Park Drive
Colchester, VT 05446
USA

AIR WAYBILL Number: _____

Description:

This insulated container contains _____ cardboard boxes and dry ice. Each box contains ____ vials of human biological substances (blood specimens) – non-infectious, non-hazardous, non-dangerous human blood serum samples for in vitro research purposes only.
This shipment is packed in compliance with IATA regulations 650 and 904.
There is no commercial value to these contents.
The shipping containers are valued at *$90.00 USD* for customs purposes.

Total weight: _____ Kg
Dry Ice weight: _____ Kg

Country of Origin: Canada

Reason for Exportation: Biological Substance (human blood, non-infectious) for in vitro research purposes only.

All the reported information is correct.

Signed: _____ Date _____
person responsible for shipping at site

Mayo Physical Activity Measurement Core Laboratory:

Manual of Procedures

James A. Levine, Principal Investigator

Gabriel Koepp, Study Director

Graham Moore, Research Assistant

The Mayo Physical Activity Measurement Core Laboratory, or AXM Core Lab, is responsible for providing object measure of physical activity (PA) during course of the INDIE-HFpEF study. AXM Core Lab will assemble, program, and distribute accelerometers to each study location, provide assistance to on-site study coordinators during the data collection process, and retrieve, clean, and analyze PA data.

CONTACTING AXM CORE LAB

AXM Core Lab is located at the Scottsdale campus of Mayo Clinic Arizona. The lab team is available by phone from 8:00 – 5:00 MST and at all times via email. To contact the AXM Core Lab:

Email: koepp.gabriel@mayo.edu

Phone: (480) 301 – 9125

Address: Mayo Clinic Scottsdale, CRB 2-208, 13400 E. Shea Blvd., Scottsdale, AZ 85259

DEVICE INFORMATION

Kinetic Activity Monitors (KAMs) will be used to collect information on subject PA patterns.

Model: AM250
Type: Tri-axial accelerometer
Sensor Model: Kionix, Model KXSD9-2050
Range: ± 4Gs
Dimensions: 5.80 cm x 4.26 cm x 1.45 cm
Weight: 20.0 g
Manufacturer: Kersh, LLC

For this study, subjects will wear two KAMs attached to an elastic belt. These accelerometers cannot be removed from the belt but are adjustable along the band. Two KAM devices are worn to provide duplicate data sets and to prevent data loss in the event of device failure. Elastic belts are 60" in length and will fit most subjects 20-40 kg/m^2.

SITE PREPARATION

Prior to study commencement, each site will receive an initial shipment of PA monitoring supplies. This will include:

1. Accelerometer Return Forms

   An Accelerometer Return Form (ARF) is a physical document study coordinators are required to fill out and return with each KAM belt. ARFs serve as a hardcopy backup for device-wear information. The time and date of device placement and removal are particularly important to the data analysis process. Be sure to indicate the correct time according to a 24 hour clock. See Page 6 for ARF template and required information.

2. A base supply of KAM belts

   Each site will start the study with a base stock of KAM belts. The initial number of belts is determined by that site's patient enrollment history in the NEAT-HFpEF study. Locations with a history of high volume recruitment will receive 8 belts; whereas those with a history of lower recruitment will receive 4. New study sites will also begin with 4 belts.

3. Sample KAM belt

This belt will be labeled "TEST" and is not to be used for data collection. Instead, the purpose of this device is to a) be used during the screening process to insure that the patient is able to wear a standard belt and tolerate the device prior to randomization and b) provide researchers with an opportunity to become familiar with the device prior to data collection.

REQUESTING ADDITIONAL DEVICES

Once recruitment begins, many sites will require additional belts to accommodate enrollment or to replace a lost, damaged, or defective device. These belts must be requested from the AXM Core Lab **at least 7 days in advance.**

To Request Additional Belts:

1. Email AXM Core Lab koepp.gabriel@mayo.edu
   Email Template
   Subject: INDIE Belt Request - site ###
   a. **Number of belts requested**
   b. **Need-by date**
   c. **Reason for request, e.g. new enrollment or lost device**

ISSUING DEVICES TO PARTICIPANTS

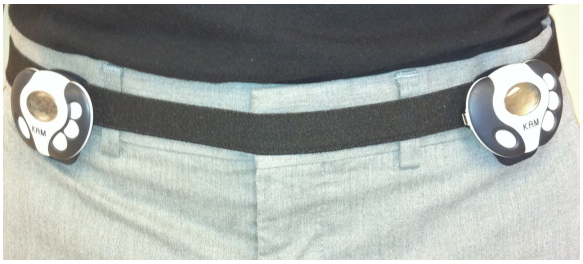
Prior to randomization, it is important to confirm that the patient can complete the protocol. Use the sample belt to insure the participant's body size allows wearing a standard KAM belt and that the patient feels they can tolerate the device. Note that some extra-large belts (>60") are available by request.

To Issue Devices to Participants:

1. Review the following instructions with the subject:
   a. KAM belts will be worn throughout the entirety of the study duration
   b. KAM belts will be worn 24 hours per day
      i. Yes, this includes during sleep.
      ii. The one exception is that belts should be removed during significant interactions with water, such as swimming or showering. KAMs are water resistant but not water proof.
      iii. If for any reason the participant does remove the belt for an extended period of time, we ask that they record the incident and report it to the study coordinator at their next visit
   c. KAMs devices should be positioned at each hip bone.
      i. However, the devices are prone to shifting position during wear time. Movement of the accelerometers on the belt will not affect accuracy of data collection but participants are encouraged to adhere to the standard positioning as often as possible.
      ii. Belts can be put on/taken off by undoing the clasp OR by stepping in/out of the belt. For those whose experience difficulty or pain when gripping objects, the stepping method may be preferable.
2. On ARF complete Fields 1 – 4
3. Activate each KAM on the belt

   a. Press the Mode and Clock buttons together twice
   b. After the second time, the letters "L-H-S" should appear on the top of the display screen and begin to blink. This means the device is now recording data.
      i. If a blinking "L-H-S" does not appear, try pressing the Mode and Clock buttons together a few more times. If that still does not work, call AXM Lab and report.
      ii. If a battery icon is visable, there is a low battery. Please contact AXM lab koepp.gabriel@mayo.edu. This is extremely rare.
   c. Once started, devices will not stop recording until memory is full.

4. Assist the subject in putting on the belt
   a. Place the belt so that the band rests around the subject's hip bones. Fasten the buckle.
   b. Tighten the elastic band, pulling until snug. Use scissors to remove excess band.
   c. Adjust KAMs so that one is positioned on each hip bone.
5. On ARF, complete Field 5



RETRIEVING DEVICES FROM PARTICIPANTS

To Retrieve Belts:

1. Ask participant to remove and return belt. Assist if necessary.
2. On ARF, complete Field 6 and 7.
3. Ask participant if they recorded or remember any instances when the device was not worn. Note any events on the ARF, Field 8.

RETURNING POST-COLLECTION DEVICES TO AXM CORE LAB

EVERY FRIDAY each site will ship all post-data collection belts back the AXM Core Lab. If no belts were returned that week, no action need be taken.

Prior to the beginning of the study, AMX Core Lab will provide each site with enough prepared FedEx return labels to cover the duration of the study. These return labels will be in PDF format and named according to the following format:

SITE#_RETURNDATE_TRACKING#

Shipping materials will be provided for each site at the beginning of the study. This includes mailing envelopes, clear return label envelopes, and protective packing material. If additional supplies are needed at any time, please email the AXM Core Lab.

To Return Belts to CORE Lab:

1. Print the return label with the correct RETURNDATE corresponding to the date of shipment
2. Enter the FedEx tracking number into the INFORM comment section for each device being returned. The tracking number is located both on the return label itself and at the end of filename (*TRACKING#*).

3. Be sure package includes **all devices and their corresponding ARFs**
4. Use standard FedEx shipping guidelines and mail back to AXM Core Lab, i.e.
    a. Fold return label, place in clear envelope, and adhere to outside a mailing envelope.
    b. Wrap devices in protective materials to prevent shipping damage
    c. Place devices and ARFs in mailing envelope and seal
    d. Drop off at nearest FedEx pickup

Following study completion, all remaining devices must be returned to AXM Core Lab **within 3 weeks.**

Accelerometer Return Form

1	**Site Number**		
2	**Participant Number**		
3	**Phase**		☐ 1 (0 to 8 weeks) ☐ 2 (8 to 16 weeks)
4	**Device Serial Numbers**	**Left KAM:**	
		Right KAM:	
5	**Device Placed**	**Date:**	
		Time (24 hr clock):	
6	**Device Removed**	**Date:**	
		Time (24 hr clock):	
7	**Is this device a replacement for a lost or damaged device?**		☐ Yes ☐ No
8	**At any time during the study did the subject remove the belt? If yes, describe**		
9	**Additional notes:**		

Massachusetts General Hospital
Cardiopulmonary Exercise Laboratory

Cardiopulmonary Exercise Testing Manual of Operating Procedures for INDIE-HFpEF

Full Title:
Inorganic **N**itrite **D**elivery
to **I**mprove **E**xercise Capacity in HFpEF

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Table of Contents

1. INTRODUCTION

1.1 Abbreviations and Glossary of Terms

BP	Blood Pressure
CPET	Cardiopulmonary Exercise Test
CV	Coefficient of variation
ECG	Electrocardiogram
f	Breathing frequency
FVC	Forced vital capacity
HF	Heart failure
HR	Heart rate
IC	Inspiratory capacity
MOP	Manual of Operating Procedures
O_2 Pulse	Oxygen uptake divided by HR
OUES	Oxygen Uptake Efficiency Slope
$PaCO_2$	Partial pressure of carbon dioxide
$P_{ET}CO_2$	Partial pressure of end tidal carbon dioxide
$P_{ET}O_2$	Partial pressure of end tidal oxygen
Peak VO_2	Peak oxygen uptake during exercise
PWC130	Physical Work Capacity at HR of 130 beats per minute
RER	Respiratory exchange ratio
SaO_2	Arterial oxygen saturation
SV	Stroke Volume
INDIE-HFpEF	**I**norganic **N**itrite **D**elivery to **I**mprove **E**xercise Capacity in HFpEF
VCO_2	Carbon dioxide output
VC	Vital capacity
V_D	Dead Space Volume
V_E	Minute ventilation
VO_2	Oxygen uptake
VThr	Ventilatory Threshold
V_T	Tidal volume
W	Watts

1.2 INDIE HFpEF OVERVIEW

- The **I**norganic **N**itrite **D**elivery to **I**mprove **E**xercise Capacity in HFpEF (INDIE-HFpEF) study will utilize cardiopulmonary exercise testing (CPET) to evaluate whether inhaled, nebulized inorganic sodium nitrite, as compared to placebo, improves maximal exercise capacity as assessed by cardiopulmonary exercise testing performed at peak drug levels.
- The primary hypothesis of the INDIE-HFpEF study is that inorganic nitrite, compared to placebo, will improve exercise capacity (peak oxygen consumption, VO_2).
- A secondary endpoint is to evaluate whether inorganic nitrite improves other measures of cardiopulmonary function such as V_E/VCO_2 slope (ventilatory efficiency) and VO_2 at ventilatory threshold (submaximal exercise capacity)

The following analyses are planned using CPET:

Peak VO_2 (ml/kg/min)
Exercise duration (min)
Peak workload (watts)
Maximum heart rate during exercise (beats/min)
Peak VE (l/min)
Respiratory Exchange Ratio (RER, VCO2/VO2)
Borg scale scores
Exercise termination reason

Only subjects who meet CPET criteria will be enrolled in the study. The inclusion criteria specific to CPET are as follows:

- **Subjects must have a Peak VO_2 ≤75% predicted (see Table 1)**
- **Subjects must have a peak respiratory exchange ratio (RER) of ≥1.0**

Table 1 Peak VO_2 Inclusion Criteria

	Normal Values for Peak VO_2*	Criteria (ml/kg/min) <75% Normal Value
20-29 M	**43±7.2**	**< 32.3**
20-29 F	**36±6.9**	**< 27.0**
30-39 M	**42±7.0**	**< 31.5**
30-39 F	**34±6.2**	**< 25.5**
40-49 M	**40±7.2**	**< 30.0**
40-49 F	**32±6.2**	**< 24.0**
50-59 M	**36±7.1**	**< 27.0**
50-59 F	**29±5.4**	**< 21.8**
60-69 M	**33±7.3**	**< 24.8**
60-69 F	**27±4.7**	**< 20.3**
> 70 M	**29±7.3**	**< 21.8**
> 70 F	**27±5.8**	**< 20.3**

***Fletcher GF et al. *Circulation*. 1995; 91:580-615.**

2. SITE ASSESSMENT AND CERTIFICATION

Previous multicenter trials evaluating exercise gas exchange have been confounded by methodological differences in exercise protocols and lack of uniformity in interpretation of gas exchange data[1-3]. The Heart Failure Network CPET Core Laboratory at Massachusetts General Hospital will work with individual centers to promote uniformity in CPET administration, reporting, and quality control measures. The CPET Core Laboratory recognizes that most participating laboratories have significant experience and expertise in administering CPETs. However, site assessment, certification, and strict adherence to this detailed protocol will be essential to ensure the consistency and validity of derived results. This manual has been divided into 8 sections, which include printable forms to guide sites through qualification procedures, patient education, and CPET administration.

2.1 CPET EQUIPMENT REQUIREMENTS

Cycle/Treadmill: Cycle ergometry will be the preferred exercise modality for INDIE-HFpEF Trial. For CPET laboratories that do not perform cycle ergometry an alternative treadmill exercise protocol has been devised (Section 3). Regardless of the exercise modality, the metabolic cart computer should be able to control the work rate of the cycle or treadmill. Electrically-braked cycles are preferred, as opposed to friction-braked cycles, based on their higher precision of work rate relative to friction-braked cycles and the ability to implement a continuous ramp protocol.[4] It will be essential to conduct the qualifying CPETs on the same equipment that will be used for the CPETs conducted for this trial.

Airflow or volume transducers: The accurate measurement of ventilation parameters during exercise is critically dependent on the accuracy of the flow-sensing device. Transducers used in exercise testing should meet established standards by the American Thoracic Society for flow and volume measurement during spirometry.[6]

Gas analyzers: Breath-by-breath analysis requires precise knowledge of gas analyzer delays and response kinetics.[7] Participating laboratories will need to follow standards for gas analyzer performance in breath-by-breath mode; these will include a transfer delay time of <1 second, a rise time <0.1 seconds, calibration stability of ± 3% over 20 min and calibration linearity ± 3% over the entire range.[8] Each site will be required to maintain a calibration logbook so that long-term trends can be monitored.

Electrocardiographic monitoring: Participating laboratories will be required to use electrodes and detection electronics designed for movement artifact rejection. Silver or silver chloride electrocardiogram (ECG) electrodes with circumferential adhesive provide good electrical contact and minimize movement artifact. Continuous display of ECG tracings with 12-lead ECG placement will be performed as described by Mason and Likar.[10] The timing of ECG monitoring must be synchronized with the timing used by the gas exchange system, preferably through an integrated ECG-metabolic cart system. ECGs will be performed for precise heart rate monitoring and for safety purposes, but will not be transmitted to the core lab for interpretation.

Metabolic measurement systems: The core laboratory encourages sites to utilize standard metabolic cart processing software. This will promote uniform generation, formatting, and acquisition of breath-by-breath data. Medgraphics Inc (St. Paul, MN) metabolic carts interfaced with BREEZESUITE software represent the most commonly used metabolic measurement systems in the United States and the primary equipment used by the Core Laboratory. Therefore, CPET data acquired and configured with BREEZESUITE software is preferred. The second most common type of metabolic cart used in CPET is Viasys (previously Sensormedics), which the core lab is equipped to interpret. For Viasys/Sensormedics equipment, sites will be strongly encouraged to use ENCORE/Vmax software formatted data to facilitate data manipulation and simple transfer of data to the core laboratory. If an alternative metabolic measurement system is utilized, it must allow real-time tabular and graphical display of exercise variables, 5-of-7 breath moving average integration of gas exchange variables, and data conversion to unencrypted format such as Excel that will lend itself to interpretation by the core laboratory.

2.2 CPET EQUIPMENT CALIBRATION

CPET equipment should be calibrated by following instructions given by the manufacturer of the equipment. Equipment calibration is not mandated prior to participation in the trial, though may become necessary if qualification studies demonstrate abnormal values for gas exchange-work rate relationships. Should calibration be deemed necessary at your site, instructions are included below.

Prior to performance of CPETs for INDIE-HFpEF the following calibration procedures are recommended.

1. Electrically braked cycle ergometers that have not been previously calibrated or newly purchased should be dynamically calibrated with the use of a dynamometer (torque meter). Because many labs do not have dynamometers, cycle ergometer manufacturers may be required to provide this service. This calibration should be repeated if the cycle is moved or jarred or if certification testing results in abnormal values for gas exchange-work rate relationships.
2. Treadmills should have belt speed verified by timing revolutions using a mark made on the treadmill belt with a subject on the treadmill. Grade may be determined by using a plumb line and tape measure.

Prior to each test

1. Record barometric pressure, temperature, relative humidity
2. Perform flow calibration with a 3L syringe (<1-15sec duration) to achieve ± 3% agreement with calculated volumes.
3. Perform gas analyzer calibration with two precision-analyzed gas mixtures.

   This is commonly done with one 6% CO_2 and 15% O_2 tank and one 0% CO_2 and 21% O_2 tank. The air baseline setting for O_2 and CO_2 should be checked before each test to correct for baseline drift since calibration.
4. Determine transport delays between the gas sampling point and each gas analyzer. This should be an automated process.

2.3 SITE QUALIFICATION PROCEDURES

Sites with sufficiently large volumes of subjects in recent trials with the MGH CPET Core Lab, along with consistent, excellent CPET data acquisition may be exempted from re-certification. However, it should be assumed that certification is required regardless of participation in other HF Network Trials, unless your site is informed otherwise.

Before baseline studies may be performed in subjects, **each site will be required to submit two incremental symptom-limited CPET tests on the same "standard normal subject".** The standard subject should be a healthy, young to middle-aged adult. These tests must be performed on separate days, preferably no more than 5 days apart, according to the protocol summarized in Section 7, page 19 or 20. Prospective CPET laboratories will be evaluated based on their ability to: (1) follow a site qualification protocol (see Section 7.1, page 19 for cycle and 7.2, pg 20 for treadmill), (2) generate reproducible CPET data, and (3) transmit data to the core laboratory. Test results will be compared to data available on normal individuals from the core laboratory and the published literature.[8, 11] Sites should await feedback from the core laboratory confirming that their site has qualified prior to scheduling study patients for testing.

Sites may subsequently use repeated studies of the "standard normal subject" to verify accuracy of their systems. The Core Laboratory will require sites to maintain a detailed log of physiologic calibration testing as described above, but this information will not need to be transmitted to the core laboratory as part of the initial qualifying procedures.

In anticipation of transmitting qualification tests to the core laboratory, the individual who will be transmitting data from the participating CPET laboratory to the core laboratory should take the following steps (also see Appendix 1, page 25): Email **cpetcore@partners.org** or Diane Cocca-Spofford BSN at dcoccaspofford@mgh.harvard.edu, CC glewis@partners.org and indicate the following:

a. Regional research site name.
b. Responsible CPET lab staff member who will primarily interact with the Core Laboratory with email address and contact information.
c. Metabolic cart manufacturer and software program that will be used.
d. Await receipt of an email from Diane Cocca-Spofford with an invitation to join Partners Research Computing network through which qualification study files and subsequent study files can be transferred via email (see Section 4 for detailed instructions).
e. Transmit the two qualification studies formatted as described below and summarized in Section 7.1, 7.2.

3.0 CPET PROCEDURES

3.1 CPET PREPARATION

Cycle Ergometry will be the preferred exercise modality for the INDIE-HFpEF trial using a modality with a ramped workload of 10 watts/min. For CPET laboratories that do not perform cycle ergometry an alternative treadmill exercise protocol has been devised that simulates cycle ergometry in terms of a linear, comparable increment in external work performed and a gradual increment in both speed and ramp that is appropriately suited to the study of HF patients.[12] In order to achieve within subject consistency, it is important that a laboratory that elects to perform

treadmill ergometry commit to doing so for all study tests that it performs. In addition, if a participating laboratory has more than one ergometer or metabolic cart, the same equipment should be used for each test in a given subject.
Each subject should use the same protocol for all of their CPET tests.

- Study Visit 1, Baseline
- Study Visit 2, (End of Phase 1, beginning of Phase 2)
- Study Visit 3, (Final)

1. **Provide patients with pre-test instructions.** The Patient Education Form, Section 6.1 is to be used by participating sites to provide uniform instructions to INDIE-HFpEF patients. Subjects should be given instructions to follow the same regimen of fasting (for at least 3 hours) and taking their medications prior to testing, particularly medications that influence heart rate.

2. **Review of contraindications to exercise testing.** Table 2 below lists contraindications to exercise testing. Questions that arise regarding exercise eligibility should be brought to the attention of the site principal investigator.

Table 2: Contraindications to Exercise Testing:

Absolute Contraindications
• Acute myocardial infarction (3-5 days) or unstable angina • Uncontrolled symptomatic arrhythmias • Active endocarditis • Acute myocarditis or pericarditis • Symptomatic severe aortic stenosis • Acute pulmonary embolism or DVT • Suspected dissecting aneurysm • Uncontrolled asthma • Uncontrolled pulmonary edema • Room air desaturation to <85% • Acute illness (i.e. infection) or orthopedic injury that is anticipated to affect exercise performance • Mental impairment leading to inability to cooperate • History of exercise-induced ventricular arrhythmia

3. **Initial patient data entry.** Upon initiating a study using BREEZESUITE or analogous software, click on the "Patient" or equivalent demographic information tab. The following information should be entered into the designated text boxes.

   Patient

   For site certification studies, identify the tests by including your Site number, substitute the word Certification for the study ID and designate the study either A or B **(**i.e. **INDIE_04Certification_A)**.

Subject Identification: For the baseline test, enter study protocol (INDIE-HF), followed by the site number, and followed by the study ID and the test description BL for Baseline.

For subsequent tests, please use the INDIE study ID and add the test description, V2 for Visit 2, and V3 for Visit 3.

Subject's identifier may look like this: INDIE301-001BL, for a baseline test and INDIE301-001V2 for a subject performing their visit 2 CPET.

Input of accurate date of birth and gender, height and weight is important to determine eligibility and to provide accurate VO_2 endpoint data. Subjects should be weighed at the time of each CPET to provide accurate data for weight-based peak VO_2 reporting. It is not permissible to rely on subject's stated weight or height.

Visit Demographics

Enter the following:
Date of Birth
Gender
Height (inches)
Weight (lbs)
Race
Referring physician (CPET lab physician)
Technician (CPET lab technician conducting the study)
Site: Indicate which site and metabolic cart are being used, if your laboratory has more than one

Patient History

Pre-test comments: Free text the name and contact information for the responsible CPET lab personnel who conducted the CPET
Name: _____Email: _____Phone #: _____

3.2 CPET PROCEDURES

3.2A. Study Drug administration:

A dose of study drug is administered immediately before the CPET under the supervision of study staff.

3.2B. Blood pressure measurement procedures: Pre-exercise blood pressure should be obtained with the subject in a relaxed, comfortable, seated position without clothing in between the cuff and the arm. Choose the correct cuff size, the bladder width should encircle 40% of the circumference of the arm and there should be at least 2 cm between the bottom of the cuff and the brachial artery. Record the blood pressure at which you hear the first Kortakoff sounds for two consecutive beats as systolic blood pressure and the pressure at which time the sounds

disappear (K5) as diastolic blood pressure. CPET laboratory staff should adhere to this protocol in measuring exercise blood pressure as well.

3.2C. 12-lead electrocardiogram recording: Skin preparation is important to ensure a high-fidelity signal, free from motion artifact and electrical interference that is sent to the electrocardiograph and the metabolic cart system where heart rate will be recorded. Proper skin preparation involves removing the hair with a disposable safety razor, cleansing the skin with alcohol or acetone to remove skin oils, followed by light abrasion to remove stratum corneum. Standard limb lead placement on the wrists and ankles must be modified for exercise by moving them to the anterior trunk in a Mason-Likar configuration (Figure 1). A 12-lead electrocardiogram should be recorded during this rest period. Heart rate at rest and during exercise will be transmitted to the core laboratory. However, interpretation of cardiac rhythms and pattern interpretation on electrocardiograms will need to be performed contemporaneously with exercise testing (and not by the core laboratory) to ensure patient safety.

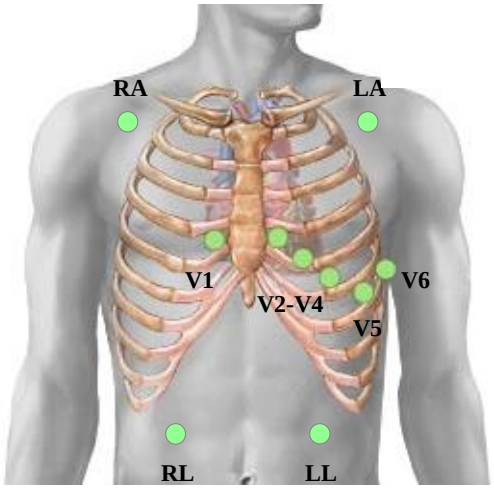

Figure 1. Electrode placement for electrocardiogram recording during exercise. RA indicates right arm, LA indicates left arm, RL indicates right leg, LL indicates left leg. Model adapted from ADAM at www.nlm.nih.gov/.../ency/imagepages/19865.htm

3.2D. Gas exchange measurements: With subjects standing on the treadmill or sitting on the cycle ergometer a nose clip is placed and the mouthpiece is inserted. At this time the importance of maintaining a tight seal around the mouthpiece should be emphasized to the patient.

3.2E. Metabolic cart, software interface preset data displays: Metabolic measurement systems should allow real-time graphical and tabular display according to the recommended format in Table 5. Because significant differences can arise as a result of time interval selection for averaging breaths in patients with HF,[9] participating laboratories will be required to provide the core laboratory with data on all breaths. However, to standardize data output across metabolic carts we will request that tabular data be displayed using **5-out-of-7 breath retrograde time averaging** which is a standard option for BREEZESUITE. An alternative is 10-second averages in Viasys and Jaeger Oxycon Pro metabolic cart software. The following

screening procedures for outlier values in Table 3 below should be programmed into BREEZESUITE or analogous software systems.

Table 3. Parameters to eliminate outlier values for breath-by-breath gas exchange variables

Measurement Variable	Minimum Value
RER	0.5
VCO_2	50 ml/min
VO_2	50 ml/min
V_T	180 ml

RER indicates respiratory exchange ratio, VCO_2 carbon dioxide output, VO_2 oxygen uptake, and V_T tidal volume.

Resting phase of CPET
A 5-minute rest period will be implemented. During this period CPET lab personnel should observe key variables in comparison to reference values to ensure proper calibration and performance of the metabolic measuring system. Table 4 below provides an example of key resting variables and their expected values.[14]

Table 4. Reference values appropriate for resting conditions.

Heart Rate min^{-1}	VO_2 (ml/kg/min)	VO_2 (ml/min)	VCO_2 (ml/min)	RER	RR min^{-1}	V_E L/min	$P_{ET}O_2$ (mmHg)	$P_{ET}CO_2$ (mmHg)
60-100	3.5	200-300	140-300	0.7-1.0	12-20	6-10	100-105	38-42

VO_2 indicates oxygen uptake, VCO_2 indicates carbon dioxide output, RER respiratory exchange ratio, RR respiratory rate, V_E minute ventilation, $P_{ET}O_2$ end tidal oxygen, $P_{ET}CO_2$ end tidal carbon dioxide. Values for VO_2, VCO_2, and VE apply to normal adults, and will tend to be lower for younger subjects.

Some clinical conditions can account for departures from expected values. However, departures can usually be explained by pre-test anxiety, leaks in the patient interface such as a poor fitting mask or failure to apply the nose clip or improper calibration of the metabolic cart. Anxiety is typified by a heart rate > 85 min^{-1}, V_E > 10 L/min, $P_{ET}CO_2$ < 35, RER > 1.0 whereas a system leak results in proportionately low V_E VO_2, VCO_2.

Warm up phase, unloaded cycling or walking on the treadmill: Following the rest period, at a verbal signal the patient should start pedaling with the cycle unloaded (i.e. free-wheeling) or walking slowly on the treadmill for 3 minutes according to the appropriate protocol outlined in Section 7.3. Subjects undergoing treadmill testing who weigh <80 kg will follow Section 7.4a, subjects weighing >80kg will follow Section 7.4b. If available, an accessory motor should be utilized to rotate the flywheel at a rate of 60 rpm in order to eliminate the inertial force needed to start the flywheel rotating and reach the desired speed. The patient should be coached to pedal at 60 rpm on the unloaded cycle to become accommodated to this pace. The pedal rate meter should be displayed in clear view of the patient to facilitate compliance with this goal pedaling frequency.

***Incremental exercise:** The pace and grade will increase every 2 minutes. Subjects will be encouraged to reach a maximal effort by monitoring the respiratory exchange ratio [goal respiratory exchange ratio (RER, VCO_2/VO_2) > 1.1] and by encouraging continued exercise until perceived exertion reaches >8 on the Borg 0-10 scale.[15] We suggest printing out the Borg scale in large font so that subjects can point to the scale during exercise or use the laminated Borg scale provided by the Data Coordinating Center. The technician and physician should work cooperatively to observe the subject's facial expression while encouraging the subject to keep their eyes open during exercise. Blood pressure, oxygen saturation, perceived exertion and dyspnea will be monitored and recorded every two minutes. ECG monitoring will be used to monitor patients during exercise testing. Heart rate, as measured by ECG, will be recorded every minute. The recognition and treatment of conditions that manifest with ECG abnormalities during exercise will be the responsibility of the on-site supervising physician due to the time-sensitive nature of such findings. If ECG abnormalities arise during testing, these should be indicated in the comment section on Section 7 worksheets. Standardized guidelines for operators to stop an exercise test are listed in Table 5 below.

Criteria for CPET Termination: The standard accepted criteria for terminating an exercise test are listed in Table 5. These are only guidelines and should be used in conjunction with clinical judgment of trained CPET lab personnel and study investigators.

Table 5. Objective criteria for termination of CPET:

Criteria for CPET Termination
Definitive ischemic ECG changes with associated chest pain
Complex ectopy (i.e. ventricular tachycardia)
Mobitz 2 Second Degree or Third degree heart block
Symptomatic fall in systolic blood pressure > 20 mmHg from the highest value during the test
Marked hypertension (systolic BP > 240 mmHg, diastolic BP >120 mmHg
Severe oxygen desaturation, SpO2<80% when accompanied by signs of severe hypoxia
Neurologic compromise such as mental confusion or loss of coordination

Recovery period: To avoid orthostatic hypotension when stopping exercise, the subject should be encouraged to slowly turn the pedals at 30 rpm to maintain venous return during the first 60 seconds of recovery. Record blood pressure during the second and fourth minute of recovery. Gas exchange and heart rate should continue to be measured for three minutes into recovery.

End of test: CPET laboratory personnel should elicit the reason for cessation of exercise. Viasys Vmax/Encore software prompts users with a "Metabolic End of Test Comments" menu. A primary reason for test cessation should be selected. Secondary reasons for cessation of exercise may also be recorded as added text. It is particularly pertinent to indicate if the test was stopped by the operator prior to the patient reaching a point of maximum exertion.

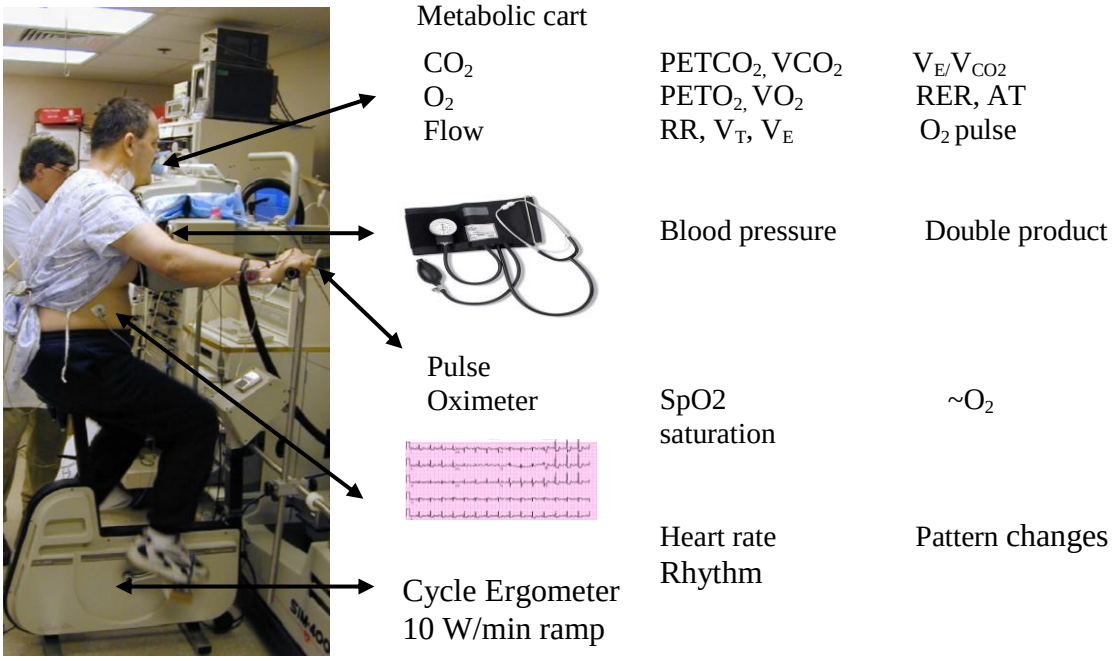

Figure 2. Measuring devices, primary, and derived measurements obtained during CPET. ET indicates end tidal, *f* breathing frequency, RER respiratory exchange ratio.

3.3 CPET PROTOCOL SCHEMATIC SYNOPSIS

Initial measurements of heart rate, blood pressure, and Borg Dyspnea Index will be conducted in subjects prior to mouthpiece insertion. The 5 minute rest period will start upon mouthpiece insertion and initiation of gas exchange collection with the metabolic cart. Blood pressure and Borg Dyspnea Index (Section 6.2, pg.18) will subsequently be obtained 4.5 minutes into the rest period, then during the last 30 seconds of every 2 minute increment during exercise (i.e. between 3.5 and 4.0 minutes into exercise). Heart rate and O_2 saturation should be recorded each minute. Peak exercise heart rate, blood pressure, O_2 saturation and Borg Dyspnea index should also be recorded. See **Figure 3**.

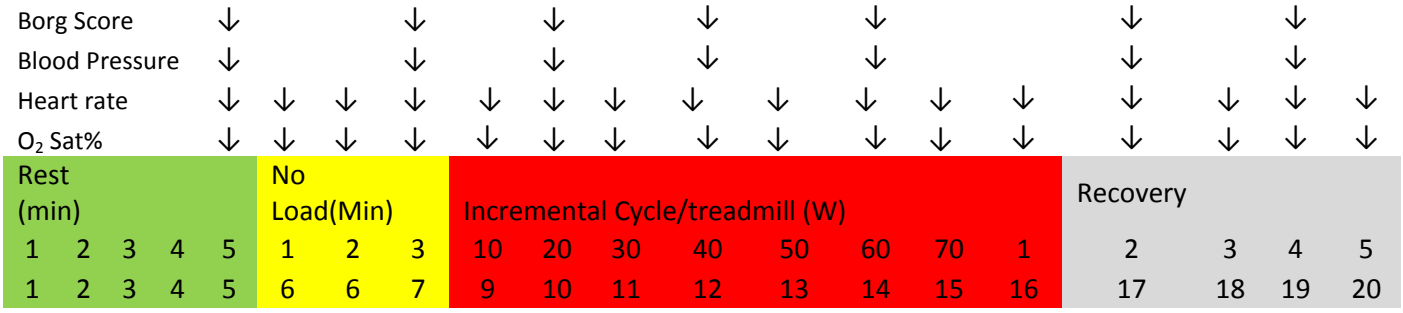

Figure 3. Schematic representation of exercise protocol

3.4 TABULAR CPET DATA DISPLAY

Table 6. Tabular data displays for cardiopulmonary exercise testing.

Time mins	Work W	VO_2 ml/kg/min	VO_2 (ml/min)	VCO_2 ml/ min	RER	RR min^{-1}	Vt (ml)	V_E L/min	$P_{ET}CO_2$ mm Hg	$P_{ET}O_2$ (mm Hg)	HR (min^{-1})	O_2 sat (%	SBP (mm Hg)	DBP mm Hg)	Borg Dyspnea (0 10)

Unshaded cells represent standard metabolic cart output. The shaded regions of this table may be automatically populated during the study or may need to have data entered depending on the degree to which other measurement equipment is interfaced with the metabolic cart. To aid in data recording for post-test completion of these data fields, please see printable data entry form (Section 7, pages 19-23). This information will need to be entered into the above tabular format either during or after the CPET, prior to transmission of the study to the core laboratory

4. DATA RECORDING AND TRANSMISSION TO THE CORE LABORATORY

Only CPET tests of subjects that have met all criteria and have been randomized will be transmitted to the MGH CPET Core Laboratory. If there is a subject in whom there is uncertainty as to whether he/she has met the inclusion criteria for peak VO_2 based on a highest 30 second median value below the cut-off threshold described in Table 1, then please transmit the test to the CPET Core Lab immediately upon completion of the study. The MGH CPET Lab will be able to adjudicate eligibility on the basis of CPET parameters within 48 hours of receipt of the tests.

4.1 CPET Data Preparation: For each CPET performed, the Core Laboratory requests that all information be integrated into a single CPET file that will be transmitted to the Core Lab. For each study, the following steps will be taken to ensure standardized data reporting.

1. Prior to exercise testing, enter patient data into BREEZESUITE or analogous software according to instructions outlined above (Section 3.1, #3).
2. Data recording during CPET testing
    - A. Breath-by-breath data should be formatted according to Table 5, page 12.
    - B. Real-time addition of heart rate every minute, and blood pressure recordings and Borg scores every two minutes during exercise is technically possible in the BREEZESUITE and Vmax/Encore programs but may be cumbersome during supervision of a CPET. Hence, we recommend recording of heart rates (from the ECG), blood pressures, and Borg scores on printed versions of Table 7, page 19 during testing. Following completion of the CPET, this data can then be entered into the BREEZESUITE "Event Entry" screen or equivalent on other software systems. To access the Event Entry screen follow these steps
        a. Enter the "Protocol/Log" tab, "Visit Log" section
        b. Select the clock/pencil icon to enter data at an appropriate time during the test (i.e. 10 minutes of exercise)

c. Select the "Gas Exchange" tab with the "Event Entry" box
d. Use the drop down variable menu to select/enter Borg Score, BP, and heart rate
e. Select "ABG" to enter current hemoglobin level
f. Confirm that the Tabular data reporting form (Table 5, page 12) is updated for heart rate, blood pressure and Borg score recordings.
g. Add "Why the patient stopped exercising". A single primary reason should be specified, though addition of a secondary reason is permissible.

C. The core lab would prefer a self-contained single file in which all data is incorporated within the CPET software program. However, if this is not possible, data may be manually entered into electronic versions of Table 7, page 19 and then transmitted to the core laboratory as a Microsoft Excel file. Files should be named as described on Section 3.1 #3, page 8, including Protocol name, study ID, and test description.

4.2 CPET Data Transfer to the Core Lab: Copy the archived file onto a computer disk immediately following the CPET. BREEZESUITE software requires entering the Import/Export Program from the Start Menu→Programs→Medgraphics→DBP Tools→Tools→Export, then select the patient file and the destination of the file under the Browse menu. This will enable labs to export the file to a local disk and to then send the file electronically as an enclosure (see below). Viasys Vmax/Encore software can be used to directly email test files using the "Special Functions" tab and selecting the "File compress/email" option. Metabolic measurement systems with software programs other that those supported by Medgraphics and Viasys/Sensormedic will be required to convert gas exchange data into Excel format for breath-by-breath data interpretation. In addition, graphical data should be generated to facilitate calculation of the ventilatory threshold by the V-slope technique. Label the computer disk with the following information: a) INDIE-HFpEF study name, b) Site number and subject ID c) date of study. This will serve as a back-up hard copy of the data to remain at the individual CPET labs. Tests will only be mailed to the core lab upon request if there is a problem with electronic submission or archiving of the data. In the event that the core lab requests disks, the participating CPET lab should retain a copy of the data in their laboratory and send a disc via Fed Ex to:

Gregory Lewis, MD
GRB 800, Cardiology Division
Massachusetts General Hospital
55 Fruit St, Boston, MA 02114

The MGH Core Laboratory will utilize the Accellion Secure File Transfer Service to exchange files with participating CPET laboratories via a web browser rather than ftp site. The service is a secure web based application with anti-virus detection built in. Attached files are encrypted and uploaded to an appliance and the recipient receives an email with a link.
When the recipient clicks the link, the file is downloaded from the appliance. This will enable rapid, readily traceable transfer of data between participating sites and the core laboratory.

CPET laboratory personnel at participating sites will receive an invitation email from Diane Cocca-Spofford with instructions on how to register for this service. Upon clicking on the attachment, the screen depicted in Figure 4 will appear with instructions on how to register for

this free service. Participants will automatically create a login and password as part of a simple authentication process which can be used to send files back to the core lab or anyone else who is registered user with an affiliate email address. Participating CPET labs will be able to send up to 10 files at a time to the core laboratory, with an overall size limit of 2GB.

Figure 4. Partners Research Computing instructions screen for registering for the file transfer service that will be used by the CPET core laboratory.

Participating labs can use the following website to login to the service in order to transfer files to MGH: https://transfer.research.partners.org/courier/1000@/mail_user_login.html. In addition to basic send and receive functions, the File Manager menu tab provides a File Cabinet, Inbox and Send History for keeping track of the files you have sent and received. The core laboratory will maintain this record of file transmissions and we will encourage participating labs to do the same. Further general information about how to use this file transfer service is available at http://www.partners.org/rescomputing/content/secureFiletransfer.asp.

4.3 Core Lab Study Processing

An Excel spreadsheet will be created to track studies. Upon arrival of each study at the core laboratory the study will be logged into our database. As each study moves through the sequence of data processing, analysis, and report generation the excel sheet will be updated accordingly. An email reminder will be sent to sites if studies do not arrive in a timely fashion.

Gas exchange data will be configured uniformly in an Excel Database. Programs will then be applied to select the highest 5-breath average VO_2 during the final minute of incremental exercise. Primary breath-by-breath data will also be used to calculate ventilatory threshold, by the V slope method.[16]

5. QUALITY ASSURANCE AND QUALITY CONTROL PRACTICES

Prior to initiation of the trials, sites will be required to conduct two initial tests on a "standard subject". These tests will serve to an indicator of appropriate calibration procedure, protocol

adherence, and appropriate data compilation and transmission to the core laboratory. Individual CPETs will be expected to demonstrate a change in respiratory exchange ratio of >0.15 and demonstrate appropriate increases in ventilation, VO_2, and carbon dioxide production during exercise.

If, at any time, the core lab questions the quality of data that they receive from a testing site, we may require the site to perform another qualifying test. In addition to recalibration of the treadmill and the metabolic cart, the core lab may request repeated studies on the "standard subject". For a normal subject, VO_2 should increase at a rate of approximately 10 ml/min/watt on a treadmill ergometer. For sites that do not meet these standards, step-by-step review of each part of the exercise test, including treadmill belt speed and angle, calibration, review of potential air leaks and verification that the metabolic cart was working properly will be performed.

6.0 PATIENT EDUCATION FORMS

6.1 Patient Education Form

Dear _____,

You have been scheduled to have an exercise test to evaluate your exercise tolerance. You will be exercising on a cycle or treadmill

Appointment Date: _____ Time:_____

Location: _____

Exercise Test

- You will have a mouthpiece in place that will enable us to measure the amount of oxygen that your body uses during exercise
- You will begin with a 5 minute period of rest with the mouthpiece in place
- The cycle will begin easy to pedal then increase in difficulty, or the treadmill will begin slowly and gradually increase its speed
- You will exercise for as long as possible
- We will measure your blood pressure periodically
- At certain times we will ask you to point to a scale which tells how much you are exerting yourself

Pre-test instructions:

- Do not eat anything for 3 hours before the test. Subjects with diabetes should have a light snack, as needed, to maintain adequate blood sugar levels during the test.
- Continue to take your regularly scheduled medications
- Avoid alcohol, caffeine, tobacco, and other stimulants within 8 hours of the test
- Avoid medications that can make you drowsy within 8 hours of the tests
- Wear loose fitting, comfortable clothing that will permit you to move your legs freely
- Wear athletic shoes appropriate for exercise
- Do not engage in strenuous exercise on the day prior to the test
- Do not exercise at all within 12 hours of the test

6.2 Borg Scale

Table 7. Borg Dyspnea Scale 0-10 to be applied to the degree of perceived dyspnea experienced during exercise.

Borg Scale	
0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight (light)
3	Moderate
4	Somewhat severe
5	Severe (heavy)
6	
7	Very severe
8	
9	
10	Very, very severe (maximal)

*Instructions for Borg Dyspnea Scale
Use this scale to rate the difficulty of your breathing.
It starts at number 0 where your breathing is causing you no difficulty at all and progresses through to number 10 where your breathing difficulty is maximal.
How much difficulty is your breathing causing you right now?

7 PROTOCOL SPECIFIC WORHSHEETS

7.1 Site Qualification Cycle Worksheet

Qualification Testing, Normal Subject, CYCLE ERGOMETRY
Referring Center Name and Number:_____
PI Name: _____
Date, Time, Study description (A or B):_____
Gas Exchange Equipment and Software Manufacturer:_____

Elapsed Time (min)	Work Rate Increase at 20 W/min	RPM (min^{-1})	HR	O_2 sat (%)	SBP (mmHg)	DBP (mmHg)	Borg Dyspnea (0-10)
-1 (Pre)	Rest	0					
0-5	Rest	0					
5-8	0 (unloaded)	60					
8-9	20	60					
9-10	40	60					
10-11	60	60					
11-12	80	60					
12-13	100	60					
13-14	120	60					
14-15	140	60					
15-16	160	60					
16-17	180	60					
17-18	200	60					
18-19	220	60					
19-20	240	60					
20-21	260	60					
21-22	280	60					
>22	+20/min	60					
Peak: t_____	**W_____**						
Rec. 1 min		0					
Rec. 2 min		0					
Rec. 3 min		0					
Rec. 4 min		0					
Rec. 5 min		0					

Rec. indicates recovery, W indicates watts, HR indicates heart rate, SBP indicates systolic blood pressure, DBP indicates diastolic blood pressure.
*Recordings should be made during the last 15 sec of each minute

7.2 Site Qualification Treadmill Worksheet

Qualification Testing, Normal Subject TREADMILL testing
Sites will only complete this protocol if a cycle ergometer is not available)

Cardiopulmonary Exercise Worksheet
Referring Center Name and Number:_____
PI Name: _____
Date, Time, Study description (A or B):_____
Gas Exchange Equipment and Software Manufacturer:_____

Elapsed Time (min)	Work Rate (Increase 20W/min)	Grade (%)	Speed (mph)	HR (bpm)	O_2 sat (%)	SBP (mmHg)	DBP (mmHg)	Borg Dyspnea (0-10)
-1 (Pre)	Rest	0	0					
0-5	Rest	0	0					
5-8	10	4	0.8					
8-9	30	9.5	1.1					
9-10	50	12.5	1.3					
10-11	70	14.5	1.6					
11-12	90	16.5	1.8					
12-13	110	17.5	2.1					
13-14	130	18.5	2.3					
14-15	150	19	2.6					
15-16	170	20	2.8					
16-17	190	20.5	3.1					
17-18	210	21	3.3					
18-19	230	21.5	3.5					
19-20	250	22	3.7					
20-21	270	22.5	3.9					
21-22	290	23	4.1					
>22		↑0.5/min	↑0.2/min					
Peak: t____								
Rec. 1 min	10	0	1.1					
Rec. 2 min	Rest	0	0					
Rec. 3 min	Rest	0	0					
Rec. 4 min	Rest	0	0					
Rec. 5 min	Rest	0	0					

*Recordings should be made during the last 15 sec of each minute

7.3 Cycle 10 Watt Ramp Worksheet for INDIE Subjects

Complete for each subject and send to MGH Core Laboratory with Gas exchange tabular data

CYCLE EXERCISE DATA WORKSHEET			
		INDIE	TRIAL
Site Name and Number:		Hemoglobin	
Subject ID:		Testing Time point:	
Date and time at start of exercise:		Dose Time:	
Technician of record:		FEV1	
Reason for cessation of exercise: Seat Height:		FVC	

Elapsed Time(min)	Work Rate Increase 10 W/min	Target Pedal Rate (rpm)	Actual Pedal Rate (rpm)	HR (bpm)	O_2 sat (%)	DBP (mmHg)	SBP (mmHg)	Borg Dyspnea (0-10)
-1 (Pre)	Rest	0						
0-5	Rest	0						
5-8	0	60						
8-9	10	60						
9-10	20	60						
10-11	30	60						
11-12	40	60						
12-13	50	60						
13-14	60	60						
14-15	70	60						
15-16	80	60						
16-17	90	60						
17-18	100	60						
18-19	110	60						
19-20	120	60						
20-21	130	60						
21-22	140	60						
22-23	150	60						
23-24	160	60						
24-25	170	60						
>25	10/min	60						
Peak: t_____	**W_____**							
Rec. 1 min	5	30						
Rec. 2 min	rest	0						
Rec. 3 min	rest	0						
Rec. 4 min	rest	0						
Rec. 5 min	rest	0						

*Recordings should be made during the last 15 sec of each minute

7.4 a Treadmill Worksheet for INDIE Subjects ≤ 80 kg

Complete for each subject and send to MGH Core Laboratory with Gas exchange tabular data

Treadmill EXERCISE DATA WORKSHEET			INDIE TRIAL	
Site Name and Number:			Hemoglobin	
Subject ID:			Testing Timepoint:	
Date and time at start of exercise:			DoseTime:	
Technician of record:			FEV1	
Reason for cessation of exercise:			FVC	

Elapsed Time (min)	Work Rate Increase ≈10W/min (W)	Speed (mph)	Grade (%)	HR (bpm)	O_2 sat (%)	SBP (mmHg)	DBP (mmHg)	Borg Dyspnea (0-10)
-1 (Pre)	Rest	0	0					
0-5	Rest	0	0					
5-8	5	0.8	2					
8-9	15	1	5					
9-10	24	1.1	7					
10-11	33	1.3	8.5					
11-12	43	1.5	9.5					
12-13	51	1.6	10.5					
13-14	60	1.8	11					
14-15	70	2	11.5					
15-16	80	2.2	12					
16-17	88	2.3	12.5					
17-18	100	2.5	13					
18-19	111	2.7	13.5					
19-20	123	2.9	14					
20-21	137	3.1	14.5					
21-22	150	3.3	15					
22-23	165	3.5	15.5					
23-24	180	3.7	16					
24-25	195	3.9	16.5					
>25		+0.2/min	+0.5/min					
Peak: t__ _____	W_____							
Rec 1min	5	1.0	0					
Rec.2min	0	rest	0					
Rec 3min	0	rest	0					
Rec 4min	0	rest	0					
Rec 5min	0	rest	0					

*Recordings should be made during the last 15 sec of each minute

7.4b. Treadmill Worksheet for INDIE Subjects >80 Kg.

Complete for each subject and send to MGH Core Laboratory with Gas exchange tabular data

Treadmill EXERCISE DATA WORKSHEET			INDIE TRIAL
Site Name and Number:		Hemoglobin	
Subject ID:		Testing Timepoint:	
Date and time at start of exercise:		DoseTime:	
Technician of record:		FEV1	
Reason for cessation of exercise		FVC	

Elapsed Time (nib	Work Rate Increase ≈10W/min	Speed (mph) (MPH)	Grade (%) %	HR (bpm)	O_2 sat (%)	SBP (mmHg)	DBP (mmHg)	Borg Dyspnea (0-10)
-1 (Pre)	Rest	0	0					
0-5	Rest	0	0					
5-8	5	0.8	1.5					
8-9	16	1	4					
9-10	24	1.1	5.5					
10-11	36	1.3	7					
11-12	44	1.5	7.5					
12-13	50	1.6	8					
13-14	60	1.8	8.5					
14-15	70	2	9					
15-16	82	2.2	9.5					
16-17	90	2.3	10					
17-18	98	2.5	10					
18-19	111	2.7	10.5					
19-20	125	2.9	11					
20-21	140	3.1	11.5					
21-22	155	3.3	12					
22-23	164	3.5	12					
23-24	181	3.7	12.5					
24-25	191	3.9	12.5					
>25		+0.2/min	+0.5/min					
Peak: t__	**W_____**							
Rec. 1 min	5	0	1.1					
Rec. 2 min	rest	0	0					
Rec. 3 min	rest	0	0					
Rec. 4 min	rest	0	0					
Rec. 5 min	rest	0	0					

8. COAPT CPET CORE LAB PERSONNEL

Primary Contact:

Core Laboratory Coordinator	Diane Cocca-Spofford BSN, MHA Tel: 617-726-8228 E-mail: dcoccaspofford@mgh.harvard.edu
Mailing Address:	MGH Core CPET Laboratory GRB 800, Cardiology Division Massachusetts General Hospital 55 Fruit St, Boston, MA 02114
MGH CPET Core Laboratory	Email: **cpetcore@partners.org**

Additional Laboratory Personnel Contact Information

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Core Laboratory Technical Support Staff	Luke Wooster Email: lwooster@mgh.harvard.edu
Core LaboratoryTechnical Support Staff	Cole Bailey Email: csbailey@mgh.harvard.edu

APPENDIX 1

Site Qualification Procedures Checklist:

☐ Verify your site's compliance with equipment requirements and calibration procedures.

☐ Email Diane Cocca-Spofford BSN at dcoccaspofford@mgh.harvard.edu or cpetcore@partners.org and CC glewis@partners.org and indicate the following:
3.1 Treatment center number
3.2 Responsible CPET lab staff member who will primarily interact with the Core Laboratory with email address and contact information.
3.3 Metabolic cart manufacturer and software program that will be used.

☐ Accept emailed invitation from Diane Cocca-Spofford to join the Partners Research Computing Secure Files Transfer Service (see Section 4 for detailed instructions).

☐ Submit two incremental symptom-limited CPET qualifying tests on the same "standard normal subject", using the Certification data worksheet, (Section 7.1, or 7.2, page 20-21) via the Partners Computing Secure Files Transfer Service.

CPET Procedures Checklist:

☐ Print and distribute patient education form (Section 6.1) to subjects at least 48 hours prior to testing and assess compliance with instructions upon the subject's arrival to the laboratory.

☐ Obtain the subject's study ID number from your site's research coordinator.

☐ Assess CPET contraindications (Table 2, page 8).

☐ Print Borg score table (Section 6.2) for use during CPET

☐ Configure gas exchange data output according to Table 6, page 13.

☐ Enter current hemoglobin level into CPET electronic file or data supplemental sheet.

☐ Complete CPET according to the appropriate protocol (Sections 3 and 7), integrate all study data into a single CPET file, and name the file according to Section 4.1.

☐ Save a backup copy of the CPET file on a disk that will be maintained in individual CPET laboratories and transmit the electronic file to the core laboratory.

☐ Transmit data file to the core laboratory. Send only subjects who meet eligibility (VO2 Max $\leq$75% predicted (Table 1, pg. 4)and RER$\geq$1, and have been randomized .

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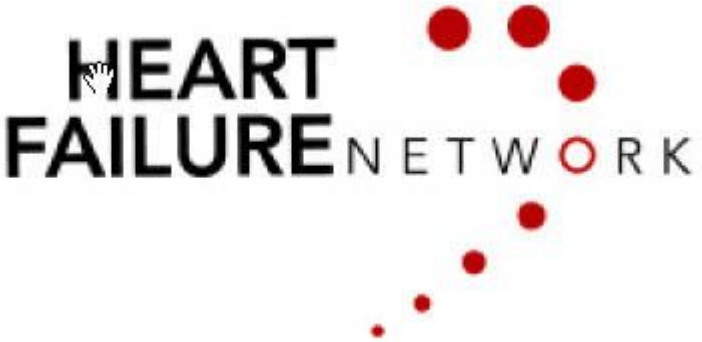
ECHOCARDIOGRAPHY MANUAL OF OPERATION (MOO)

FOR INDIE-HFPEF



Grace Lin, MD, Barbara G. Manahan, RDCS, Jae K. Oh, MD

Echocardiography Core Laboratory
Mayo Clinic Rochester
May 2016

CONTENTS

A. ECHOCARDIOGRAPHY FOR HFN TRIALS

1. INTRODUCTION

INDIE-HFpEF is a randomized, double blind, placebo controlled crossover study to investigate the effect of inorganic nitrite on aerobic capacity in heart failure with preserved ejection fraction (EF >50%). The study requires two limited echoes at visit 2 (42-49 days from visit 1) and visit 3 (42-49 days from visit 2) to assess secondary endpoints of the trial which include change in E/e' ratio, left atrial volume index, and estimated right ventricular systolic pressure.

This updated and abbreviated "**Echocardiography Manual of Operation**" produced by the Echo Core Laboratory will serve as a reference for the required echocardiographic measurements for INDIE-HFPEF. Each clinical site is encouraged to identify a core group of sonographers who will be responsible for acquiring echocardiographic studies for the HFN trials to minimize variability and ensure standardization of image acquisition. A lead sonographer should also be identified to serve as a liaison with the Echo Core Laboratory.

Digital images from echocardiograms performed at participating clinical centers should be uploaded on to the HEART IT server whenever possible. If unable to upload to HEART IT, the digital images should be transferred to a CD and shipped to the Mayo Echo Core Lab. ***Please note that the Echo Core Lab no longer accepts videotaped studies.***

All measurements and analyses will be performed by the Echo Core Lab without knowledge of other clinical or laboratory data. For Doppler data, an average of 3 cycles will be used for sinus rhythm and 5 cardiac cycles for atrial fibrillation. In atrial fibrillation or in frequent ectopic rhythms, only cardiac cycles with an adequate R-R interval will be used. It is not necessary to provide measurements when submitting digital echo images to the Echo Core Lab. However, if measurements are required the clinical site and marked on the echo images, please acquire and include duplicate digital clips without marked measurements to facilitate accurate analysis in the Echo Core Lab.

2. INDIE-HFPEF LIMITED ECHO PROTOCOL

The INDIE-HFPEF limited echo protocol will focus on acquisition of data required for the secondary endpoints as well as measurements for left ventricular (LV) ejection fraction, mass, and dimensions. Limited measurements will also be obtained for quantitative assessment of right ventricular (RV) systolic function.

ECHO IMAGE ACQUISITION PROTOCOL

1. LV ejection fraction (2D linear), dimensions, and mass
    a. LV end-diastolic and end-systolic dimensions from parasternal long axis
    b. LV posterior and septal wall thickness measured at end-diastole in parasternal long axis views
2. LV filling pressure estimate
    a. Mitral inflow pulsed wave Doppler (E wave, A wave) measured at leaflet tips
    b. Mitral annulus tissue Doppler velocity from lateral and medial mitral annulus
    c. E/e' calculation
3. Right ventricular (RV) systolic function

a. Apical 4 chamber focused on RV (RV centered in window)
b. Tricuspid annulus tissue Doppler velocity (s') from RV lateral annulus
c. Tricuspid annular plane systolic excursion (TAPSE)

4. Left atrial (LA) volumes
    a. Area-length method from the apical 4 chamber and 2 chamber views
5. Pulmonary artery systolic pressure estimate
    a. Tricuspid regurgitation peak continuous wave Doppler velocity
    b. Right atrial (RA) pressure estimate from inferior vena cava size
    c. Mid ascending aorta level

3. MEASUREMENTS

LV dimensions

Acquistion (clinical sites): Obtain a parasternal long axis view with the imaging sector width and depth optimized to include the mid left ventricle but excluding the apex.
Analysis (Echo Core Lab): LV end diastolic and systolic dimensions and wall thicknesses will be measured from the 2D parasternal long axis view, at the papillary muscle and chordae junction, perpendicular to the long axis of the LV (Figure 1).

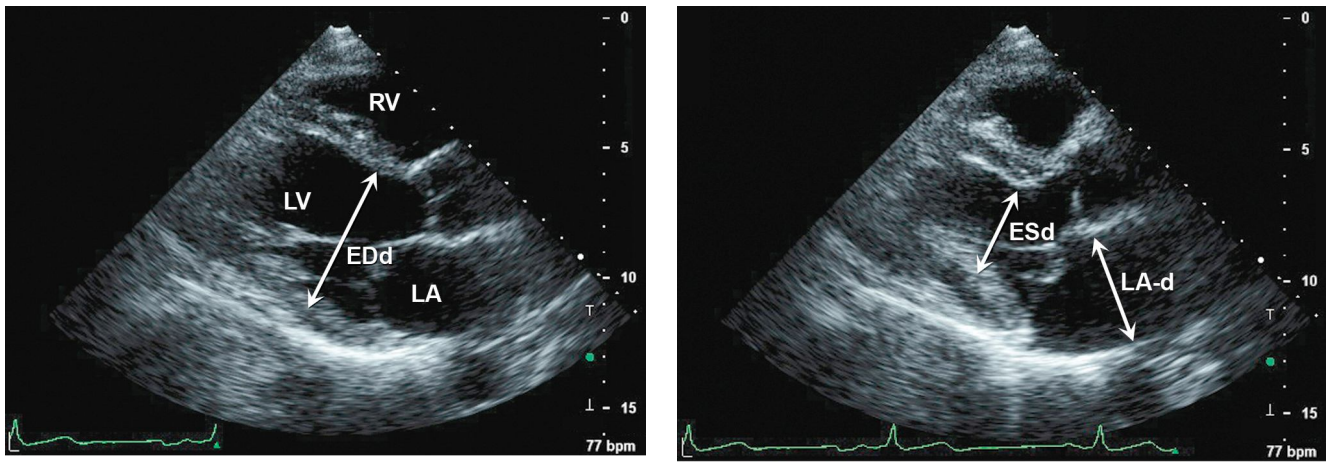


Figure 1. Left ventricular dimensions measured from parasternal long axis and short axis views. Examples of fundamental compared with harmonic imaging.

Left atrial volumes

Acquisition (clinical sites): Obtain an apical four chamber and 2 chamber view with the sector depth and width optimized to include the left atrium.
Analysis (Echo Core Lab): When tracing the left atrial areas, care should be taken to exclude the pulmonary vein from the left atrial trace. The posterior wall of the atrium should be carefully defined to ensure an accurate trace of the left atrium in the two chamber view. Left atrial volume can be determined from two orthogonal views of the left atrium (apical four and two chamber

views, figure 2). Left atrial volume is then calculated by the area length method and indexed to body surface area:

$$\text{Left atrial volume} = (0.85 \times \text{Area}_{4ch} \times \text{Area}_{2ch}) / \text{Length}$$

The area of the left atrium is traced in both the apical four and two chamber views and the length of the atria are measured in both views and the shortest length is used in the calculation.

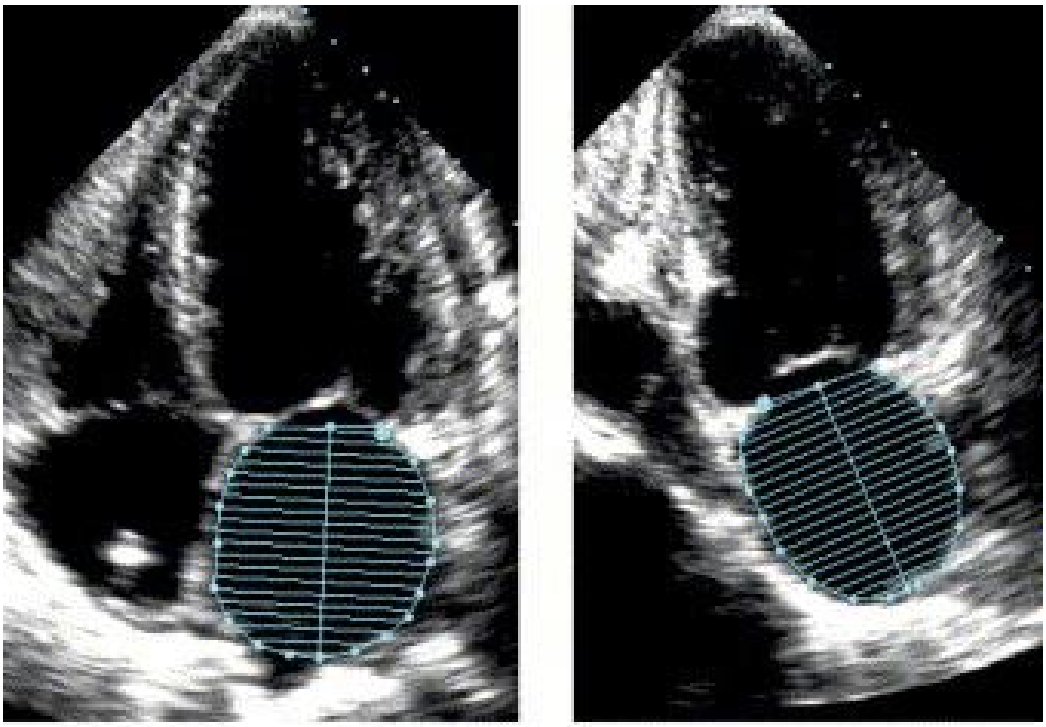


Figure 2. Apical four (A4C) and two chamber (A2C) views for LA area measurements which are in turn used to calculate LA volume.

Mitral inflow velocity for diastolic function and filling pressure assessment

Acquisition (Clinical sites): Using pulsed wave Doppler, place a small sample volume (1 to 2 mm) should be placed at the tip of the mitral leaflet during diastole. The direction of the ultrasound beam should be parallel with the jet of the mitral inflow direction which is usually directed laterally especially when the left ventricle is dilated. In these cases, transducer position should be shifted laterally when mitral inflow velocity is required (figure 8). The filter should be lowered to record and show low velocities.

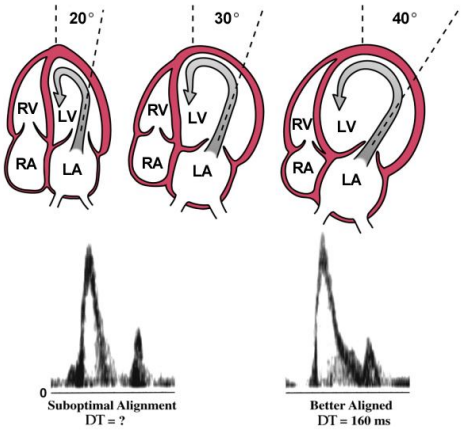


Figure 3. Alignment of pulsed wave Doppler for mitral inflow

Analysis (Echo Core Lab): Mitral inflow velocity will be used for assessment of LV filling pressure. The E velocity (peak early filling), A velocity (peak late filling), and deceleration time of E velocity will be measured. Examples of optimized mitral inflow Doppler recordings of mild diastolic dysfunction with delayed myocardial relaxation (left), normal (center), and advanced restrictive filling (right) pattern with respective E velocity, A velocity, E/A ratio, and deceleration time (DT) measurements are shown.

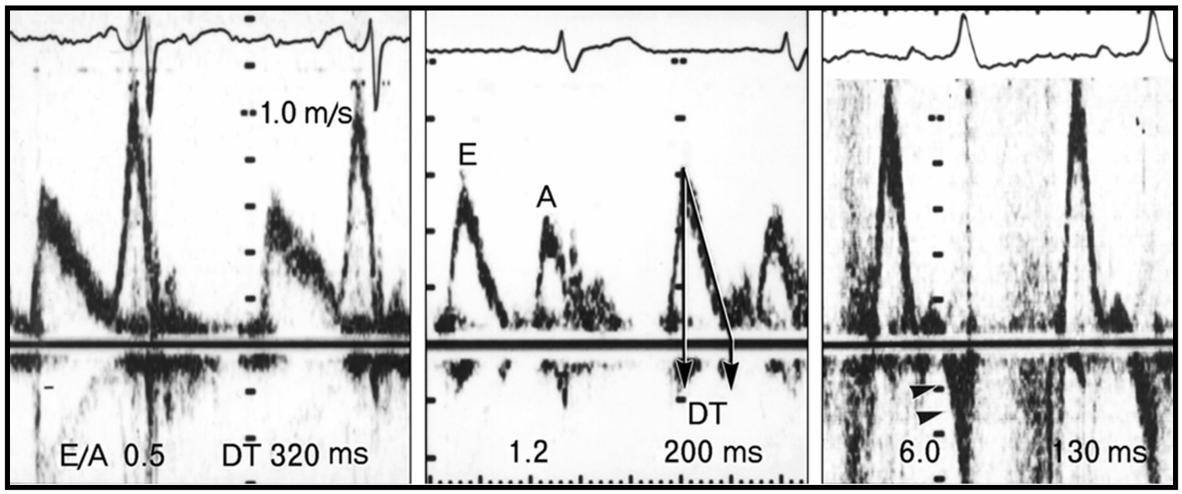


Figure 4. Recordings of mitral inflow velocities of mild diastolic dysfunction with abnormal relaxation (left), normal or pseudo-normal (middle), and severe diastolic dysfunction with restrictive filling and increased filling pressure (right).

Tissue Doppler of mitral and tricuspid annulus velocity

This is a simple recording of the mitral annulus velocity using pulsed wave Doppler.

Acquisition (clinical sites): Usually, preset or program option should be selected for obtaining tissue Doppler (DTI function after selecting preset, or program function), and then a sample volume should be placed both at the septal and lateral portion of the mitral annulus to record the E' and A' velocity of the mitral annulus. Another recording should be done with the sample volume at the lateral tricuspid annulus (Figure 5).

Analysis (Echo Core Lab): The early and late mitral annular velocities will be measured at the lateral and medial annulus. An E/e' ratio can be calculated for the septal and lateral annulus separately, or the early mitral annular velocity (e') can be averaged (recommended) to determine the E/e' ratio for diastolic function assessment.

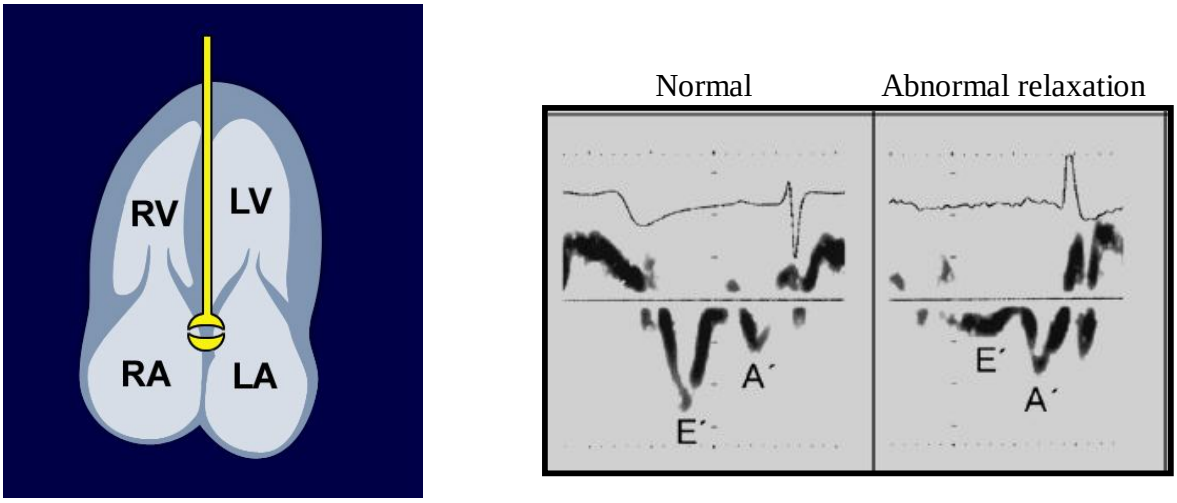


Figure 5. Sample volume placed at the mitral annulus for recording of DTI (left). Examples of normal and abnormal relaxation patterns (right).

Tricuspid regurgitation velocity

Tricuspid regurgitation velocity (continuous wave Doppler) can be obtained by the duplex imaging probe or non-imaging probe from the right ventricular inflow view, apical four chamber view, or parasternal short axis view at the basal level or even the subcostal view. Since there is a respiratory variation in tricuspid regurgitation velocity, the velocity should be recorded at end – expiration. When there is a significant variation, the highest velocity should be used.

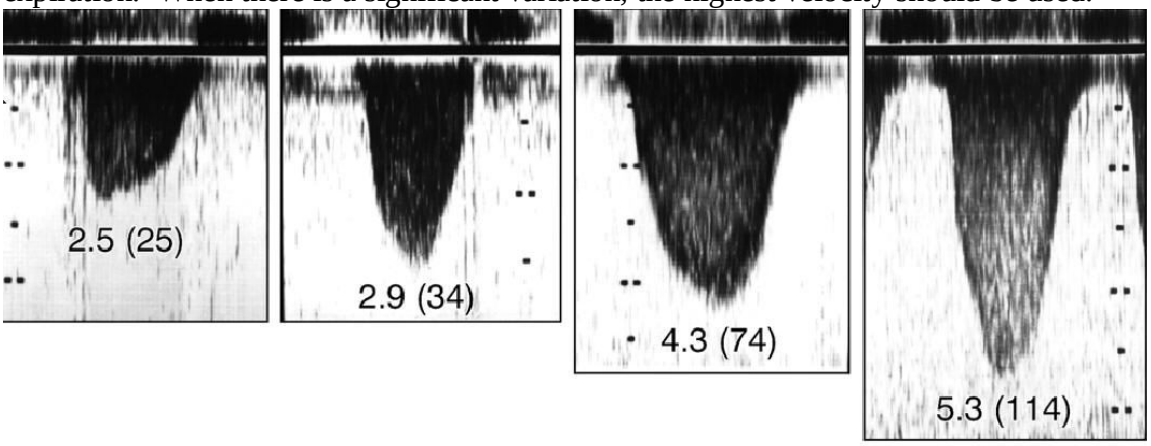


Figure 6. Tricuspid regurgitation velocity recordings of 2.5 m/s to 5.3 m/s. The numbers in parentheses indicate trans-tricuspid gradients derived from the velocities using the Bernoulli's equation.

Pulmonary artery systolic pressure: Systolic PAP will be estimated from the peak tricuspid regurgitation (TR) velocity obtained with continuous wave Doppler echocardiography. It is usually obtained from the right ventricular inflow view, parasternal short-axis view or, most frequently, the apical view. After peak TR velocity is measured, systolic PA pressure is calculated as the following:

$$\textit{Systolic PAP} = 4 \times \textit{TR velocity}^2 + \textit{right atrial pressure (RAP).}$$

RAP is estimated from the inferior vena cava (IVC) caliber response to inspiration. If the IVC dimension decreases 40% or greater with inspiration, RAP is assessed to be 5 mm Hg. If IVC caliber decreases 10-39% with inspiration, RAP is estimated to be 10 mm Hg. If the IVC dimension decreases less than 10%, RAP is assumed to be 15 mm Hg. If the TR velocity signal is weak or not adequate, intravenous administration of Definity (0.2 - 0.3 cc followed by saline flush) will improve the signal

RV size and systolic function assessment

RV size will be measured as shown below. RV systolic function will be visually assessed as "normal", "mild-moderate dysfunction", or "severe dysfunction." Tricuspid lateral annulus velocity (see Tissue Doppler section) will also be used to assess RV systolic function. Tricuspid annular plane systolic excursion (TAPSE) can also be obtained by placing an M-mode cursor through the tricuspid lateral annulus in the apical four chamber view. TAPSE is measured from the maximal ascent of the annulus to the maximal descent in ventricular systole.

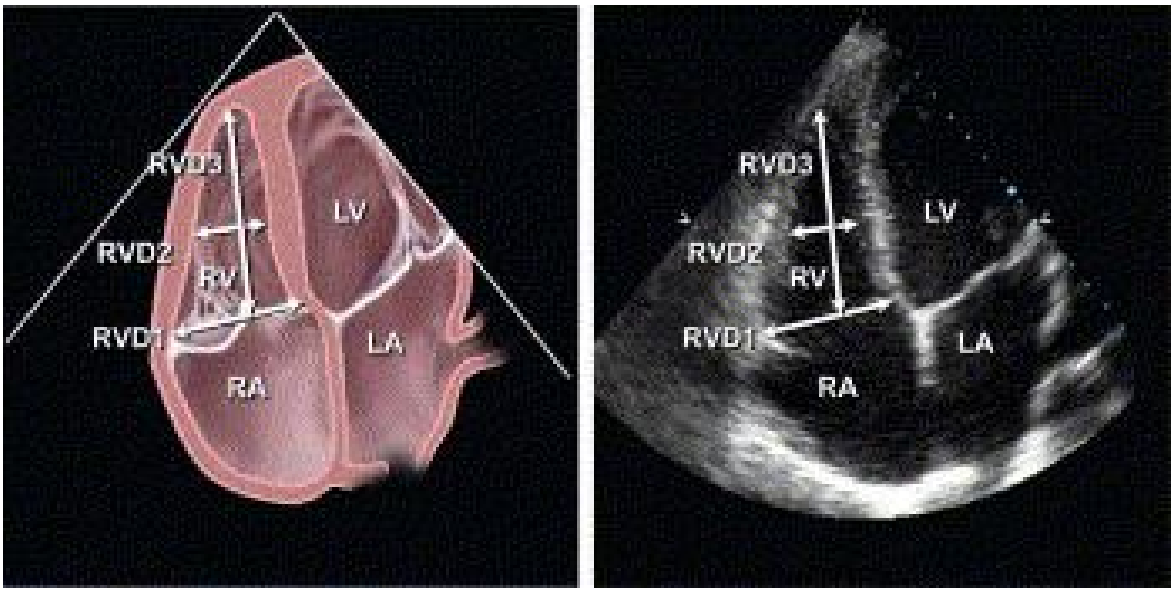


Figure 7. Measurement of right ventricular size

Tricuspid annular systolic velocity

Tricuspid lateral annular systolic velocity (see Tissue Doppler section) should be obtained by placing a sample volume at the lateral tricuspid annulus (Figure 8).

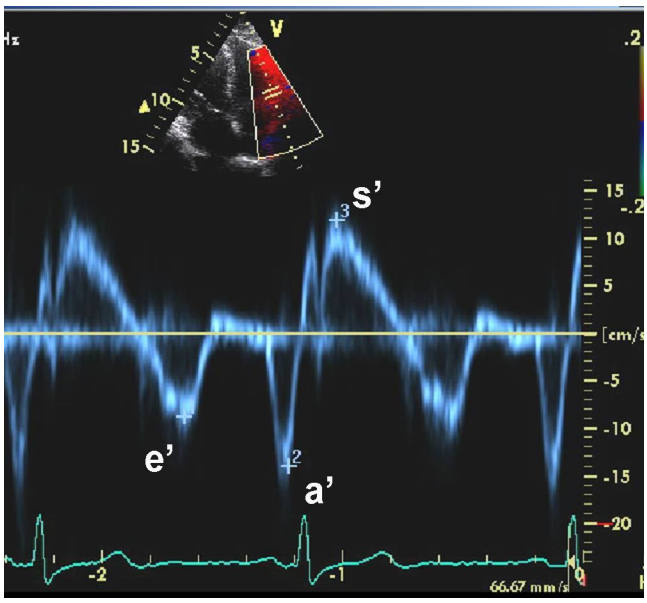


Figure 8. Measurement of tricuspid lateral annular systolic velocity in a patient with normal right ventricular function.

Tricuspid annular plane systolic excursion

Tricuspid annular plane systolic excursion (TAPSE) should be obtained by placing an M-mode cursor through the tricuspid lateral annulus in the apical four chamber view. TAPSE is measured from the maximal ascent of the annulus to the maximal descent in ventricular systole (Figure 9).

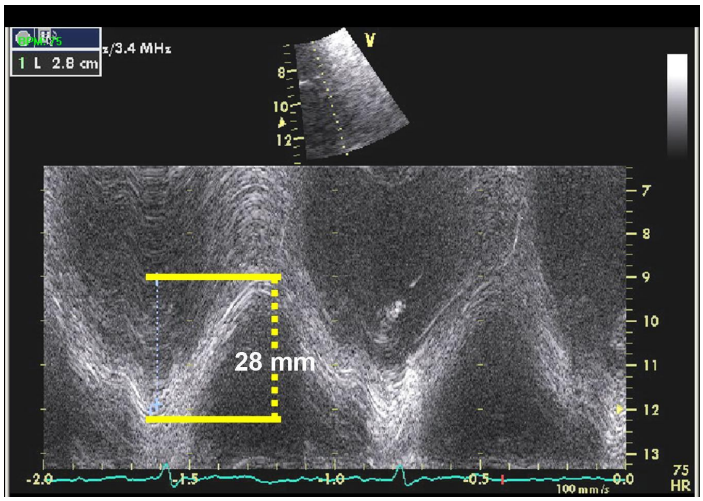


Figure 9. Sample TAPSE in a normal patient. Measure from maximal ascent to maximal descent.

B. QUALITY CONTROL

1. QUALITY CONTROL

To maintain the quality of echocardiographic examinations for the HFN trials, the following steps will be taken:

- Distribution of a Manual of Operations
- Pretrial review of echocardiograms of one or more patients from each clinical center demonstrating satisfactory acquisition of required images (certification).
- Continuous evaluation of echocardiographic quality and techniques to improve subsequent examinations.

2. *REQUIREMENTS FOR CERTIFICATION*

The Echo Laboratory at each clinical site will need to be certified prior to the enrollment of the first patient in the HFN Trial. For certification, each center is required at minimum to send one or more studies with the following information;

1) ECG recording on each study
2) Satisfactory apical and parasternal long axis views
3) Mitral inflow velocity and annular velocities for LV filling pressure assessment

Subsequent measurements may be required for certification if further echocardiographic data are requested by other trials in the HFN.

3. DATA TRANSFER

Digital images can be uploaded directly to the HEART IT server by the clinical sites and accessed by the Echo Core Laboratory personnel. Please follow HEART IT instructions for image upload and de-identify all images. Each site will be assigned a login and password to upload the digital images to the website. If unable to upload to HEART IT server, studies can be mailed directly to the Echo Core Laboratory on a CD. Unless otherwise specified, the CD will not be returned to the clinical centers. If submitting via CD or DVD, copies of the shipment and patient information forms (included at the end of this MOO) should also be enclosed with the media at the time of shipping. If uploading via HEART IT, please fax the shipment and patient information forms to the Core Lab. Suggested data formats for CD/ DVD shipments:

*All CD or DVD Dicom data must be submitted in True or Pure Dicom format.
Suggested codes:
Indeo 5.x
Microsoft Video 1
Cinepak
CDs or DVDs may also be submitted using AVI and JPEG files.
For NTSC:
All video files must be in the (.avi) format
All video files must be 640x480 pixel size

All still images must be in the (.jpeg) format
All still images must be 640x480 pixel size

C. MAYO ECHO CORE LAB PERSONNEL AND CONTACT INFORMATION

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SONOGRAPHER COORDINATOR	Barbara G. Manahan, RDCS
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Instructions for Shipment Form

Instructions for **Echo Core Laboratory Shipment Form**:

1. One copy faxed to Echo Core Lab with each study.
2. If mailing CD, include a copy with shipment.
3. Record Echo site, date of study, shipment date, contact information (phone and fax, name of contact person) for site
4. Sign and date each form before shipping

The Shipment Form includes the address, phone number and fax number of the Echo Core Lab.

HFN ID Number, **Patient Initials**, **Echo Date** and **Scan Description** (e.g. baseline, 6, 12, or 18 month echo, 180 day, or end of study echo) should be completed for each echo included in the shipment. A comment section is available and may be used as needed. (More than one shipping form may be used if necessary.)

Fax a copy of the completed shipment form to the Echo Core Lab.

Fax number: 507-266-2532

Make a copy of the shipment form for your records.

Enclose the completed shipment form as the packing slip with the shipment of echocardiograms.

Remember to include your Patient Information Form(s) with the shipment.

HFN ECHO CORE LAB SHIPMENT FORM

To: HFN Trials
Echo Core Lab
Plummer 115
200 First Street, SW
Rochester, MN 55905
Attn: Barbara Manahan

Fax: 507-266-2532

Telephone: 507-266-0072

From: _____
Site Name/Number

Contact Person: _____

Fax: _____

Telephone: _____

Date: ____/_____/_____

HFN ID#	Patient initials	Echo Date	Scan description	Number of Clips

Instructions:
If using Heart IT transfer system:
Fax a copy of completed form to the Echo Core Lab, 507-266-2532.
Keep this form for your records.
Include a completed study specific Patient Information Form for each study in the shipment.

If NOT using Heart IT transfer system:
Fax a copy of completed form to the Echo Core Lab, 507-266-2532.
Make a copy of this form for your records.
Enclose this form as packing slip with shipment of echocardiograms.
Include a completed study specific Patient Information Form for each study in the shipment.

_____ _____
Signature of Sender **Date**

Instructions for Patient Information Form

A Patient Information Form is to accompany each echocardiographic study sent to the Echo Core Lab. Please be as thorough as possible when filling out these forms.

Please indicate the name and site number of the medical facility in the **Site** section of the form.

This form contains the **Patient Initials** and **HFN ID Number.** The **HFN ID Number** should be the patient's randomized study ID number, not their hospital or clinic ID number. Example: HFN ID for EXACT is EX100-001 (site 100, 1st patient). TEST 1, TEST 2 etc. may be used for the certification echoes instead of an ID number and/or initials.

Please indicate the type of exam (scan description) performed. **Baseline** should be checked for the echo performed during the run-in period. **6 month** should be checked for the 6 month follow-up echo exam. **12 month** should be checked for the 12 month follow-up exam. **18 month** should be checked for the 18 month follow-up exam. **Other** should be checked if the echo is obtained within any other time frame. If **Other** is used please document the time frame from randomization in the free form section.

The **Echo Date** should be listed with the day first. This is followed by the first 3 letters of the month and the year. Months should be listed as:

January	Jan
February	Feb
March	Mar
April	Apr
May	May
June	Jun
July	Jul
August	Aug
September	Sep
October	Oct
November	Nov
December	Dec

A brief patient history section is included on the form.

Gender should be indicated by a check in the appropriate box.

Birthdate should be entered in the same manner as the echo date with day first, first 3 letters of the month, followed by the year.

Height can be entered as either centimeters or inches. A check mark should be placed in the appropriate box indicating whether the measurement is in centimeters or inches.

Weight can be entered as either kilograms or pounds. A check mark should be placed in the appropriate box indicating whether the measurement is in kilograms or pounds.

A resting **Blood Pressure** should be obtained at the time of the echo exam. This value should be recorded on the Echo Core Lab Information Form.

Whenever possible, record the **Heart Rate** at the time the left ventricular outflow tract pulsed-wave Doppler exam is performed.

Please specify the patients dominant **Rhythm** at the time of the echocardiogram by placing a check mark in the box. An "other" category is included with space for specifying and should be used as needed.

Please print the name of the **Sonographer** and the **Physician** involved with the echo exam on the form.

A section has been included for **Comments to Reviewer**. This section may be used to indicate any additional information to the Echo Core Lab personnel.

Please use "**NA**" for information that you are unable to obtain. Record this in the designated area on the form.

Patient Information Form

Site Name	Site Number (first three digits of subject ID number)

Study ID Number	Patient Initials (first, middle and last)	Date of Birth (example: 22/ DEC/2003)
__ __ _____ __ __	__ __ __	__ __ Day __ __ __ Month __ __ __ __ Year

ECHO Date (example: 22/ DEC/2003)	ECHO Visit (check one)
__ __ Day __ __ __ Month __ __ __ __ Year	☐ **Baseline** ☐ **Other (describe)**_____

Patient History

Gender	Height	Weight
☐ **Male** ☐ **Female**	_____ ☐ **cm** ☐ **inches** *(check 1)*	_____ ☐ **kg** ☐ **pounds** *(check 1)*

Heart Rate	Blood Pressure	Rhythm:
__ __ __ **bpm**	__ __ __ / __ __ __	☐ **NSR** ☐ **A Fib** ☐ **Other, specify** _____

Sonographer (print name): _____

Physician (print name): _____

Comments to Reviewer:

Echo Certification Submission Form

Site/Number

Certification Number

Echo Submitted by (please print)

_____

e-mail _____
Phone number _____

Sonographer (print name)

_____

Physician (print name)

_____

Would you like this echo returned? YES NO

Comments to Reviewer:

INDIE-HFpEF

Addendum to HFN Site Manual of Operations

Inorganic **N**itrite **D**elivery to **I**mprove **E**xercise Capacity in HFpEF

Version 1: 21April2016

Table of Contents

1 INTRODUCTION & CONTACTS

The purpose of Addendum is to describe the study-specific operational plan and procedures to comply with the INDIE-HFpEF protocol and HFN Network policies and procedures generally outlined in the HFN Site Manual of Operations.

1.1 DCRI Coordinating Center Contacts

Name	Role	E-Mail	Phone
Mary Ann Sellars	Program Manager	maryann.sellers@duke.edu	(919) 668-8544
Pamela Monds	Project Leader	pamela.monds@duke.edu	(919) 668-5590
Teresa Atwood	Lead Clinical Research Associate	teresa.atwood@duke.edu	(919) 668-8841
Ryan Stults	Clinical Research Associate	ryan.stults@duke.edu	(919) 668-8149
Judy Toutant	Clinical Research Associate	judy.toutant@duke.edu	(919) 668-8314
Lagia Davis	Clinical Research Associate	lagia.davis@duke.edu	(919) 668-8748
Melissa Brockington	Clinical Trials Assistant	melissa.Brockington@duke.edu	(919) 668-7809
Shrabani Sharma	Clinical Trials Assistant	shrabani.sharma@duke.edu	(919) 668-9215
Medical Monitor	Refer to HFN Website		(919) 970-4433
Trevorlyn Haddock	Safety Surveillance	trevorlyn.haddock@duke.edu	(919) 668-8552
Kristy Hwang	Data Management	kristy.hwang@duke.edu	(919) 668-8059
Jane Winsor	Data Management	jane.winsor@duke.edu	(919) 668-8645
HFN RightFax #:	N/A	hfn.rightfax@dm.duke.edu	(919) 668-9871

1.2 Core Laboratories

BIOMARKER - University of Vermont, Dr. Russell Tracy
208 South Park Drive
Colchester, VT 05446
Rebekah Boyle- Project Manager
Office: 802-656-8938
Email: rebekah.boyle@med.uvm.edu

GENETICS - Montreal Heart Institute, Dr. Michael Phillips
740 Penfield Avenue
Montréal, Québec H3A 1A4
Hélène Brown RN B. Sc. CCRP Team leader
Montreal Heart Institute
Tel: (514) 376-3330 ext 3931
Fax: (514) 593-2575
E-mail: helene.brown@icm-mhi.org

CPET – Mass General Hospital, Dr. Greg Lewis
Heart Failure and Transplant Section
Massachusetts GeneralHospital, Bigelow 800
Fruit Street
Boston, MA02114
Tel: 617-724-6158
Fax: 617-726-4105
E-mail: glewis@partners.org

ACCELEROMETRY – Mayo Clinic, Dr Levine
13400 Shea Blvd.
CRB 2-208
Scottsdale AZ 85259
Gabe Koepp
E-Mail: Koepp.Gabriel@mayo.edu
Phone: 507-990-2187

ECHOCARDIOGRAM – Mayo Clinic
200 First Street SW
Mayo Echo Core Lab
Rochester MN 55905
Barb Manahan, RDCS
E-Mail: manahan.barbara@mayo.edu
Phone: 507-266-0072
Fax: 507-266-2532

Please refer to the Core Lab specific MOP for further requirements and details.

1.3 InForm EDC (eCRF)

Questions regarding technical problems using the eCRF or to reset your password:
U.S. 1-866-999-DCRI [3274]
E-mail: edchelp@dm.duke.edu
Coverage: 6am to 12am Monday through Friday
Questions regarding entering subject data, queries, etc., contact the DCRI Data Management Team. (Contact information listed in Section 1.1.)

1.4 Clinical Helpline and IXRS

Almac Clinical Technologies	Telephone Number
Clinical Helpline	919-970-4433
Almac Helpline	1-800-923-3209

1.5 HF Network Clinical Helpline

A physician will be available 24 hours a day to answer urgent questions about recruitment, enrollment, and patient management by calling the HFN Clinical Helpline at 1(919)970-4433. For non-urgent or general questions please contact your CRA

Sites are encouraged to call the Clinical Helpline to seek assistance on unclear issues that arise, after consultation with your site PI/Investigator, when applying the research plan to specific patients. Use of the Clinical Helpline is especially important before randomization of patients with any question regarding eligibility and when a patient is considering discontinuing the study for any reason.

The HFN Clinical Helpline is for questions related to:

- Enrollment
- Urgent clinical questions related to patients and the trial (most should be addressed initially by the site PI)
- Unblinding
- Discontinuations

The HFN Clinical Helpline is not for questions related to:

- Operational or procedural questions re: an HFN trial or Core Lab

- Re-supply orders should not go through the clinical helpline. Study Coordinators should contact the DCRI Coordinating Center (CC) or logon to the HFN website for site materials.

HFN Fellows, will take the role as the Medical Monitor on the HFN Clinical Helpline. Study Coordinators should email the HFN Fellows if they do not receive a call back after paging the helpline. When placing a call to the HFN Clinical Helpline: listen carefully to the instructions on the phone. The prompter will either say a) enter your call back number or b) note the telephone number to call (cell phone). **It I important to listen to the whole message**, do not punch a number in right away or the message will not be delivered. If the Study Coordinator still does not receive a response from the Medical Monitor then CC Site Management should be contacted.

2 HF NETWORK CLINICAL CENTER SITE ACTIVATION PROCESS

Site activation by the CC will occur once the following has been completed:

- Site contract has been executed
-
- Regulatory documents are complete and approved by CC (refer to table 1)
- Study/Protocol training has been completed by PI & Primary Study Coordinator (minimum)
- Inform Training completed by those requiring access
- CPET certification complete (unless documented exemption is provided)
- Study drug/device & Core Lab supplies have been received
- Site Initiation Phone Visit Conducted (and any required action items closed)

All HF Network and study specific site manuals, documents and tools will be provided by the CC. Additional copies or resupply requests are available via the HFN Website resupply request form under the study folder on the HFN website at www.hfnetwork.org. Please review and organize all study specific materials and required documents prior to the Site Initiation Phone Visit with the CC. Please refer to the HFN MOP for details regarding preparation for patient enrollment.

Once all activation activities have been completed, a site activation notification from the DCRI CC will be distributed notifying the PI, study coordinator, and other appropriate personnel of approval to initiate study enrollment. This notification should be filed in the regulatory binder as documentation of the date in which the site was activated.

TABLE 1 - Documents for Completion by each Clinical Center to be collected: (Please refer to the regulatory document completion guidelines provided with the regulatory packet for start-up documents.)

1.	Study Site Staff Delegation and Signature Log (SDSL)	Sites send to the DCRI project team at start-up. The SDSL is a living document and should be updated upon changes in personnel at site. The original is maintained at the site.
2.	Investigator CV / Bio sketch	RCC PIs and site PI must provide current CV. Site sends to the DCRI project team prior to activation.
3.	Medical License	RCC PIs and site PI licenses must be current and available to the DCRI project team prior to activation.
5.	COI Form	RCC PIs, site PIs and Sub-Investigators listed on the 1572 must sign a COI at the start of the study and update it annually or upon change in COI status.
6.	FDA Form 1572	Upon completion, the site PI must sign and date and send to the DCRI Project team prior to site activation. (Once a site is activated if there is a change in PI or information included on the 1572, an updated form must be sent to the DCRI Project team.)
7.	Federal Wide Assurance (FWA) Number	FWA Number is updated according to the expiration date. Sites send to the DCRI project team prior to activation and as needed according to expiration date.
8.	IRB Approval for Protocol, Informed Consents, & Patient Materials	IRB approval must be updated yearly. Sites send IRB approvals to the DCRI project team prior to activation and throughout study as IRB renewal and amendment approval is obtained. The CC will review and approve all ICFs prior to submission to their local IRBs.
9.	Documentation of Human Subject Protection Training	This documentation is required for all key personnel listed on the SDSL. Sites send completed training to the DCRI project team prior to activation and provides updates to the form when new key personnel are added.
10.	Signed Contract with CC	Each RCC and Enrolling Center in the U.S. needs an executed Rapid Start Network-Federally Funded Grants Participation (RSNG) and Contract Addenda prior to activation.
11.	Protocol Training	Training Certifications for each individual protocol will be provided by the DCRI project team. The PI and primary SC (at minimum) must receive protocol training prior to activation. Ongoing training and training of new study personnel must be documented at the site.
12.	InForm Training	Each site user, including the PI must complete InForm training.
13	CPET Certification	All sites are required to complete CPET certification regardless of participation in other HF Network Trials, unless site is informed that they're exempt. (refer to current CPET MOP for more details)

A change in study PI requires the collection of new or updated documents including CV, medical license, updated ICF(s), IRB approval/acknowledgement, and contract amendment.

Evidence of qualifications including any licensures or credentials and documented protocol training for those listed on the SDSL should be maintained on file at the site.

3 SCREENING

Screening/Recruitment Plan

A protocol specific Recruitment Plan will be completed by each enrolling center to define strategies to increase protocol awareness, delegate personnel and adequate resources, identify competing studies and prioritization plans, and list potential recruitment barriers and actions to mitigate barriers. This plan will be reviewed and discussed during the site initiation phone visit. Based on recruitment, it is expected that PI and study team will review the effectiveness of their plan on a periodic basis throughout the study and update strategies as needed to meet the HFN recruitment goal of 1 subject per month.

Screen Failure Log (for non-consented subjects)

Sites are to maintain a record of subjects screened but not consented for the study. This screening log data will be entered by the research team into SharePoint or the screening logs can be sent to the CC on a weekly basis at the beginning of the study, and then at minimum, monthly, as specified by the CC. Screening log data includes: date of prescreening, failed code(s), reason description, and chart status of failed. Note: All consented subjects will be entered in the IXRS and InForm.

Screening Process

Patients with a HFpEF diagnosis are screened for basic entry criteria, including those designed to ensure that HF symptoms are the primary limitation to activity and ability and willingness to wear the accelerometer belt.

Patients meeting eligibility criteria will be approached regarding participation in this study. Potentially eligible research participants will be identified by investigators reviewing the patients' medical records. The primary physician will be contacted with full explanation of the protocol and consultation regarding the suitability of the patient. If the primary physician agrees, patients will be approached for study participation. Suitable candidates will be approached by investigators (or their designee) regarding the study and be provided with detailed study information and a copy of the informed consent form. Patients who fulfill entry criteria will undergo the consent process and written informed consent will be obtained prior to any study specific procedures

All participants who sign informed consent to continue further assessment of entry criteria will be screened in IXRS and assigned a Subject ID. For detailed instructions on screening procedures, please refer to the Almac User Guide on the HFN website.

- A screening question to assess whether their ability to be active is related to their heart failure symptoms (versus orthopedic, neurologic or behavioral factors) will be completed. If patients indicate that reasons other than heart failure symptoms limit their ability to be active, they will be considered a screen failure and will not continue.
- Subjects will be assessed for their ability to wear the accelerometer belt confirmed with a "test belt" provided to each center. Willingness to wear the accelerometer belt and to participate in all study procedures is confirmed.
- Baseline blood draws: CBC, complete chemistry panel, biomarkers, biorepository and genetics (if consented) – since the exertion of the CPET will impact the cGMP biomarker, it's necessary to obtain the baseline biomarkers blood prior to the CPET. **Please note:** for this reason, we've proposed that all bloods are obtained at this time point to avoid having to stick the patient more than once. If for any reason the biomarkers are not obtained prior to the CPET then the biomarkers cannot be obtained until 3 hours post CPET and prior to run-in test dose.

- An HFN study-specific, baseline CPET will be conducted to ensure patient meets the entry criteria. Patients that do not meet the CPET criteria will be considered a screen failure and will not continue.

If the subject does not pass the above criteria then the subject is a screen failure and will not continue.

Once these criteria have been confirmed, patients will undergo the remaining baseline studies (below) and undergo single dose unblinded run-in receiving 80 mg nebulized sodium nitrite followed by observation for 60 minutes.

Please note: Once subjects are consented, they must be registered in the IXRS system, assigned a study patient number and followed for adverse events. It is important to assess and rule out as many inclusion/exclusion criteria as possible, prior to consent, in order to avoid unnecessary activities. In addition, the sequence of screening / baseline activities are important to avoid exposing the patient to unnecessary tests and/or procedures.

Remaining baseline studies and procedures include:

- Complete Medical History and Medication Review
- NYHA class assessment and Physical Examination
- ECG
- KCCQ

Run-In Test Dose (post completion of baseline studies):

- Verify SBP $\geq$ 115 mmHg seated and $\geq$ 90 mmHg standing and resting HR $\leq$ 110 just prior to test dose (if not, the participant is considered a screen failure and will not receive the test dose).
- Participants passing the SPB and HR criteria receives open label run-in with sodium nitrite (80 mg) given once, with monitoring for AE for 60 minutes following dosage for adverse effects or symptomatic hypotension.
  - Systemic blood pressures (seated and 3 minutes after standing) will be monitored every 15 minutes for 60 minutes. Results should be documented in the participant's source documentation.
- Participants developing hypotension (SBP < 90 seated or standing), lightheadedness or who otherwise do not tolerate the single-dose run-in will be considered failures and will not be randomized. Any events experienced or tolerability issues should be documented in the participant's source documentation.

Participants that fail screening after signing informed consent and are not randomized, their screen failure status and reason for screen fail should be updated in IXRS. For detailed instructions on screen fail procedures, please refer to the Almac User Guide on the HFN website.

Pocket Reference Cards will be provided for key inclusion/exclusion criteria and scheduling. This reference card includes the study flow diagram and the schedule of activities table which will be a useful reference at the time of each patient assessment.

Master Subject Log

Per GCP, sites will maintain a log of all subjects who signed informed consent along with their study ID, name, and medical record number. This information is confidential and should be files in a secure location.

4 OBTAINING INFORMED CONSENT

Informed consent will be obtained as required by the local institutional review boards. Written informed consent must be obtained from the patient prior to beginning any activities that are not part of the patient's routine care. Patients should be encouraged to have supportive family member(s) and other advocates present during the consent process. Additionally, they should be given full access to all physicians involved in their care to aid in their deliberation on trial entry. Patients should be informed that their consent can be withdrawn at any time and for unstated reasons. A copy of the signed consent should be given to the subject, and the consent process should be documented in the patient's source.

5 RANDOMIZATION

After providing informed consent and signing the ICF, all subjects who fulfill all the inclusion criteria and none of the exclusion criteria will be randomized.

Participants will be randomized by using Almac's IXRS randomization system. For detailed instructions on randomization procedures, please refer to the Almac User Guide on the HFN website. All study personnel who wish to access the system must attend training or read the training manual. Once completed, the study personnel should document training. Upon confirmation of the training, the project team will then request access. Training documentation should be kept in the regulatory binder.

A Randomization Worksheet with evidence that the patient meets inclusion/exclusion criteria and a signed Informed Consent Form should be completed prior randomization.

Following randomization, study staff will dispense the accelerometer, nebulizer device and supplies, nebulizer study drug guide, medication chambers (PURPLE for administration of 46 mg and WHITE for administration of 80 mg), and washing basket. Then will educate participant on how to use the nebulizer device and how to wear the accelerometer using the patient instructions provided by the CC. Participants will be required to demonstrate independent proficiency with the nebulizer prior to dismissal from Visit 1.

Study staff will dispense Phase 1 study drug and participant must demonstrate proficiency with the instructions on how to administer both the 46 mg dose (with the PURPLE study drug guide) and the 80 mg dose (with the WHITE study drug guide).

Study staff will define optimal times and phone numbers for the protocol specified phone contacts to encourage compliance with study procedures. A plan for the weekly phone visits will be established with the participant. Participant-specific strategies such as sign posting, bathing-time accelerometer placement, and alarms may be used to enhance compliance with accelerometry and study drug administration.

All participants will receive the following documents or materials at Study Visit 1:

- A copy of the signed informed consent and contact numbers for the research team in the event of questions or to report any concerns related to the study
- A patient wallet card to present to a hospital, emergency room or urgent care facility following randomization into the trial
- An I-Neb Device User Guide (this will be included in the nebulizer kit)
- I-Neb Quick Start Guide
- Subject Manual for Plastic Ampules Quick Reference Guide
- Participant Diary to document study drug dosing and device following randomization
- Accelerometer Instructions
- An HFN Tote Bag – to transport study materials/supplies
- An HFN Sharpie Pen – to record date each foil packet is opened

IRB approval is required for all subject documents/materials prior to distribution to participants.

6 FOLLOWUP EVALUATIONS

Clinic Visits 2 and 3

All participants will receive study clinic visits at the end of each treatment phase. Participants will return no sooner than 42 days (but up to 49 days) for each phase. Participants requiring extra time beyond the 42 day window must remain on study drug during this period with no interruption in therapy. The following Study Visit 2 and 3 procedures will be performed:

- Confirm study drug held the day of the visit (confirmed by study staff)
- Interim History and Medication Review
- Physical Examination and NYHA class assessment
- KCCQ questionnaire
- Obtain blood draws prior to study drug administration and CPET (in the event this is not feasible, then bloods must be obtained at least 3 hours post to study drug administration): CBC, Complete Chemistry Panel (Sodium, Potassium, Chloride, Carbon Dioxide, BUN, Creatinine, Glucose, AL, AST, Alkaline Phosphatase & Total Bilirubin) and HFN biomarkers (cystatin C, NT-proBNP, cGMP and nitrosothiols), and biorepository samples (if consented)
- Limited Echocardiogram (Obtain echo prior to study drug administration and CPET); in the event, the echo cannot be completed prior to study drug administration, it cannot be obtained until at least 3 hours post study drug administration)
- Administer a dose of Phase 1 study drug just prior to CPET
- Perform cardiopulmonary exercise test
- Collect Phase 1 study drug and perform accountability/compliance
    - review subject diary (assess if information is consistent with returned drug)
    - download nebulizer compliance report (refer to the I-Neb AAD Insight System User Instructions)
    - assess and evaluate study drug administration compliance
    - re-educate subject as needed to support/improve compliance

  The following procedures will be performed at Visit 2:

  - Compliance plan: A plan for the phone visits will be established with the Participant. Participant-specific strategies such as sign posting, bathing-time accelerometer placement, and alarms may be used to enhance compliance with accelerometry and study drug administration.
  - Collect and return phase 1 accelerometer
  - Issue Phase 2 accel accelerometer
  - Assign Phase 2 study drug via ALMAC IXRS and dispense to subject

  The following procedures will be performed at Visit 3:

  - Collect and return accelerometer
  - Collect Phase 2 study drug
  - Participants are asked to indicate the study phase during which they felt better (Patient preference secondary endpoint); results should be documented in the participant's source

Routine Phone Follow-up

All participants will receive weekly study phone calls post-randomization to monitor compliance with the accelerometer belt, nebulizer device and study drug dosing and tolerance. During the follow-up phone visits, the participant will receive:

- Review and assess accelerometer and study drug compliance (utilizing the subject diary content)
    - Reminder of appropriate study drug dose for the stage of the protocol.
    - Reminder regarding appropriate use of nebulizer device, dose specific study drug guides and compliance
    - Encouragement of compliance with accelerometry use
- Encouragement of activity within the limits of their HF symptoms
- Confirm plans for future study visits
- Confirm need to bring study drug and accelerometers to future study visits
- Reminder to hold study drug on days of Study Visit 2 and 3.

Specific queries regarding dosing and tolerance of study drug will be reviewed during these calls to determine whether adjustment is required. In case of study drug intolerance due to headache, participants will be encouraged to treat with acetaminophen and continue the study drug. Study staff will also inquire about any changes to general health. A study visit calculator will be provided to sites to assist with scheduling the visits within the appropriate time frame per the protocol. Refer to the protocol for additional details.

2 Week Phone Follow-up (Final Visit)

A final phone visit is conducted 2 weeks after Study visit 3 to assess clinical stability and any adverse events.

7 STUDY DRUG

7.1 Study Treatment

On February 11, 2016 an IND number was granted by the FDA. See protocol for additional details.

7.2 Study Drug an Device Supplies

Drug dispensation will be managed by the CC in collaboration with Almac Clinical Services. Upon site activation, sites will receive an initial supply of the following study drug and device supplies:

- Open label drug for administration of run in test dose
- Study drug kits for dispensation to randomized participants
- I-neb AAD kit which includes the nebulizer device a purple and white drug guide, with medication chamber and mouthpiece, and I-Neb User Guide Instructions.
- Insight device (including power cord, battery charger and disc) for downloading administration compliance for randomized participants
- Extra Purple and White study drug guides, medication chambers, and mouth pieces for dosing per protocol (purple administers the lower dose and white administers the full dose) for randomized participants
- Washing Basket for randomized participants

Study drug kits for dispensation will be automatically resupplied when site has reached low threshold. Resupply for devices and other materials will need to be manually managed/requested by the site. Therefore, it is important for sites to monitor available supplies and utilize the Device Accountability Logs (DAL) or equivalent documents to track receipt, dispensation, return, and destruction per section 7.4.

7.3 Study Drug & Device Dispensing

At the study visit 1 (Phase 1), study staff will dispense a sufficient supply of inorganic nitrite or placebo ampules to permit three doses a day until the second study clinic visit, realizing that participants may not be able to return at the exact 42 day window due to scheduling conflicts

(range 42-49). To account for potential damage or loss of ampules after opening the pouches and to account for unavoidable (for example adverse weather, family illness, etc) further delays in returning for study visits beyond the 42 day (+ up to 7 days) timeline, additional study drug doses will be supplied in the kits. Similarly, study staff will receive enough inorganic nitrite or placebo ampules at the second study visit to last until the third (final) study visit as above.

Study staff will instruct participants to take the medication as required by the protocol. Compliance will be assessed at each study visit and by telephone contact as described in the protocol: inhaled, nebulized placebo or inhaled nebulized sodium nitrite starting at 46 mg and titrated up to 80 mg 3 times daily, at a minimum of 4 hours apart, with the first dose starting at the beginning of the active part of the day (for example, 8:00, 12:00, and 16:00). The participant should disperse the doses by at least 4 hours and deliver them over their normal active day time. The doses do not need to be given at the same time each day if the participant's active period varies by day, for example if the participant arises later on the weekends. Inhaled, nebulized sodium nitrite or placebo will be administered utilizing the Phillips I-neb AAD nebulizer over 10-15 minutes for each dose. Participants are instructed to open one pouch at a time and utilize each ampule before opening another pouch. Ampules not used within 7 days of opening should be discarded and a new foil pouch opened.

For permitted dose adjustments for adverse events, refer to section 10 of the protocol. In case of study drug intolerance due to headache, participants will be encouraged to treat with acetaminophen and continue study drug. For other intolerable side effects, the dose of study drug is reduced or discontinued. If participants cannot tolerate the 46 mg dose they will discontinue the study drug, but continue with all study procedures and visits. If participants cannot tolerate the 80 mg dose, they will return to the previously tolerated 46 mg dose and continue all study procedures and visits.

7.4 Drug and Device Storage, Accountability and Destruction

7.4.1 Storage

Trial products (both unused and in-use) should not be exposed to moisture but can be stored at room temperature (15-30°C or 59-86°F). Investigators should ensure proper storage conditions and record and evaluate the temperature. Study drug should be stored in a locked and secure environment.

7.4.2 Drug and Device Accountability

All study supplies must be accounted for in written documentation that must be maintained by the investigator. Forms to record dispensing of study drug, nebulizer device and supplies, open label run in drug as well as accelerometers will be provided prior to the initial shipment of the study drug and device. A copy of the completed study drug accountability record will be provided to the CC as part of the study closeout activities. Shipments from Almac will be confirmed in the system prior to the kits being available for dispensation.

7.4.3 Study Drug and Device Accountability Logs (DAL)

The DAL enables tracking of how much drug and devices are available on site. The Study Drug and Device Accountability logs or equivalent documents should be used to document when a shipment of study drug, devices and open label run in test drug is received, dispensed, lost/damaged, returned and the balance. The site should initial by each entry. A separate DAL will be provided to track how much open label run in test drug is available on site. In order to maintain study blind, the pharmacy will have to receive, dispense and maintain accountability for the open label study drug.

After a participant is randomized and dispensed study drug, nebulizer device and other supplies, **a Subject Accountability Log** or equivalent document should be completed. The subject number/ID, amount, date dispensed, and amount and date returned must be documented on the subject drug accountability log (DAL). The site should initial by each entry.

The following study drug records should be maintained by the designated site personnel:

- All study drug shipment invoices
- All IXRS confirmations documenting allocation for each subject number
- Study Drug Accountability Logs or equivalent
- Subject Accountability Logs for study drug, devices and supplies dispensed to participants

Participants can be instructed to discard used ampules. But will be reminded to bring the nebulizer and unused study drug to each visit. Returned trial product should be stored separately from the non-allocated trial product(s) until drug accountability has been reconciled. Counts should be done to assess subject compliance at study visits 2 and 3. All study drug and devices must be collected from the subjects at the final visit.

7.4.4 Destruction

Upon notification from the CC, excess or unused study drug as well as nebulizer devices can be be destroyed per institution policy upon occurrence of either of the following:

- Conclusion of the study
- Study is stopped prematurely

Partially used and unused study drug should be destroyed at the site according to accepted pharmacy practice, local and national guidelines, using the site's destruction procedure. A copy of the drug destruction SOP should be maintained in the pharmacy section of the Regulatory Binder. In addition, study drug destruction should be documented in the comments section of the Study Drug and Open Label Accountability Log.

Used nebulizer devices can also be destroyed per institutional policy and documented accordingly. All unused nebulizer devices must be returned to MAST Therapeutics. Instructions will be provided by your CRA at close-out.

7.4.5 Randomization, Stratification and Blinding

Randomization will occur at the first study visit. Randomization to active drug/placebo during the first phase of the crossover study (1:1 allocation ratio) is stratified by site. Blinding is ensured by preparation of identically appearing placebo and active drug ampules. Participants will be randomized using procedures determined by the CC to one of 2 sequences. A permuted block randomization method stratified by site will be used to ensure relatively equal distribution of participants to each sequences within each clinical site.

7.4.6 Unblinding

Unblinding should be a very rare occurrence. The investigative sites will be given access to the treatment code for their participants for emergency unblinding ONLY by contacting the medical monitor at the CC. Decisions about un-blinding will be made at the discretion of the site PI and the CC Medical Monitor. Randomization data are kept strictly confidential, accessible only to authorized persons until the time of unblinding.

7.4.7 Concomitant Medications

Participants should be treated with standard HFpEF strategies as per recommended guidelines. Participants should be on stable medications and with adequate blood pressure control prior to entry as outlined in the entry criteria. Further adjustment of diuretics or blood pressure medications during the study period is discouraged and should be performed according to new and clinically compelling worsening of clinical status. As above, therapy with organic nitrates, phosphodiesterase-5 inhibitors or soluble guanylyl cyclase activators is contraindicated during the study period.

8 DISCONTINUATIONS/WITHDRAWALS

INDIE-HFpEF is an intent-to-treat (ITT) study which means that all enrolled/randomized subjects will be included in the analysis as stated in the protocol. It is expected that every attempt is made to have the subjects complete all study treatment, assessments, evaluations and lab work per protocol.

If the patient states that they no longer want to participate in the study please clarify exactly what aspects of the study the subject wishes to discontinue. It's expected that the Study Coordinator notify and involve their PI/Investigator in these decisions:

- If a patient wishes to stop study drug/device and/or intervention – clarify if he/she will continue to complete all other study assessments.
- If a patient wishes to stop study assessments and/or clinic visits – clarify if he/she will allow you to continue to follow him/her via phone, contacting his/her physician and allowing you access to the medical records.
- If a patient will not allow phone follow-up, then clarify if he/she will allow medical record review.
- If a patient will not allow medical record review - if he/she no longer wants anything to do with the study and withdraws consent then do not contact him/her further.

After internal consultation with Site PI/Investigator, the site is required to contact the HFN helpline anytime that a patient wishes to discontinue any or all study participation, as described above, for additional guidance/instruction. In addition, sites must document these discussions and decisions on the HFN Subject Discontinue/Withdrawal Worksheet, or institutional equivalent document. (Refer to HFN Recruitment and Retention Guidance for more details).

8.1 Lost To Follow-Up Procedures

It is essential to have safety and mortality status on all patients within the HFN studies. Below are the procedures for locating a lost to follow-up patient:

Five Telephone Contact Attempts

There should be at least 5 attempts to contact the patient at home. 2 of these contacts should be in the evening hours and 1 attempt should be on the weekend. In addition, these calls should be spread over a 2 week period.

Call Primary Care Physician

Should the above attempts fail, a call to the primary care physician should be made. Often times the primary physician may have additional information that may not have been relayed to the Study Coordinator.

Send Certified Letter

If these attempts fail, then a certified letter should be sent to the patient with return receipt requested. Any information completed on the return receipt will at least give you a date the patient was last known to be alive.

Other Methods for tracking patients:

- Hospital medical records – further assistance in obtaining patient information admission/discharge dates with treating physician names
- Hospital demographic database – encounter dates (office visits, ER visits, admissions)
- Vital records/register of deeds – verify and obtain actual date of death (verify copy of death certificate), location of death

- Public libraries – assistance in determining/locating death information via obituaries.
- National Death Index – may be used to determine if a patient is alive
- Internet – may be used to find patients who may have moved.

9 ELECTRONIC CASE REPORT FORMS (eCRF)

This study will use InForm, a web-based electronic CRFs developed through a validated, electronic records and electronic signatures (ERES)-compliant platform (21 CFR Part 11). Site staff who will be entering data will receive training on the system, after which each person will be issued a unique user identification and password. Qualified study staff at each site will perform primary data collection from source-document reviews.

See INDIE-HFpEF eCRF instructions document for entering data into the eCRF. Data should be entered within 7 days of the completed study visit and queries should be resolved within 5 days of issue.

Data management expectations include:

- Maintain a minimum of 90% on-time data entry and cleaning
- Enter all visit data into InForm within 7 business days of visit completion
- Comply with protocol windows and schedule of assessments
- No visit has missing items for more than 30 business days
- No queries are open for more than 30 business days
- Requests for source documentation are submitted via secure fax email (to the HFN right fax inbox at hfn.rightfax@dm.duke.edu) or via FTP site within 5 business days; when sending source to the CC via email, it must be sent securely
- Requests for PI signature on safety events occur within 5 business days
- PI signature is obtained on all subject data in InForm at trial completion

10 SAFETY MONITORING

The reporting of information from an adverse experience (AE) can lead to important changes in the way a new treatment is developed, provide integral safety data, and foster awareness of new and important information concerning serious adverse events (SAE) among regulators, investigators and other appropriate people. The Site Investigator is responsible for monitoring the safety of patients enrolled in this study at each study site.

10.1 Safety Definitions

AE, SAE and endpoints: Refer to INDIE-HFpEF protocol adverse events section for study specific definitions.

Business Day: Any day which is not a Saturday, Sunday or public holiday. Business hours are 08:00 to 17:00 Eastern Standard Time.

Calendar Day: Any 24-hour day of the seven day week.

Receipt Date: The date when DCRI becomes aware of safety related information. The date of receipt of each initial report and follow-up report will be clearly marked on all documents. If information is received on a non-business day or after normal working hours on a business day, the receipt date will be the next business date. Additional information received during processing of the initial version of a case (prior to reporting to the INDIE-HFpEF DCRI trial team/designee) does not reset the regulatory reporting clock, based on receipt of follow up information at this point; however, new information will be incorporated within the initial case.

Day 0: The calendar day that DCRI Safety Surveillance is notified of an SAE or, if different from day received by DCRI Safety Surveillance, the date the medical monitor has determined the event qualifies for expedited reporting to the regularity authorities.

Safety Medical Monitor: A physician assigned to the study to perform a review of serious adverse events, review the investigator's brochure or product labeling for listedness, and to confirm the MedDRA coding for the event.

Study Medical Monitor: A physician assigned to answer clinical questions regarding the protocol.

Valid Case: A case that includes each of the following minimum criteria for the purposes of reporting:

- an identifiable patient
- the name of the suspect medicinal product(s) or clinical study if considered related to a clinical study or procedure/design
- an identifiable reporting source
- a serious adverse event

Screen Failures: A screen failure subject is defined as a one who signs an informed consent document and does not become randomized. Screen failure subjects who experience SAEs from signing of the informed consent until the point of screen failure must have the event reported via InForm or the backup paper SAE form, if InForm is down.

10.2 Anticipated Disease Related Events

The following AEs are anticipated, disease-related events in patients with HF with preserved EF (HFpEF):

- **Arrhythmias:** This refers to both atrial and ventricular arrhythmias.
- **Acute coronary syndrome:** This refers to unstable angina, non ST segment elevation myocardial infarction (NSTEMI), and ST segment elevation myocardial (STEMI).
- **Unplanned hospitalization, ER visit or clinic visit for worsening HF:** This refers to treatment for acute heart failure such as receiving intravenous diuretics.
- **Cerebrovascular event:** This refers to cerebrovascular accidents (stroke) of any cause (hemorrhagic, ischemic, or embolic) and transient ischemic attack (TIA).
- **Venous thromboembolism:** This includes both deep venous thrombosis and pulmonary embolus.
- **Worsening renal function:** This refers to acute kidney injury, typically defined as a rise in creatinine > 0.3 mg/dL over 48 hours, or progressive loss of renal function over time.

Anticipated disease related events will not be captured as AEs/SAEs during the study, but will be entered on the appropriate electronic case report form (eCRF) module ("Events of Interest" page).

10.3 Recording and Reporting of Adverse Events

The site investigator is responsible for monitoring the safety of participants enrolled into the study at the study sites. For this study, AEs/SAEs (except for those events reported as anticipated disease related events) occurring from signing of the informed consent through 2 weeks post visit 3 will be captured on the AE/SAE eCRF. Patients that have screen failed will be followed for AES/SAES until two weeks post last study drug dose. Unless exempted (anticipated, disease-related events, as described, per INDIE-HFpEF protocol, all SAEs whether or not deemed drug-related or expected must be reported by the investigator or

qualified designee within 1 business day of first becoming aware of the event. The investigator or qualified designee will enter the required information regarding the SAE into the appropriate module of the eCRF. If the eCRF system is temporarily unavailable, the event, including the investigator-determined causality to study drug should be reported via the back-up paper SAE form to DCRI Safety Surveillance at 1-866-668-7138. Upon return of the availability of EDC system, the SAE information must be entered into the eCRF.

10.4 Follow-up

When additional relevant information becomes available, the investigator will record follow-up information according to the same process used for reporting the initial event as described above. It is understood that complete information about the event may not be known at the time the report is submitted. The Investigator must provide causality assessment for the study drug and device, based on the current available information. As additional information pertaining to an SAE becomes available, the eCRF should be updated. It is the responsibility of the Investigator to follow all reportable SAEs until resolution, stabilization or the event is otherwise explained.

DCRI Safety Surveillance will follow all SAEs until resolution, stabilization, until otherwise explained, or until the last subject completes the final follow-up, whichever occurs first. DCRI Safety Surveillance will forward all SAEs to the CC Study Medical Monitors, DCRI INDIE-HFpEF Clinical Operations Team, and notify the DCRI Safety Medical Monitor and NHLBI designee of all related SAEs within 1-2 business day(s) of receipt. Investigators are also responsible for promptly reporting adverse events to their reviewing IRB/EC in accordance with local requirements. The DSMB will review detailed safety data approximately every 6 months throughout the study.

10.5 Suspected Unexpected Serious Adverse Reaction

AEs that meet the criteria of serious, related to study drug, and unexpected per investigator brochure, qualify for expedited reporting to the regulatory authorities. DCRI will notify the FDA and all participating investigators in a written IND safety report of an SAR that is serious and is unexpected, based on the opinion of the investigator and DCRI safety medical monitor, as soon as possible, but not later than 15 calendar days after the event is confirmed to be a serious, unexpected SAR and qualifies for expedited reporting. DCRI will identify all safety reports previously filed with the IND concerning a similar SAR, and will analyze the significance of the SAR in light of the previous, similar reports. Follow-up reports will be sent to investigators to inform and update them about an important suspected adverse reaction if it significantly affects the care of the participants or conduct of the study.

10.6 Study Site Responsibility

1. The site will identify an AE/SAE.
2. Site will determine whether the event is an anticipated disease related event (See Section 10.2).
3. Enter anticipated disease related events on the **EVNTINT** eCRF page, as these events will not be captured as AEs/SAEs, regardless of relationship to study drug.
4. Enter AEs/SAEs on the AE/SAE eCRF page.
5. When information is entered on the SAE page within InForm, this will generate an SAE email notification to DCRI or site will complete a paper SAE form and fax/email to DCRI if InForm is down.
6. Sites will enter all patient deaths on the **Death and SAE eCRF page,** except, anticipated disease related deaths will not be reported on the SAE eCRF page.

10.7 DCRI Safety Surveillance

1. Will be notified of SAEs via InForm generated emails.
2. Will generate the SAE report from InForm as a PDF document.
3. Will perform a clinical review of all SAE forms to verify that all sections are complete and consistent.

4. Will write a clinical narrative of the event in sufficient detail to enable event processing for all serious, related and unexpected adverse events.
5. Will enter the data into the DCRI safety database
6. Will code the event using the current MedDRA dictionary
7. Will independently issue queries on the SAE eCRF in InForm or will fax/email queries for incomplete or inaccurate information for the following fields:
    - Serious adverse event term
    - Event onset date and time
    - Event stop date and time
    - Severity
    - Relationship to study drug including rationale (if positive assessment provided)
    - Serious criteria
    - Outcome
    - SAE narrative
    - Relevant concomitant medications
    - Relevant labs/diagnostic test data
    - Study Drug start date and dose
    - Action taken with Study Drug
    - PI verification
8. Will email the SAE reports to the CC Study Medical Monitors, DCRI INDIE-HFpEF Clinical Operations Team, and notify the DCRI Safety Medical Monitor and NHLBI designee of all related SAEs, including any queries generated for the site within 1-2 business days of initial receipt.
9. Will assist with SAE data reconciliation of the INDIE-HFpEF safety database with the InForm database on the following data variables: Subject ID, Verbatim Term, MedDRA Preferred Term, Onset date, Outcome and Causality.
10. DCRI Safety surveillance will generate a MedWatch for all events, the DCRI safety medical monitor deemed as serious, related to study drug and unexpected per investigator brochure or product labeling.
11. DCRI Safety surveillance will forward the MedWatch to DCRI Regulatory Services for submission to the FDA, within 7 calendar days for death or life-threatening events, and 15 calendar days for all other events, assessed as serious, related to study drug and unexpected.
12. A copy of the voluntary MedWatch/CIOMS-I report will be filed in the master project file at DCRI.

10.8 DCRI Safety Medical Monitor

1. Will review all study drug related SAEs
2. Will review the MedDRA coding for the event
3. Will review the site reported causality assessment
4. Will assess and verify the event for causality assessment and listedness per the product labeling.
5. Request additional follow-up, as needed

10.9 Coordinating Center

1. Will be responsible for reviewing all SAEs/SUSARS for MedDRA coding, and evaluating the event for reporting to the regulatory authorities.
2. Will send any additional queries to DCRI Safety Surveillance, as needed, to be entered into InForm.
3. Will assess the event for listedness per the current documents or product labeling.

10.10 Data and Safety Monitoring Board (DSMB)

The DSMB will be provided safety data approximately every 6 months throughout the study including all SAE data in accordance with the HFN DSMB charter.

10.11 Pregnancy

Pregnancy will be ruled out prior to randomization. Thus pregnancy occurrence during the study period is not expected and only those patients who are either post-menopausal or surgically sterile or have a negative pregnancy test will be included in this study. Pregnancy occurring during a clinical investigation, although not considered a serious adverse event, must be reported to DCRI within the same timelines as a serious adverse event. The pregnancy will be recorded on the appropriate paper pregnancy tracking form. Any associated SAEs that occur to the mother or fetus will be recorded in the SAE eCRF, within InForm.

10.12 Study Site Responsibilities

The site will follow the pregnancy and report the status to DCRI Safety Surveillance, once a trimester, via the paper INDIE-HFpEF Pregnancy Tracking Form, or until final outcome has been determined. If there is an unknown pregnancy outcome by the end of the study, the site will provide follow up via the INDIE-HFpEF Pregnancy Tracking Form, directly to the INDIE-HFpEF trial Team.

10.13 DCRI Safety Surveillance Responsibilities

1) Will receive paper Pregnancy Tracking Form via fax or email from the site.
2) Will forward the pregnancy form to the INDIE-HFpEF trial team within 2 business days of receipt.
3) Will follow the reported pregnancy until final outcome has been reported.
4) If there is an associated SAE with the reported pregnancy, or regarding the infant, DCRI Safety will forward the SAE report according to SAE reporting timelines.

10.14 SAE Reconciliation

The clinical data, including all SAEs will be housed in the InForm database. A separate safety database will be maintained in Argus. DCRI Safety Surveillance will assist with reconciling the data within InForm, with the information within the Argus safety database to ensure that the data matches and/or is clinically consistent.

10.15 Disposition of Safety Records

DCRI Safety Surveillance will forward the safety files to the INDIE-HFpEF trial team Project Leader or designee at the end of the study. The electronic safety files will be saved as portable document formats (PDFs) and provided to the HFN Team or designee via compact disc (CD).

11 RECORD RETENTION

Records relating to this study, including receipt and disposition of the study materials will be retained for at least 2 years from the date the marketing application is approved.

Source documents, such as patient charts, will be retained for not less than five years.

12 ABBREVIATIONS

ADR Adverse Drug Reaction
AE Adverse Event
CFR Code of Federal Regulations
CRA Clinical Research Associate
CRF Case Report Form
DAL Drug or Device Accountability Log
DCRI Duke Clinical Research Institute
EC Ethics Committee
eCRF Electronic Case Report Form
EDC Electronic Data Capture
FDA Food and Drug Administration
IA Investigator Alert
IXRS Interactive Web Response System
MedDRA Medical Dictionary for Regulatory Activities
NA North America
SAE Serious Adverse Event
SAR Suspected Adverse Reactions
SUSAR Suspected Unexpected Serious Adverse Reaction

13 REFERENCES

(Refer to HFN Website: INDIE-HFpEF Protocol Manuals, Protocol, Regulatory Documents, Study Coordinator Materials, and Subject Materials)

14 REVISION HISTORY