D3-CREATINE DILUTION

1. Background and Rationale

The goal of this project is to understand whether a new method of measuring skeletal muscle mass is related to strength, physical performance, falls, fractures, disability and mortality in older men. The new method of determining skeletal muscle mass is called the "creatine dilution method." This method involves several steps. First, the participant ingests a small amount of a special kind of creatine called deuterated creatine. In skeletal muscle, this special kind of creatine is converted into a special kind of creatinine called deuterated creatinine. Then, a few days after ingesting the deuterated creatine, the participant provides a urine sample. The amount of deuterated creatinine in the urine is determined. From this value we can accurately estimate the participant's total amount of skeletal muscle. We will then determine if the amount of skeletal muscle is related to strength, physical performance, falls, fractures, disability and mortality. Appendix A lists frequently asked questions and answers about this assay.

Section 3.1 includes information for clinical centers administering the dose in clinic and collecting urine by mail, including details for equipment and supplies, providing the dose, producing the specimen, timing of the specimen collection, returning the specimen to the clinic and the receipt of the specimen in the clinic.

Section 3.2 includes information for clinical centers administering the dose by mail and collection urine in the clinic, including details for equipment and supplies, providing the dose, producing the specimen, and timing of dose administration.

2. Exclusion criteria

Participants must have provided informed consent to complete this measure and be scheduled for a clinic visit. Participants who use a urine bag should not be included in this measure. There are no other exclusions.

3. Dose and Administration

3.1 Method A: Sites Administering dose in clinic and urine collection by mail or drop-off

3.1.1 Equipment and supplies (Method A)

- Dose:
  - Labeled creatine dose in bulk packaging
  - Dixie cups 3 oz bathroom cup or other water cup (Amazon or other retailor)
- Collection:
  - Urine collection kit (Starplex B952-1O, Fischer Scientific 22-146-535)
  - Label for urine collection container (provided by Coordinating Center)
- Shipping material (to return the specimen to the clinical center)
  - Styrofoam container (Polar Tech Industries #205F)
  - Outer cardboard shipping box (Polar Tech Industries #KD205)

  - USPS label for Priority Overnight shipping
  - Exempt Human Specimen sticker (Polar Tech #BMH311)
  - Freezer gel packs (Polar Tech #IB6)
  - Tape strips (available on Amazon, product: Decker RT150 Return Tape: 2 in. wide)
  - Absorbent pads (Therapak, Fischer Scientific #22-130-039)
  - Instructions and worksheet for providing the specimen by mail
- Processing
  - Two 2.0 mL capped cryovials (Fischer Scientific#: 10-500-26)
    - Fill with 1.0 mL urine
  - -20○C freezer
  - Labels for cryovials and data collection form (provided by Coordinating Center)
- Shipping material (to send the specimen to the laboratory)
  - Freezer boxes with 8 x 8 cell (for 2.0 mL tubes)
  - Electonic manifest and hard copy manifest
  - Rubber bands
  - Paper towels
  - Styrofoam shipper and cardboard box
  - Dry ice

3.1.2 Providing the dose (Method A)

Place one pill into a Dixie cup, and fill the other Dixie cup with water. Have the participant take the dose (with or without water to swallow) and record the date and time the dose was taken. The participant may take the dose in a fasting or non-fasting state. Provide the dose as early in the morning as possible, ideally right after the specimen collection for the main study visit.

Provide instructions regarding taking the dose.

The instructions inform the participant

- The dose may be taken with or without water or other liquid.
- The dose may be taken with a meal, or between meals.
- The clinic staff should write down the time and date the dose was taken on the dose Creatine Dilution Teleform.

3.1.3 Producing the specimen (Method A)

The labeled creatine dose will not interfere with any other assays that may be competed in the collected urine.

Participants will be asked to provide a fasting urine sample 3-6 days after ingesting the dose. A sample provided before 3 days (72 hours) or after 6 days (144 hours) will be considered invalid. The participant should be instructed not to eat or drink anything EXCEPT WATER for at least eight hours before the urine collection. Tea, coffee and other liquids are not allowed.

The sample collected should not be the first void. Generally, many participants will be able to provide a 2nd or 3rd void. However, some participants will urinate multiple times overnight; thus, voids after the 3rd void are acceptable as long as the sample is fasting and collected in the morning before 12:00 Noon.

Instructions for home are provided in Appendix B.

Participants should urinate directly into the specimen collection container.

Instructions for the participant:

1. Have all equipment ready.
2. Void directly into the collection container until half full, starting midway through urination.
3. Remove funnel cap if the participant is provided a funnel. Note using a funnel is optional.
4. Carefully seal the cap of the container so that it is tight and leak proof.

3.1.4 Timing of urine collection (Method A)

In the event that a participant forgets to produce a sample, the test should not be re-started. An early or late sample (one that is provided before 72 hours or after 144 hours) is not acceptable, so that participant will have missing data for this measure. If a participant calls to report that he forgot to produce a sample, instruct him that a sample can no longer be accepted and the shipping and collection materials can be discarded.

Use the chart below to determine the preferred day for the urine collection & specimen mailing, and the day the sample will be received in the clinic. Alternative schedules are listed in Appendix G.

Table. Specimen collection timing for participants who will be administered the dose in the clinic and return urine by mail or drop-off

Day of clinic visit and dose ingestion				
Monday	Tuesday	Wednesday	Thursday	Friday
Day of reminder call (to produce and ship/deliver specimen the next business day)				
Wednesday	Friday	Monday	Monday	Tuesday
Day specimen produced and delivered/shipped to clinic				
Thursday*	Monday**	Tuesday**	Tuesday	Wednesday
Day sample received in clinic***				
Friday	Tuesday	Wednesday	Wednesday	Thursday

*Specimen produced on Thursday must be produced LATER than the time of day the dose was administered at the clinical center the previous Monday. For example, if the dose was administered at 8 AM on Monday, then the specimen should be collected AFTER 8 AM on Thursday.

** Specimens produced on Monday must be produced EARLIER than the time of day the dose was administered at the clinic center the previous Tuesday. For example, if the dose was

administered at 9 AM on Tuesday, then the specimen should be collected BEFORE 9 AM on Tuesday.
***For participants who drop of their specimen in the clinic, they should drop it off the day the sample is produced.

Clinic visits that that precede a holiday when mailing service is not available should contact the Coordinating Center for alternative schedules.

Suggested reminder phone call script for participants dropping the specimen off at the clinical center:
"Please remember to do the following for this study:

- Provide a urine specimen in the morning tomorrow, the second or third time that you urinate, but before you've had any food. You may drink water before you provide the urine specimen.
- Follow the instructions for collecting the urine. Fill the container half-full.
- Place the specimen container in the plastic bag we provided.
- Return the specimen and the provided instruction worksheet to the clinical center during working hours."

Suggested reminder phone call script for participants shipping the specimen to the clinical center:
"Please remember to do the following for this study:

- Provide a urine specimen in the morning tomorrow, the second or third time that you urinate, but before you've had any food. You may drink water but not black coffee, tea or any other beverage before you provide the urine specimen.
- Follow the instructions for collecting the urine. Fill the container half-full.
- Place the specimen container in the plastic bag we provided.
- Place the specimen, freezer packs and the provided instruction worksheet in the shipping box and seal the box.
- Please place the box where your mail is regularly collected. If you have questions, please review the shipping instructions that we provided at the clinic visit.
- As a reminder, please place the gel packs in your freezer if you haven't done so already."

Appendix C lists the instructions for dropping off the specimen to the clinical center.

Appendix D lists the instructions for shipping the urine to the clinical center.

3.1.5 Returning sample to clinic for sites administering dose in clinic and urine collection by mail (Method A):

Participants who are willing and able to return to the clinical center to drop off the specimen will be asked to produce their sample at home, and drop the sample off at the clinical center the same day. The specimen should remain at room temperature (avoiding hot cars, etc.). It is anticipated that between 10-30% of participants will return the specimen in this manner.

Participants who are unable or unwilling to return to the clinical center will mail the sample to the clinic in packaging provided to the participant at the time of their clinic visit. The package will be shipped via postage paid packaging by USPS Priority Express Mail with Commercial Base pricing, which is overnight shipping from the US Postal Service. Clinical centers will schedule a package pick-up from the USPS for participants via the USPS website with costs for Commercial Base pricing (that is, through Stamps.com, usps.com or other business vendor) when creating the pre-paid shipping label for the package. Instructions for coordinating a package pick-up are listed on the USPS website here: https://tools.usps.com/go/ScheduleAPickupAction!input.action, but instructions may vary if a third party postage service (such as stamps.com) is used.

3.1.5.1 Packing the specimen by the participant for shipment to the clinical center, for sites administering dose in clinic and urine collection by mail or drop off (Method A):

Packaging of the shipment by the participant involves a few steps.

The urine sample does not need to be frozen by the participant prior to mailing. The urine sample will be placed into the shipping container with the frozen gel packs to maintain a cool temperature during shipping.

First, the clinic should prepare the return shipping container. Provided no urine has been spilled during previous use, and the material is in good working order, the Styrofoam box and freezer packs can be reused. If contaminated by urine, these materials should be discarded. The outer cardboard box will be used only once per shipment and then recycled.

To prepare the return shipping container, the clinic shall:

1. Assemble the cardboard box and seal three sides with tape.
2. Place the Styrofoam box inside of the cardboard box.
3. Place absorbent material inside the Styrofoam box (2 sheets)
4. Create mailing label for the box and arrange package pickup.
5. Adhere the USPS postage label to the cardboard box
6. Adhere the EXEMPT HUMAN SPECIMEN label to the cardboard box
7. Label the urine collection container with label provided by Coordinating Center
8. Provide participant with:
    a. Assembled shipment box
    b. Urine collection kit (container cup, funnel (optional), lid and transport zip-top bag)
    c. Tape strips. The tape strips should be cut in half by the clinical center so that each strip is approximately 11.5” long.
    d. Collection Worksheet & Instructions (Appendix B)
    e. Shipping Instructions for Urine Sample (Appendix D)

When the participant returns home from the clinic visit, the participant should place the freezer packs in their home freezer. Highlight this instruction when reviewing the shipping instructions with the participant.

3.1.6 Receipt of the specimen by mail or drop off from participant (Method A)

Record date and time specimens received. Record condition of material. Below are the conditions to be recorded:

- Gel packs (frozen/partly thawed/completely thawed or not included)
- Urine (no leakage/partly leaked/completely leaked or not included)
- Other problem Yes/No (list other problem)

Upon receipt of the specimen, the clinics will aliquot 1 ml of urine into each of two cryovials (so that each cryovial has 1 ml of urine.)

The cryovials will have a unique ID number and label; these ID numbers should be recorded on the data collection form, and a label should be placed on the data collection form. Write the date of specimen collection on the cyrovial labels. (The MrOS ID will not be sent to the laboratory completing the assays.)

The cyrovials should be frozen at -20C until shipment to the laboratory.

3.2 Method B: Sites administering dose by mail and urine collection in clinic

3.2.1 Equipment and supplies (Method B)

- Dose:
  - Labeled creatine dose in bulk packaging (except Portland which will receive individually packaged dose)
  - Snap Cap vial (Total pharmacy supply #7720, 6 dram amber)
  - USPS priority mail packaging or first class mail to mail dose
  - Instructions and worksheet for taking the dose by mail
- Collection:
  - Urine collection container (can be same container as main study urine collection)
- Processing
  - Two 2.0 mL capped cryovials (Fischer Scientific#: 10-500-26)
    - Fill with 1.0mL urine
  - -20°C freezer
  - Labels for cryovials and data collection form (provided by Coordinating Center)
- Shipping material (to send the specimen to the laboratory)
  - Freezer boxes with 8 x 8 cell (for 2.0 mL tubes)
  - Electonic manifest and hard copy manifest
  - Rubber bands
  - Paper towels
  - Styrofoam shipper and cardboard box
  - Dry ice

3.2.2 Providing the dose (Method B)

Send the participant the dose via USPS mail 3-6 days before their scheduled clinic visit. The dose should be packaged in a snap cap pharmacy vial (6 dram).

Clinical centers must adhere to local IRB regulations for obtaining consent for this procedure prior to the clinic visit.

Provide instructions regarding taking the dose.

The instructions inform the participant

- The dose may be taken with or without water or other liquid.
- The dose may be taken with a meal, or between meals.
- The time and date the dose should be ingested (See section 3.2.4) The dose should be taken 3-6 days before the clinic visit, at around the time the visit is scheduled. Sites will call the participant the day before the dose should be ingested as a reminder.
- The participant should write down the time and date the dose was taken on the dose administration worksheet. This information should be recorded on the Teleform when the participant comes into the clinic.
- Sample instructions and worksheet are provided in Appendix E.

3.2.3 Producing the specimen (Method B)

The labeled creatine dose will not interfere with any other assays that may be competed in the collected urine.

Participants will be asked to provide a fasting urine sample 3-6 days after ingesting the dose. A sample provided before 3 days (72 hours) or after 6 days (144 hours) will be considered invalid. The participant should be instructed not to eat or drink anything EXCEPT WATER for at least eight hours before the urine collection. Tea, coffee and other liquids are not allowed.

The sample collected should not be the first void. Generally, many participants will be able to provide a 2nd or 3rd void. However, some participants will urinate multiple times overnight; thus, voids after the 3rd void are acceptable as long as the sample is fasting and collected in the morning before 12:00 Noon.

Instructions for the urine collection specific for this project are not different from the instructions for the urine collection as part of the overall clinic visit. For both, we are collecting a fasting sample. The already planned urine collection at Visit 4 can be used to collect the urine specimen for this project. The urine for this sub-study will be allocated from the urine collected from the main study.

3.2.4 Timing of dose administration (Method B)

In the event that a participant forgets to take the dose, the test should not be re-started unless the clinic visit can be rescheduled.

To read this chart, start with the first line which is the day of the week of the clinic visit. Then read down the chart to determine the day for a reminder call and the day the dose should be ingested. The participant should take the dose in the morning so that the time of day of the dose ingestion is before the time of day of the clinic visit when the specimen will be produced. For example, if the clinic visit is scheduled for 8 am, the dose should be ingested before 8 am on the day of dose ingestion. The participant should be instructed not to take the dose until the prescribed time. The participants should write the date and time the dose was ingested on the worksheet that is sent to the participants with the dose. The participant should bring this worksheet with them to their clinic visit.

Appendix E lists instructions for participants who will take the dose by mail.

Table. Dose timing for participants who will be administered the dose by mail, with reminder call the night before the dose ingestion

Day of clinic visit (and urine sample production)				
Monday	Tuesday	Wednesday	Thursday	Friday*
Day of reminder call (to take the dose the **next** day)				
Tuesday prior to clinic visit	Wednesday prior to clinic visit	Thursday prior to clinic visit	Friday prior to clinic visit	Monday prior to clinic visit
Day of dose ingestion (at home)				

Wednesday prior to clinic visit	Thursday prior to clinic visit	Friday prior to clinic visit	Saturday prior to clinic visit	Tuesday prior to clinic visit

Alternatively, if the clinics want to call the participant on the day he should take the dose, the following table should be used

Table. Dose timing for participants who will be administered the dose by mail

Day of clinic visit (and urine sample production)				
Monday	Tuesday	Wednesday	Thursday	Friday*
Day of reminder call (to take the dose the **same** day) and day of dose ingestion (at home)				
Wednesday prior to clinic visit	Thursday prior to clinic visit	Friday prior to clinic visit	Saturday prior to clinic visit	Tuesday prior to clinic visit

*For clinic visits on Fridays, the time of the urine collection must be after the time of day of the dose ingestion the previous Tuesday. For example, if the participant took the dose at 9 AM on Tuesday, then the urine sample should be collected AFTER 9 AM on Tuesday.

3.2.5 Aliquoting of specimen (Method B)

From the urine collected as part of the main study collection procedures, the clinics will aliquot 1 ml of urine into each of two cryovials (so that each cryovial has 1 ml of urine) for the creatine dilution study (in addition to the other aliquots for the main MrOS study.)

The cryovials for this study will have a unique ID number and label; these ID numbers should be recorded on the data collection form, and a label should be placed on the data collection form. Write the date of specimen collection on the cyrovial labels. (The MrOS ID will not be sent to the laboratory completing the assays.)

4. Maintaining a Creatine Dose Tracking Log

A log sheet should be maintained at the clinic to keep count of doses remaining and track the doses that have been distributed. Please contact the Coordinating Center when there are 10 doses remaining to receive another batch.

See Appendix I for example logs of tracking doses administered by mail and in clinic. The tracking logs are for internal use only and can be modified by individual clinics as needed. They do not need to be sent to the Coordinating Center.

5. Shipment of the specimen to the laboratory for all sites (regardless of administration method)

Only of the 2mL vials collected for this study should be sent to the central laboratory for processing. The other vial should be stored locally until further notice from the Coordinating Center. If any sites have concerns about storage of the second vial, please contact the Coordinating Center.

The specimens will be sent to the laboratory (PPD International, http://www.ppdi.com/) for processing on the second Tuesday of each even numbered month. The first shipment should take place once your clinic has at least 10 samples to send to the laboratory. Subsequent shipments should take place regardless of the number of samples available.

Samples should be shipped to:
Maria Edwards
PPD
2244 Dabney Road
Richmond VA 23230

Before shipping, create an electronic manifest.

The electronic manifest should list at the top:
"MrOS Visit 4 urine specimen" and your clinic's name and contact information

For each specimen, the electronic manifest should list:
Tube ID (not the MrOS ID)
Date of specimen collection
The electronic manifest is just a simple list. An example is in Appendix F.

On the day of shipping, email Maria Edwards (Maria.edwards@ppdi.com) and Aaron Wheeler (Aaron.Wheeler@ppdi.com) the following information:

- Electronic manifest
- The shipment tracking number (e.g. FedEx or UPS tracking number)
- date of shipment
- expected arrival date
- number of styrofoam mailers shipped

A grid for inventory is not required.

Shipments to the laboratory are charged to your local Federal Express or other company's account number.

This shipping protocol follows the procedures mandated by the International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions 650 and 904.

Samples should be prepared for shipping to the laboratory as follows:
- Wrap each freezer box in paper towels to absorb possible leakage. Put a rubber band around the towel-wrapped box or bag. Using two rubber bands, put a rubber band in each direction (horizontally and vertically), forming a cross with the rubber bands.
- Put the individual freezer boxes containing the samples into a leakproof zip-lock plastic bag and seal the bag.
- Place approximately one third of the dry ice on the bottom of the mailer.

- Carefully place the freezer box(es) into the styrofoam mailer. Place no more than a total of 4 L of sample into the styrofoam shipping container. Although unlikely, use two or more styrofoam mailers for the shipment when necessary. (In this case, label the mailers “1 of 2” and “2 of 2”).
- Place the remaining dry ice (approximately 7 - 14 lbs. total) on top and around the samples to fill the styrofoam container.
- Enclose the styrofoam container in the outer cardboard box.
- Enclose the completed hard copy of the manifest
- Ensure that the date of the specimen collection is written on the cryovial

Appendix A: Frequently asked questions about the D3-creatine dilution method

What is creatine?
Creatine is a molecule that is found in skeletal muscle. It is made from amino acids by the kidney and the liver and also found in animal protein. Most people eat about 1 gram/day of creatine. Creatine is sometimes used by athletes and body builders as a nutritional supplement at very high doses (2-5 grams).

What is "labeled creatine"?
Labeled creatine is just like the regular protein creatine. The only exception is that the regular hydrogen atoms have been replaced by deuterium. Regular hydrogen has only a single proton in its nucleus. Deuterium (also called D or ^{2}H or heavy hydrogen) is a stable isotope of hydrogen that has one proton and one neutron in its nucleus. By replacing the hydrogen with deuterium, the labeled creatine molecule can be traced in experiments.

Is there a trade or generic name for labeled creatine?
This is not a drug or biological agent. The dose of labeled creatine is used for research purposes only and has no generic or trade name.

Is labeled creatine radioactive?
As a stable isotope, deuterium is not radioactive.

How is the labeled creatine stored?
The labeled creatine is stored at room temperature.

What is creatinine?
Creatine is converted into creatinine at a constant rate (~1.7%./day) in skeletal muscle. Creatinine diffuses from the muscle into plasma and urine.

What is the timing of the measure?
The dose is given 3-6 days before the urine sample is provided.

How much labeled creatine is given?
Participants take a dose of 30 mg. This is much lower than the 1 gram (1000 mg) of creatine that is typically ingested by most people in a typical day.

How do you determine skeletal muscle mass from this measure?
We measure the amount of labeled creatinine in urine 3-6 days after the labeled creatine is ingested. We enter this data into an algorithm to estimate the total amount of creatine in the body (also called "creatine pool size"). Creatine pool size is directly proportional to total body skeletal muscle mass.

Does this have an IND?
No, this is not a drug or a biologic. No IND is needed.

How will the labeled creatine get to the participants?

The labeled creatine will be packaged in bulk, sent to the Coordinating Center and then delivered to the clinical centers. The clinical centers will then provide the dose to participants directly. Due to specific state laws in Oregon, the creatine in Portland will be individually packaged.

Is this being used for an "unapproved use"?
No. Labeled creatine is not a drug or biological product; therefore, it cannot be considered "unapproved use."

What risks are associated with labeled creatine?
There are no known risks. Labeled creatine is a stable isotope; there is no radioactivity. Deuterium is a naturally occurring isotope of hydrogen; about 1 gram of deuterium exists naturally in the human body. Creatine is a normal component of animal protein; the average person consumes 1 gram of creatine per day which is a few orders of magnitude greater than our dose. Deuterium is commonly used in human research, most often in metabolic studies which use doubly labeled water. Doubly labeled water ($D_2{}^{18}O$) is comprised of deuterium and oxygen-18 instead of regular hydrogen and oxygen.

How is GSK involved?
GSK is providing the labeled creatine and funding the assays on the urine specimens. They will not receive participant level data, and the samples sent for urine analysis will be a non-identifiable ID (that is, an ID that isn't the MrOS ID or other PHI). GSK is not directly providing funds to the Coordinating Center or the clinical centers. The NIH grant funds the analysis of the data; the collection, shipping and handling of the specimens; providing the dose to the participant; and other clinical administrative tasks (such as providing informed consent).

Who manufactured the labeled creatine?
Both the labeled creatine as well as the filled capsules were made by GSK approved Contract Manufacturers. Following the manufacture of each, representatives of GSK reviewed all documentation, resulting in GSK QA-Approved product for human use.

Is labled creatine (d3-creatine) vegan and vegetarian friendly?
Yes. The D3-Creatine was made from starting materials/reagents that did not originate from animals. The capsule is made from, HPMC (hydroxypropylmethyl cellulose) and not from animal origin.

Appendix B: Collection Worksheet Instructions for ***dose administered in clinic and urine specimen mailed or dropped off at clinic (Method A)***

Dear Mr. _____:

Thank you for agreeing to participate in this study.

Please provide your urine sample before __:____ a.m. on ____/____/_____

You should not eat anything before you provide your sample. You may drink water but do not drink coffee, tea or other liquid before providing your sample.

You should provide your urine sample the second or third time you urinate on the date listed above.

When you are ready to prepare the urine sample, please do the following:

1. Have all equipment ready.
2. Starting midway through urination, void directly into the collection container until the container is half full
3. Remove the funnel *(not required if funnel is not provided)*
4. Carefully seal the cap of the container so that it is tight and leak proof.
5. Follow the directions on the next page to return the urine to the clinic.

Please record the time and date you provided your urine sample:
Time: _____ a.m. or p.m.
Date: ____/_____/_____

Were you fasting when you produced your urine sample?
○ Yes ○ No

When is the last time you ate or drank anything before producing your urine sample?
Time: _____ a.m. or p.m.
Date: ____/_____/_____

If you provided your dose at a different time or day than listed above, that is okay. We need this information for our records.

Instructions on returning the urine to the clinic are on the next page. If you have any questions, please contact <<list clinic contact>>

<<Clinic staff please highlight the information below>>
PLEASE RETURN THIS PAPER WITH YOUR SPECIMEN

Appendix C: Instructions for dropping off the urine specimen to the clinical center, ***for sites with dose administered in clinic and urine specimen mailed or dropped off at clinic (Method A)***

Instructions for dropping off the urine sample:

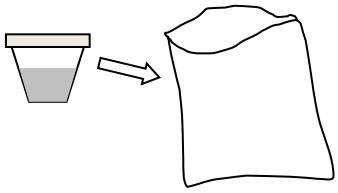
1. Place the urine specimen container in the plastic bag provided.
2. Keep the urine specimen at room temperature. Avoid hot cars and other heat or cooling sources.
3. Return the specimen during clinic hours, <<X a.m. to X p.m.>> on __/____/_____
4. Return the specimen to:
   <<list clinic address>>
5. Return the Collection Worksheet with your specimen.

If you have any questions, please contact <<list clinic contact>>

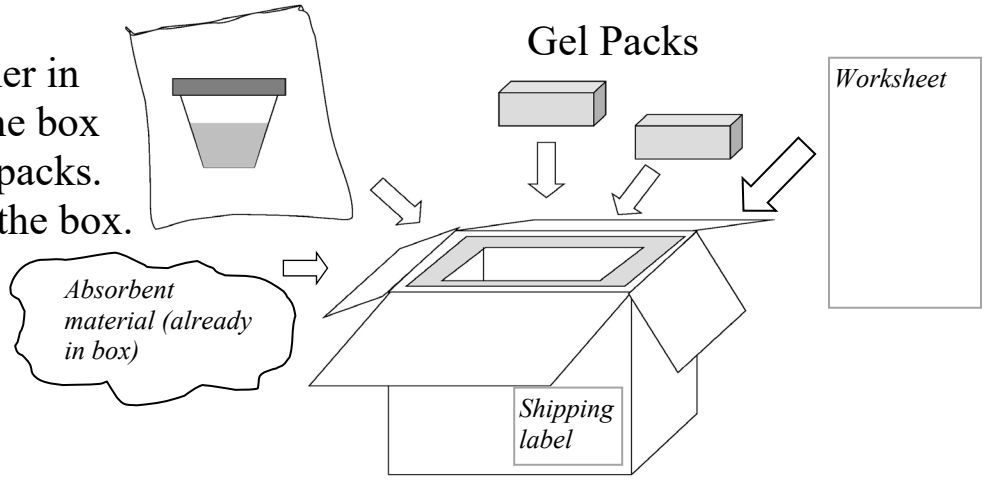
Appendix D: Shipping Instructions for Urine Sample ***for sites with dose administered in clinic and urine specimen mailed or dropped off at clinic (Method A)***

The clinic has arranged to have your urine sample picked up by the US Postal Service. Please prepare your sample for shipping using the following directions. If you have any questions please call <<list clinic contact>>>

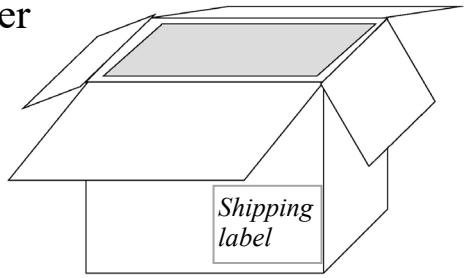
1. Place the sealed urine container in the zip-top bag



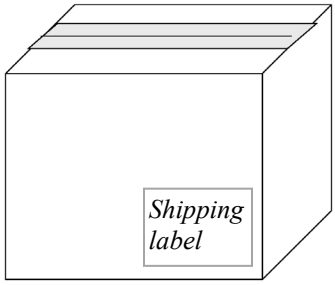
2. Place the container in the zip-top bag in the box with the frozen gel packs. Place worksheet in the box.



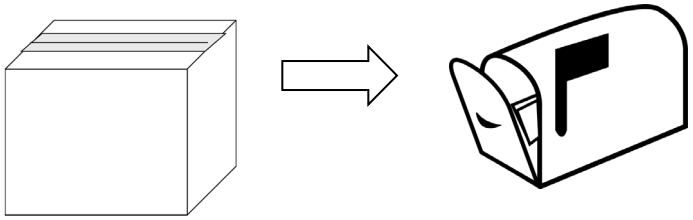
3. Place the lid on the inner Styrofoam box.



4. Seal the outer cardboard box with tape provided.



5. Place the box where your mail is collected. If it won't fit in your mailbox, leave it as close to your mail box as possible.



Appendix E: Worksheet Instructions for ***administering dose by mail and urine collection in clinic (Method B):***

Dear Mr. _____ :

Thank you for agreeing to participate in this study.

The single pill included in this packet is labeled creatine.

<<picture will be inserted here by Coordinating Center>>

Please take your dose before __:_____ a.m. on _____/_____/_____

You may take your dose with a meal, before a meal, or with or without water or other liquids.

On ____/____/_____, someone will call you from the MrOS study to confirm with you that you ingested the creatine pill and to confirm your MrOS clinic visit 4 time and date.

When you attend your clinic visit on _____ you will be asked to provide urine sample before you've had any food, coffee or other liquids. You may drink water prior to the visit. Some of this urine sample will also be used in this study.
<<Clinic staff please highlight the information below>>

Please record the time and date you actually took your dose:
Time: _____ a.m. or p.m.
Date: ___/_____/_____
If you took your dose at a different time or day than instructed, that is okay. We need this information for our records.

If you have any questions, please contact <<<list clinic contact>>>
PLEASE BRING THIS PAPER TO YOUR MROS CLINIC VISIT

Appendix F: Sample Electronic Manifest

Tube ID	Date of Collection
X123456	5/27/2014
Y325434	5/28/2014
Y325435	5/28/2014
Q325434	5/29/2014
Q326434	6/01/2014
Q325934	6/05/2014

Appendix G
Alternative schedule for Method A: Dose <u>in clinic</u> and urine collection by mail or drop off.

Follow the preferred schedule for dose administration and specimen timing whenever possible. If needed, the following alternative schedule may be used. The clinic staff should provide the participant a specimen date and time to produce the specimen even when an alternative schedule is being used; the participant should not be allow to pick which time to produce the specimen following an algorithm.; this decision should be made by the clinic.

If the participant is given the dose to take at a later point (following the clinic visit), then refer to the day the dose is ingested below (rather than the day of the clinic visit).

<u>Monday</u> dose ingestion
Preferred specimen production: Thursday(AFTER time of day dose was ingested on Monday)
Preferred specimen drop-off: Thursday
Preferred specimen mailing: mailed Thursday for Friday receipt

Alternative mailed return: None
Explanation: The specimen must be produced Thursday-Sunday. Specimens produced Friday-Sunday cannot be mailed because the clinic is not open to receive them, or the mail service is not operating, or both. Thus, Thursday is the only day allowed for urine collection with mailed return of specimen.

Alternative drop-off or pick-up return: Friday
Explanation: The specimen must be produced Thursday-Sunday. Specimens produced Saturday-Sunday cannot be dropped off because the clinic is not open to receive them. If the clinic will be open on Saturday or Sunday for processing, it is acceptable to arrange drop off/pick-up on those days. However, the clinic MUST process the sample the day it is received. If the sample is provided on Sunday, it must be produced BEFORE the time the dose was ingested on Monday.

<u>Tuesday</u> dose ingestion
Preferred specimen production: Monday (BEFORE time of day the dose was ingested on Tuesday)
Preferred specimen drop-off: Monday
Preferred specimen mailing: mailed Monday for Tuesday receipt

Alternative mailed return: None
Explanation: The specimen must be produced Friday-Monday. Specimens produced Friday-Sunday cannot be mailed because the clinic is not open to receive them, or the mail service is not operating, or both. Thus, Monday is the only day allowed for urine collection with mailed return of specimen.

Alternative drop-off or pick-up return: Friday (AFTERthe time of day dose was ingested on Tuesday)
Explanation: The specimen must be produced Friday-Monday. Specimens produced Saturday-Sunday cannot be dropped off because the clinic is not open to receive them, thus only Friday and Monday specimen production is allowed. If the clinic will be open on Saturday or Sunday for processing, it is acceptable to arrange drop off/pick-up on those days. However, the clinic MUST process the sample the day it is received.

<u>Wednesday</u> dose ingestion:
Preferred specimen production: Tuesday(BEFORE time of day the dose was ingested on Wednesday)

Preferred specimen drop-off: Tuesday
Preferred specimen mailing: mailed Tuesday for Wednesday receipt

Alternative mailed return: Monday (any time so long as specimen is fasting) specimen production with shipment arriving on Tuesday
Explanation: The specimen must be produced Saturday – Tuesday. Specimens produced Saturday-Sunday cannot be mailed because the clinic is not open to receive them, or the mail service is not operating, or both. Specimens produced on Monday can be mailed for receipt on Tuesday. However, if participants require a phone call the night before the specimen is to be produced as a reminder, this phone call would need to take place on the Sunday before the specimen is to be produced (a day when the clinic is likely closed).

Alternative drop-off or pick up return: Monday (any time as long as the specimen is fasting)
Explanation: The specimen must be produced Saturday – Tuesday. Specimens produced Saturday-Sunday cannot be picked up/dropped off because the clinic is not open to receive them. Specimens produced on Monday to be dropped off the same day. However, if participants require a phone call the night before the specimen is to be produced as a reminder, this phone call would need to take place on the Sunday before the specimen is to be produced (a day when the clinic is likely closed). If the clinic will be open on Saturday or Sunday for processing, it is acceptable to arrange drop off/pick-up on those days. However, the clinic MUST process the sample the day it is received. If the specimen is produced on Saturday, it must be produced AFTER the time the dose was ingested on Wednesday.

Thursday dose ingestion:
Preferred specimen production: Tuesday
Preferred specimen drop-off: Tuesday
Preferred specimen mailing: mailed Tuesday for Wednesday receipt

Alternative mailed return: Monday specimen production with shipment arriving on Tuesday; OR Wednesday specimen production (BEFORE the time of day the dose was ingested on Thursday) for shipment arriving on Thursday
Explanation: The specimen must be produced Sunday-Wednesday. Specimens produced on Sunday cannot be mailed because there is no mail service this day. Specimens produced on Monday can be mailed for receipt on Tuesday. However, if participants require a phone call the night before the specimen is to be produced as a reminder, this phone call would need to take place on the Sunday before the specimen is to be produced (a day when the clinic is likely closed). Specimens produced on Wednesday can be mailed for receipt on Thursday, however, that specimen must be produced before the time of day the dose was administered on Thursday.

Alternative drop-off/pick-up return: Monday; or Wednesday (BEFORE the time of day the dose was ingested on Thursday)
Explanation: The specimen must be produced Sunday-Wednesday. Specimens produced on Sunday cannot be dropped off because the clinic is closed this day. Specimens produced on Monday can be dropped off that day. However, if participants require a phone call the night before the specimen is to be produced as a reminder, this phone call would need to take place on the Sunday before the specimen is to be produced (a day when the clinic is likely closed). Specimens produced on Wednesday can be dropped off the same day, however, that specimen must be produced BEFORE the time of day the dose was administered on Thursday. If the clinic will be open on Saturday or Sunday for processing, it is acceptable to arrange drop off/pick-up on those days. However, the clinic MUST process the sample the day it is received. If the specimen is produced on Sunday, it must be produced AFTER the time the dose was ingested on Thursday.

Friday dose ingestion:
Preferred specimen production: Wednesday
Preferred specimen drop-off: Wednesday
Preferred specimen mailing: mailed Wednesday for Thursday receipt

Alternative mailed return: Monday (AFTER time of day the dose was ingested on Friday); Tuesday, Thursday (BEFORE time of day the dose was ingested on Friday).
Explanation: The specimen must be produced Monday-Thursday. Specimens produced on Monday can be mailed for receipt on Tuesday. However, if participants require a phone call the night before the specimen is to be produced as a reminder, this phone call would need to take place on the Sunday before the specimen is to be produced (a day when the clinic is likely closed). Specimens produced on Thursday can be mailed for receipt on Friday, however, that specimen must be produced BEFORE the time of day the dose was administered on Friday.

Alternative drop-off/pick-up return: Monday (AFTER time of day the dose was ingested on Friday); Tuesday, Thursday (BEFORE time of day the dose was ingested on Friday).
Explanation: The specimen must be produced Monday-Thursday. Specimens produced on Monday can be dropped off that day. However, if participants require a phone call the night before the specimen is to be produced as a reminder, this phone call would need to take place on the Sunday before the specimen is to be produced (a day when the clinic is likely closed). Specimens produced on Thursday can be mailed for receipt on Friday, however, that specimen must be produced BEFORE the time of day the dose was administered on Friday.

Appendix H
Alternative schedule for Method B: Dose by mail and urine collection during clinic visit

Follow the preferred schedule for dose administration and specimen timing whenever possible. If needed, the following alternative schedule may be used. The clinic staff should provide the participant a time for the dose ingestion an alternative schedule is being used; the participant should not be allow to pick which time to take the dose following an algorithm.; this decision should be made by the clinic.

Note that the reminder call to the participant can either be the night before the dose is to be taken at home, or the same day (morning) before the dose is to be taken at home.

Monday clinic visit
Preferred dose administration: Wednesday prior to clinic visit
Reminder call either Tuesday or Wednesday prior to clinic visit

Alternative dose administration: Tuesday – Friday prior to the clinic visit. If the dose is taken the Tuesday prior to the clinic visit, then the specimen must be produced BEFORE the time of day the dose was taken the previous Tuesday, and the reminder call on must be made on Monday. If the dose is taken on the Friday prior to the clinic visit, then the specimen must be produced AFTER the time of day the dose was taken the previous Friday.

Tuesday clinic visit
Preferred dose administration: Thursday prior to clinic visit
Reminder call either Wednesday or Thursday prior to clinic visit

Alternative dose administration: Wednesday – Saturday prior to the clinic visit. If the dose is taken the Wednesday prior to the clinic visit, then the specimen must be produced BEFORE the time of day the dose was taken the previous Wednesday. If the dose is taken on the Saturday prior to the clinic visit, then the specimen must be produced AFTER the time of day the dose was taken the previous Saturday, and the reminder call must be made the night before the dose should be taken (call on Friday).

Wednesday clinic visit
Preferred dose administration: Friday prior to clinic visit
Reminder call either Thursday or Friday prior to clinic visit

Alternative dose administration: Thursday – Sunday prior to the clinic visit. If the dose is taken the Thursday prior to the clinic visit, then the specimen must be produced BEFORE the time of day the dose was taken the previous Thursday. If the dose is taken on the Sunday prior to the clinic visit, then the specimen must be produced AFTER the time of day the dose was taken the previous Sunday, and the reminder call must be made the night before (or two nights before) the dose should be taken (call on Friday for Saturday or Sunday dosing).

Thursday clinic visit
Preferred dose administration: Saturday prior to clinic visit
Reminder call either Friday or Saturday prior to clinic visit

Alternative dose administration: Friday – Monday prior to the clinic visit. If the dose is taken the Friday prior to the clinic visit, then the specimen must be produced BEFORE the time of day the dose was taken

the previous Friday. If the dose is taken on the Monday prior to the clinic visit, then the specimen must be produced AFTER the time of day the dose was taken the previous Monday. The reminder call for doses taken on Saturday, Sunday or Monday must be made the night before (or two nights before) the dose should be taken, or on Monday morning (for Monday dosing).

Friday clinic visit
Preferred dose administration: Tuesday prior to clinic visit
Reminder call either Monday or Tuesday prior to clinic visit
For clinic visits on Fridays, the time of the urine collection must be AFTER the time of day of the dose ingestion the previous Tuesday. For example, if the participant took the dose at 9 AM on Tuesday, then the urine sample should be collected AFTER 9 AM on Friday.

Alternative dose administration: Saturday– Tuesday prior to the clinic visit. If the dose is taken the Saturday prior to the clinic visit, then the specimen must be produced BEFORE the time of day the dose was taken the previous Saturday. The reminder call for doses taken on Saturday, Sunday or Monday must be made the night before (or two nights before) the dose should be taken, or on Monday morning (for Monday dosing).

Appendix I

Creatine Dose Tracking Log (dose by mail)

Batch #	Count	ID	Scheduled Visit Date	Date Mailed	Date participant reminded to take dose	Date dose taken	Date participant reminded to produce sample	Date sample produced	Protocol compliant Y/N?	Comments

Appendix I

Creatine Dose Tracking Log (dose in clinic)

Batch #	Count	ID	Acrostic	Visit Date	Date participant reminded to produce sample	Date sample produced	Protocol compliant Y/N?	Comments