Body Composition Measures

Ultrasound

Assessor Type:	**Time to complete assessment:**
Blinded Outcome Assessor	10-15 minutes

#

The ultrasound assessment will acquire the following measurements on the right leg. If the measurement is not possible on the right leg, then the left leg can be used:

1) Muscle Linear Depth (LD) – at time of exam
2) Cross sectional Area (CSA) – at time of exam
3) Echogenicity – post exam

Timeline of Ultrasound Testing

Ultrasound assessment will occur at multiple time points:

1. baseline assessment (with 24 hours of randomization)
2. 10 Days post randomization (± 2 days)
3. Hospital Discharge

Equipment

1) Tape measure
2) Ultrasound Equipment- Gel, Machine, Probe
3) Marking Pen/Marker
4) Tegaderm (or other dressing) to obtain marking

Each site will use their own equipment. It will be the same as that which is used for clinical ultrasound assessment. The protocol requires documentation of the type of equipment used. It is very important that all measurements used on a given patient are done with the same machines and probes by the same operator- to minimize noise. We ask that linear probes are used for all assessments if possible. The use of a curvilinear probe makes getting good skin contact between the probe and the thigh very difficult without applying pressure. You end up losing the outer portions of your field of view. The probes should have a minimum of a 38 mm FOV width, but greater than that is better for obtaining CSA. The machine and probe type will be recorded on the CRF (See Appendix 1). The identifying number/code used locally at each site from both the unit and the probe should be recorded at baseline as well to ensure the same ones are used during follow-up measures. Changing the machine may introduce a measurement error.

Personnel:

1) A designated clinician/study staff who has been trained on the US measures

Staff performing the assessment should be trained and pass quality assurance evaluation (See more detail below). The person doing the ultrasound will need to communicate with clinical personnel and schedule the procedure at a time that is convenient for the patient and clinical staff.

Quality Assurance

All people conducting US evaluations (assessors) will participate in a training session conducted by CERU or a local expert. All assessors must pass a quality assessment as part of their training to ensure they can obtain high quality images that will allow for the extraction of our muscle mass and quality measures. This will be done in 2 parts.

1) In person quality assessment – At the end of the in person training, a trainer will oversee the trainee while they execute all phases of obtaining high quality images. This will include, patient positioning, land marking and obtaining an image. Feedback will be provided at this time. (See Appendix 2)
2) Quality assessment by Central Review Committee – Once assessors have completed the in person training and they have ethics approval and CERU approval to begin enrolling patients in the EFFORT trial, they will begin enrolling patients into the EFFORT trial as per the study protocol. For the first 10 patients enrolled, we will require assessors to undergo assessment of image quality and reliability. If there are more than 1 assessor at each site, we ask that 1 person be designated the primary person. All other assessors will be compared to the primary person by having both assessors (Primary person and the additional assessor) conduct the measurements on a patient on the same day and send their measurements and images for central adjudication. If there is only one assessor at each site, we ask that they repeat the image acquisition and measurements a few hours apart and send both for central adjudication. These images will be reviewed and feedback returned within 24-48 hours. If images are not of high enough quality, sites may be asked to repeat image collection. After the first 10 images, if the Central Review Committee is satisfied with the quality of the images, they will no longer be required to do reliability assessments or be reviewed in real time. If there are quality issues, the Central Review Committee will determine if more training and/or quality assurance is required or whether the operator will be discontinued from the trial.

This is a two part quality assurance because the in-person training will not likely be conducted using an ICU population, which comes with challenges not found in a healthy population.

Timing of Assessment

Baseline assessment: should be done within the first 24 hours of entry into the study.

Note: If you are conducting a cycling intervention: *The assessment should ideally be completed as soon as possible and prior to any exercise sessions.) If an intervention participant has already*

received a cycling session, this should be noted on the CRF— either by the ultrasound assessor (if he/she is aware of group assignment) or by the research coordinator.

Study Day 10: The assessment should be as close to study day 10 as possible, but it can be ± 2 days. Obtaining the images takes precedent over exact timing. This will allow for weekends or unavailability of staff.

Hospital Discharge: It will be very difficult to obtain images after discharge so it is important that we complete scans before participants leave the hospital. This will mean completing the assessment on a Friday before the weekend if there is a moderate to high chance that a participant will be discharged over a weekend.

If the patient is discharged prior to Day 10: If a patient is discharged from hospital prior to Study Day 10, complete the hospital discharge assessment at the time of discharge.

If Study Day 10 and Hospital Discharge are within 72 hours: If hospital discharge occurs within 72 hours of Study day 10, the study day 10 images and measurements may be used at hospital discharge as well. We do not need to conduct 2 assessments if they are going to be performed within 72 hours of one another.

Preparing to Begin the Test

Location

- For the duration of this testing procedure, participants will remain in their hospital bed.

Patient positioning

- Patient should be supine during assessment. **If this is not possible**, make a note of the head of the bed angle (ideally <15 degrees), foot of bed angle, pillows and mattress firmness (if possible) in the notes section of the data collection forms. These will have to be duplicated for future assessments.

It is very important that the patient be positioned in the exact same way for all measurements.

- The patient's leg should be ***straight*** *(Knee in full extension)*, with hip in 0-10 degrees of flexion. The leg should be in neutral external rotation ***not rotated inward or outward*** since this will affect the image. One important landmark that you need to make sure is straight and facing the ceiling is the patella. Especially with ICU patients, they might not be able to keep their leg straight (e.g. sedated participants); in that case you need to put rolled towels on both sides of the foot to keep their foot perpendicular to bed.
- Patient's leg should be completely ***relaxed***, because if the patient is contracting the muscle, it will affect the muscle bulk in the image, this is especially important if the patient is agitated and keeps moving and does not relax their muscles.

Land Marking Study Site:

With the patient and limb positioned as described above, mark a point two-thirds of the distance distally between the anterior superior iliac spine and the superior border of the patella. Record the exact distance from the superior border of the patella to the site of measurement. This will be used in subsequent assessments.

For most patients, it should be possible to measure LD and CSA at the two-thirds site. **Only if it is not possible to use the 2/3 site** (for example, obese participants, participants with edema), an alternate site can be used. In order to reduce time and because you will not know this prior to acquiring images, landmark this alternative site at the same time they are marking the two-thirds distance site whether it will be used for image acquisition or not. Use a different marking (e.g. circle thcross) so that the positions are not mixed up accidentally.

Alternate Site: Taking care not to move the patella as you do this measurement, measure 10cm proximal to the superior pole of the patella. Please take both measurements (LD and CSA) at the same site. Measurement site(s) should be noted so that repeat assessments can be conducted at the same site(s) as previous assessments for each patient (i.e. if edema resolves, do NOT change to using the 2/3 point).

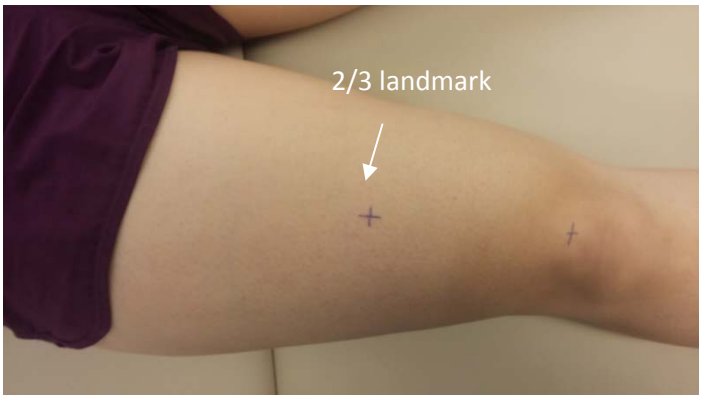


Figure –Landmarking

Ultrasound Machine Settings

Pre-programmed protocols: many ultrasound machines allow for programmed protocols to ensure settings are consistent, that all images are obtained and that each study is labelled appropriately. It is suggested this method be employed.

For Example:

- Go to "patient" key on your keyboard, and then type ***subject id number, Image label and time point***
- Make sure that you hit ***"new/end"*** first, if you don't do this, it will add the images to the previous record.

Then type ***subject id number as described above*** (S# - KE – image type (ECHO,LD) and image # (01 or 02))

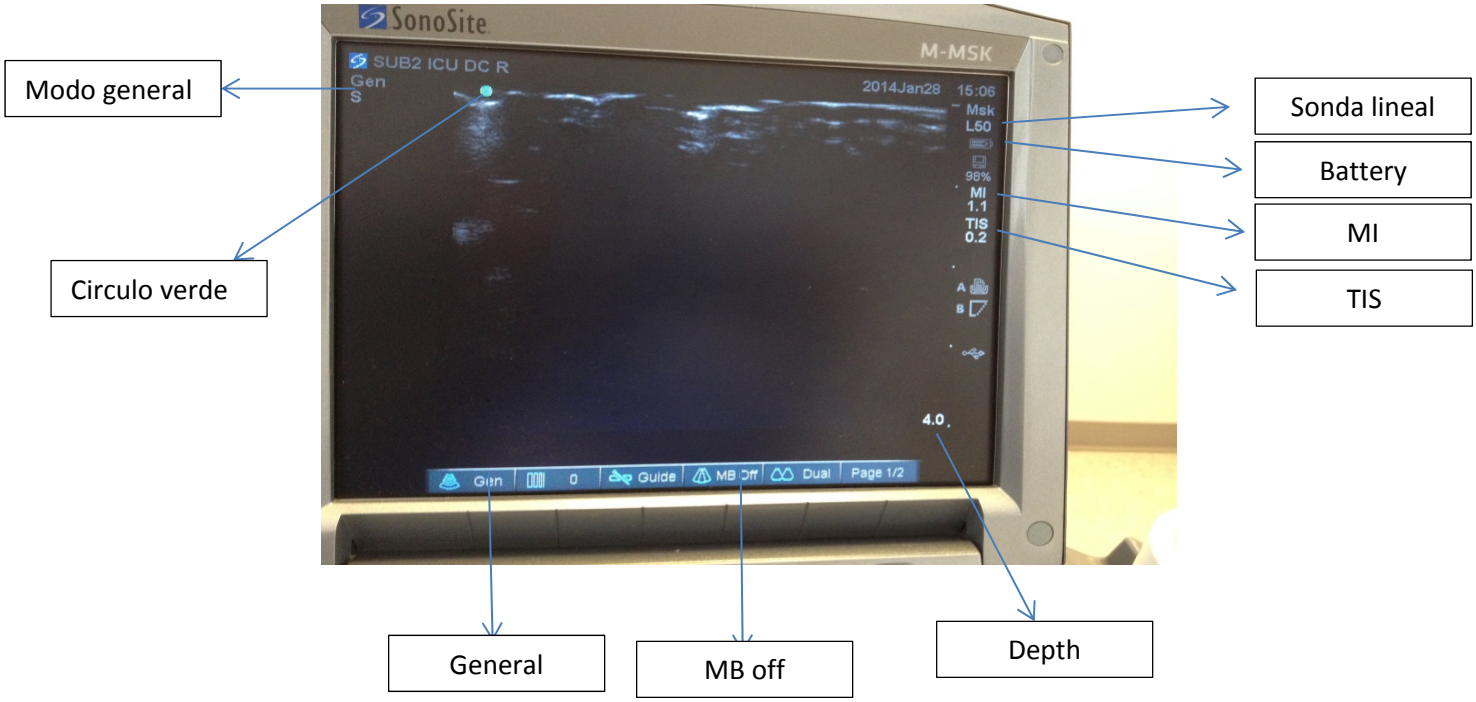
- Select Musculo-Skeletal setting. This will have pre-set settings for a musculo-skeletal ultrasound image acquisition.

U/S Settings

Make sure that all the following U/S settings are correct:

- General mode: make sure that you are in general mode; it should appear as ***Gen S*** on upper left side of your screen. This will change the focal depth to a mid-range focal depth.
- Always use ***2 dimensional modes***.
- Make sure that multiple beam feature (***MB) is off***, since this will distort the image.
- Check to see that the ***green circle*** is on upper left side of your screen.
- Then put the tip of your finger on your probe and find which end appears on the left side of the screen, so that it corresponds to the left of the probe.
- We will be using the **linear probe**; you can check that also on the upper right side of the screen appearing as L50.
- Always check the ***battery icon*** on top right of the screen, you will need to be plugged in during session if the battery is low, otherwise you may potentially lose data.

The image below provides a general example of the settings mentioned about. The displays and terms will differ based on the equipment used at each local institution. The goal is to keep settings consistent across all patients within each site.



For measurements of muscle echogenicity: The images obtained to evaluate echogenicity should use uniform study settings that are constant across subjects and across time points (Depth, time-gain, frequency, line density, mechanical index, and power). **It is critical that assessors use a standardized gain, which must be the same across all images for a participant. Use the default/autogain (brightness) for the unit when acquiring images for echogenicity and record this value to be used while images at subsequent time points. If the brightness is altered this will affect the images.**

For measurements of linear depth and cross sectional area: Once measures of echogenicity are obtained at each site, device settings including gain can then be modified to obtain optimal measurements of cross sectional area and linear depth as needed. Keep in mind that with most us-devices the width decreases with increasing depth.

-Avoid other factors which may deform the muscle being measured: examiner contact with the study site is the most common source of error, but contact with linens, IV lines or other objects can cause errors as well. Aberrant positioning will result in changes to study site.
-Pay careful attention to probe angle as small deviations from perpendicular can result in significant measurement error. This can be seen by the echogenicity of the femur. It will be most echogenic when the probe is perpendicular to it.

Outcome Assessor Procedures

Patient Instructions

- Once you have the patient in the proper position (see above),
    - Ask participant to remain completely still and to completely relax their leg.
- Tell the patient that you must landmark the proper location.
    - Explain the landmarks in lay terms
    - Landmark the location that the images will be taken (use instructions above)

Conducting the Test

Each of these assessments must be performed systematically and according to the detailed protocol that follows below

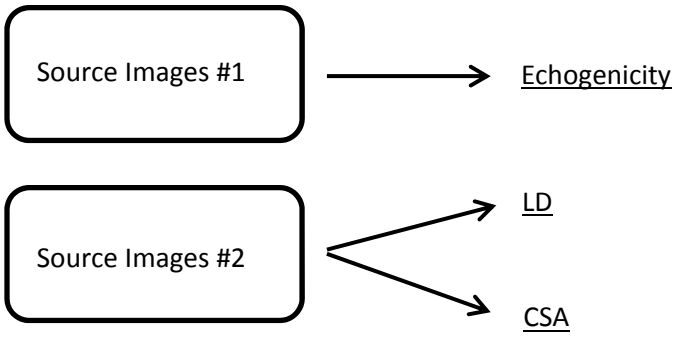
Obtaining Image:

- Apply an excess of gel to the head of the probe and make sure that the full surface is covered with gel.
- Make sure that you are not pushing down the probe too hard; it should just feel like a ***light touch.***
- The surface of the probe should not be in direct contact with the skin, there should be always gel between probe and skin.
- Make sure that you are keeping the ***probe horizontal and perpendicular*** to the patient's thigh, this is especially important before you capture the image, since you might be looking at the

screen and not paying attention to your probe's position. Always make sure to check your hand and probe's position again at the end, before hitting the freeze button. It is often useful to have a second person available to 'freeze' the image if possible.

- You should be able to see all borders of rectus femoris muscle; if you don't see the margins it may be due to the setting of depth or due to the probe not being in complete contact with the patient's skin, so make sure that you are using enough gel.
- There is a fine line between putting too little pressure and losing the borders and putting too much pressure and compressing the muscle bulk.
- Femur border is a very good landmark; you should be able to see it as a crescent line in the middle of the screen (unless the patient has an anatomical variation).
- You can change the depth depending on the patients muscle mass, but most commonly you ***remain between 4-4.9*** and ***record that*** on data collection forms, so **that all U/S measures for that patient are performed with the same depth**. It is really important that you keep this at the same depth for every measurement taken from the same patient. This is because we want to be able to compare them and see what is happening to the muscle bulk.
- After you hit "freeze", if you are satisfied with your image, press the "text" button on the keyboard to and type in "R" or "L", make sure you hit the "save" button, if you don't do this it is not going to save.
- Save first image for measurement of echogenicity
- Hit "freeze" again. This will unfreeze the image, and you can continue with taking the next image.
- Acquire the next image for the purpose of obtaining LD and CSA. Follow the instructions below for measuring LD and CSA.
- Repeat this process (see diagram below) so you have saved three images for each of the 3 measures.

Image acquisition (Repeated 3 times)

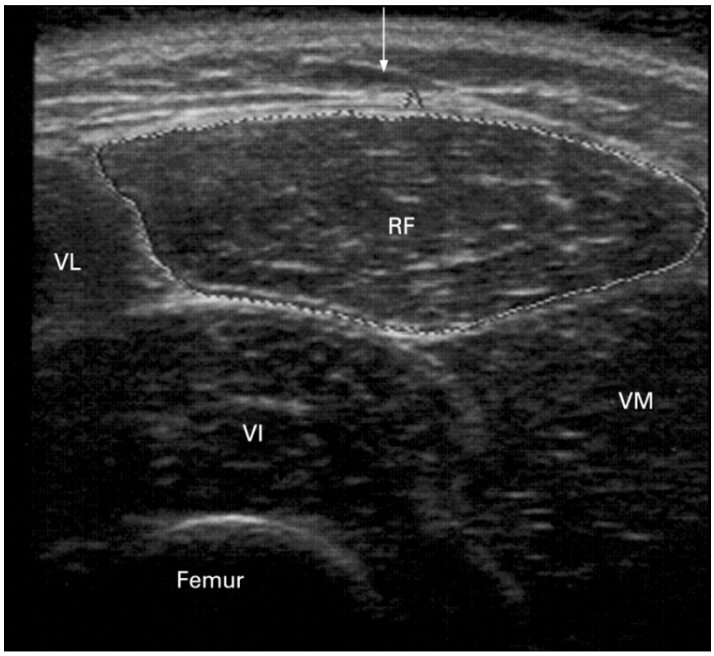


Acquiring values for Linear Depth and CSA in real time:

Measures of linear depth will be obtained at the time of the exam using digital calipers.

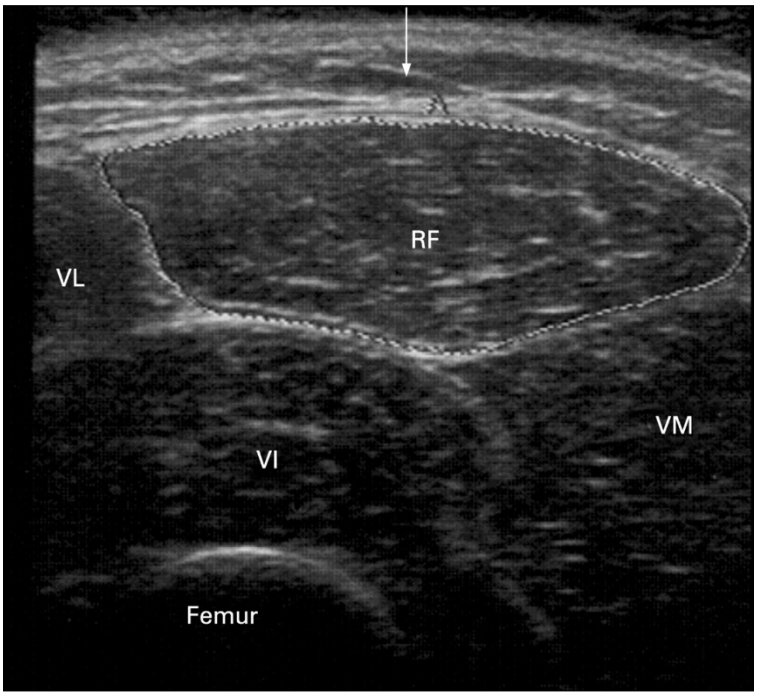
Linear depths:

1. Take measurements at 2 points as indicated below in the image.
    - Measurement (roller ball or cursor); highlight caliper on screen
    - Mark depth @ surface of femur (press Set)
        - Make sure measurement line is from the center of the proximal side of the RF to the femur. You should measure the shortest distance here.
    - Mark proximal depth of muscle (press Set) up the fascia below the adipose tissue
    - Check top left of screen for measurements.
    - Measurement should be recorded to 2 decimal places.
    - Save image and label accordingly.
    - Delete this measurement once it is recorded and saved and move on to the CSA.



Cross Sectional Area:

2. Take measurements around the outer edge of the RF as indicated below in the image.
    - Select the calcs tool (or equivalent)
    - Mark a position at the inside of the fascia of the RF, below the adipose tissue.
    - Measure around the outside border of the Rectus Femoris inside the fascia using the cursor
    - Check top left of screen for measurements. (Location may differ between machines)
    - Measurement should be recorded to 2 decimal places.
    - Save image and label accordingly.



Echogenicity:

This will be done centrally.

Image Saving / Labeling Measurements

Images and reports should be saved in DICOM format if able. JPEG format is acceptable.

Measurement of LD and CSA should be apparent on the image stored.

-Label images with file name.

Images:

Study Site	Image Number	Measurement (s)	Measurement obtained at time of exam	Modification of Standard Settings Permitted?	File name / Image Label/Time Point
Knee Extensor	**1**	Echogenicity	No	No	S# - KE – ECHO1-BAS
	2	Knee Extensor LD	Yes	Yes	S# - KE – LD1 -BAS
	3	Rectus Femoris CSA	Yes	Yes	S# - KE - CSA1-BAS

	4	Echogenicity	No	No	S# - KE – ECHO2-BAS
	5	Knee Extensor LD	Yes	Yes	S# - KE – LD2 -BAS
	6	Rectus Femoris CSA	Yes	Yes	S# - KE – CSA2-BAS
	7	Echogenicity	No	No	S# - KE – ECHO3-BAS
	8	Knee Extensor LD	Yes	Yes	S# - KE – LD3 -BAS
	9	Rectus Femoris CSA	Yes	Yes	S# - KE – CSA3-BAS
LD = Linear Depth, CSA = Cross sectional area, S# = Study number, BAS = baseline, ICU = ICU discharge, HOS = Hospital Discharge; KE= knee extensor					

Once all images have been exported to a USB, re-name the files as described in the above table. Then save to your research drive.

Example guide for saving image as DICOM (applicable to Sonosite m-turbo)

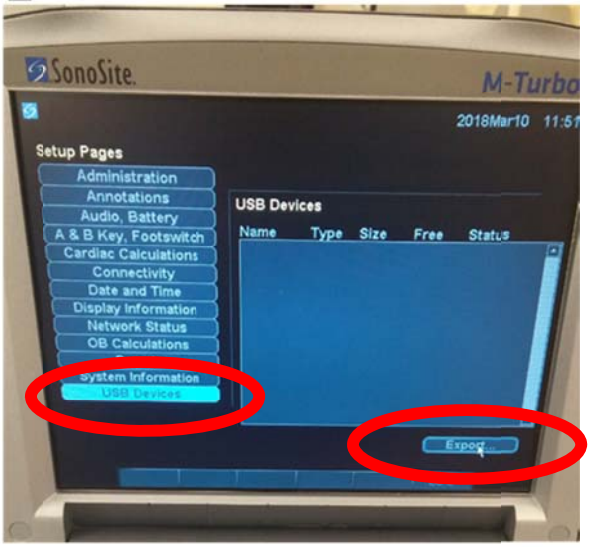
Images and reports should be saved in DICOM format

Instructions for Sonosite m-turbo to download DICOM files (See Fig 4.)

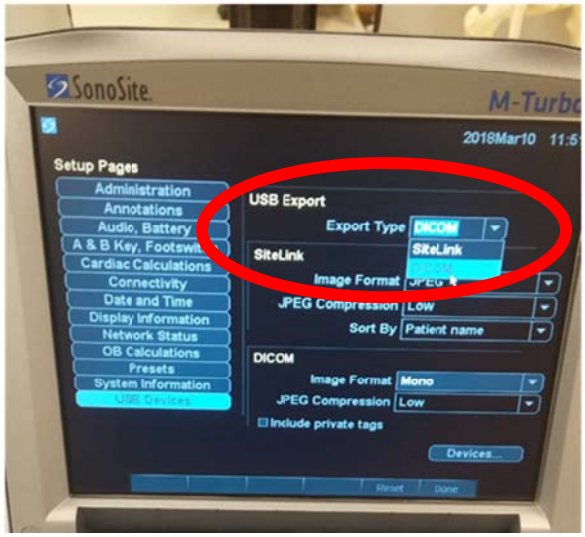
- Once the ultrasound has been powered up and hard-drive loaded (floppy disk stops blinking), press the "**Setup**" button, located on the top right side of keyboard/console
- Using the on screen mouse, click "**USB DEVICES**", which is located at the bottom of the setup page
- Within the "USB DEVICES" page, click on **"Export"**, located at the bottom right side of the screen
- Under "USB Export" section, select **"DICOM"** as the export type
- Under the "DICOM" section, select **"Mono"** as the image format

1

2



3



4

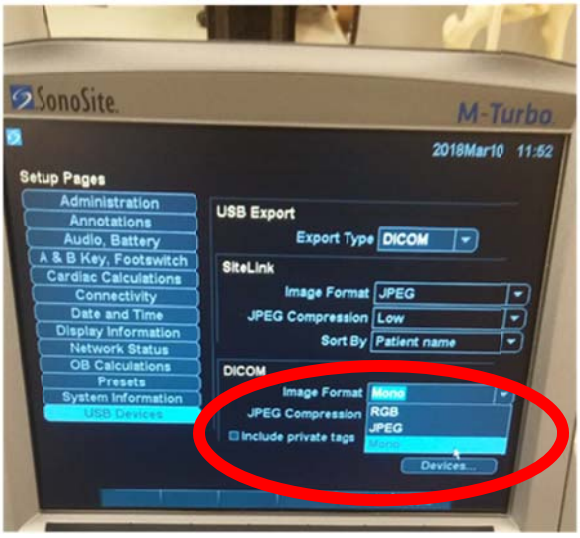


Image Transferring

All ultrasound images obtained will be de-identified prior to being transferred. The images will be sent to RTWH Aachen University in Aachen, Germany via a secure, electronic file transfer. The commercial system will be in accordance with the European Union General Data protection Regulation (GDPR). Each site will be provided with a login and password to access the system and upload images. Images will be accessed through this electronic portal by the Central Review Committee.

References:

1.Cartwright, M.S. *et al.* Quantitative Neuromuscular Ultrasound in the Intensive Care Unit . *Muscle Nerve.* **47,** 255–259 (2013).

2. Zaidman, C.M., *et al*. Qualitative and Quantitative Skeletal Muscle Ultrasound in Late-Onset Acid Maltase Deficiency. *Muscle Nerve*.44(3), 418-423. (2011).

3. Gruther W et al. Muscle wasting in intensive care patients: Ultrasound observation of the M. Quadriceps Femoris Muscle Layer, J Rehabil Med 2008; 40 Campbell et al. Muscle thickness, measured with ultrasound, may be an indicator of lean tissue wasting in multiple organ failure in the presence of edema, Am J Clin Nutr 1995; 62.10/25/2010

Appendix

Appendix 1

Effort – Ultrasound CRF

Assessor Name:_____ Participant ID:_____ Date:_____

Refer to *Study Procedures Manual* for detailed instructions.

Time Point:	☐ Day 1	☐ Day 10 Post Randomization	☐ Hospital Discharge
Was the Ultrasound attempted:	☐ Yes	☐ No	☐ No, 72 hrs between ICU and HC discharge
If you have indicated 'No', select the reason why below: ☐ Participant deceased ☐ Participant withdrew ☐ Not able to complete due to illness or physical limitation ☐ Participant refused ☐ Missed due to hospital discharge ☐ Missed due to RC unavailable ☐ Other (specify): _____			

Machine Used: **Probe Used:**

Leg Assessed: ☐ Right ☐ Left

(Use Right leg unless there is a specific reason otherwise)

Landmarks:

☐ 2/3 site →

☐ 10cm from Patella

Distance from Superior Border of Patella (1/3 distance): _____ cm (only measured at baseline)

Measurement should be recorded to 1 decimal place

Head of Bed Angle: _____ (Must be the same at all time points – Refer to Baseline)

Ultrasound Settings:

Gain (For Echogenicity): _____ (Must be the same for all images for a participant- Refer to Baseline)

Depth (For LD and CSA): _____ (Must be the same for all images for a participant – Refer to Baseline)

Measurement should be recorded to 1 decimal place

Image 1: Depth Used (must be same at all assessments)

Linear Depth: _____ cm ☐ Not Available

RF CSA: _____ cm ☐ Not Available

Effort – Ultrasound CRF

Assessor Name:_____ Participant ID:_____ Date:_____

Refer to *Study Procedures Manual* for detailed instructions.

Image 2: Depth Used (must be same at all assessments)

Linear Depth: _____ cm ☐ Not Available

RF CSA: _____ cm ☐ Not Available

Image 3: Depth Used (must be same at all assessments)

Linear Depth: _____ cm ☐ Not Available

RF CSA: _____ cm ☐ Not Available

NOTES:_____

_____

_____

_____

Appendix 2

Quadriceps Linear Depth and Rectus Femoris CSA QA

Instructions to QA Reviewer:

When conducting the quality assurance review, consider the following:

1. **Patient Positioning:** Does the operator position the patient appropriately (inclusive of angle of the head of the bed as well as positioning of the leg to be examined)?
2. **Use of the Ultrasound Machine:** Does the operator demonstrate an understanding of the ultrasound machine? Does he/she confirm use of the linear probe (or use the curvilinear probe if necessary)? Does he/she confirm the settings (eg on MSK for the M-Turbo)
3. **Appropriate images:** Does the operator obtain the appropriate images?
4. **Troubleshooting:** Does the operator troubleshoot properly? Eg non-contact artifact, inability to see the femur due to obesity/edema, measurements that are not within 10% of each other.

Quadriceps Linear Depth and Rectus Femoris CSA

Machine Used:	**Probe Used:**

Leg Assessed: Right ☐ Left ☐

Landmark:

2/3 site	☐	Distance from Superior Border of Patella:	cm
10cm from Patella	☐		
Architecture site (5 cm lateral 2/3)	☐		

Image 1:

Linear Depth: _____ cm

RF CSA: _____ cm^2

Image 2:

Linear Depth: _____ cm

RF CSA: _____ cm^2

Echogenicity

Image 1:

Heckmatt _____

Grey-Scale SD _____

Grey-Scale mean _____

Image 2:

Heckmatt _____

Grey-Scale SD _____

Grey-Scale mean _____

Architecture images captured:

Image 1 ☐ Image 2 ☐

- ☐ Correctly positioned the patient and recorded necessary values.
- ☐ Assessed the landmarks of the superior border of the patella and the ASIS correctly.
- ☐ Measured the distance between them and marked at the 2/3 site.
- ☐ Correctly identified and marked the architecture landmark.
- ☐ Was able to setup the ultrasound machine, input patient information, and set correct parameters (i.e. gain)
- ☐ Applied sufficient gel to the probe
- ☐ Minimal pressure and correct tilt of the probe was applied
- ☐ Was able to identify the femur and the vastus and rectus femoris muscles.
- ☐ Was able to measure the quadriceps linear depth. Had 2 measurements that were within 10% of each other.
- ☐ Was able to measure the rectus femoris cross-sectional area. Had 2 measurements that were within 10% of each other.
- ☐ Correctly captured images for muscle architecture

- [ ] Was able to save the appropriate images.
- [ ] Demonstrated an understanding of how to save images and export them to a USB.
- [ ] Form filled out completely and clearly.

Overall QA: ☐ **OK** ☐ **Needs improvement**

COMMENTS: