

Appendix 2: Instructions for ultrasound of rectus femoris muscle

- A. Ensure the patient is lying comfortably, with leg extended in neutral position. The head of bed should ideally be inclined at 30 degrees.
- B. Locating position
- Choose the right leg wherever possible. Use the same leg for measurements throughout the study.
 - Locate the base of the iliac crest and the top of the patella. Measure the distance and mark the mid-point (children < 6 years) or 1/3 the distance from the patella (children >6 years).
- C. Ultrasound measurement
1. Use the linear probe with the largest footprint available.
 2. Ensure that the settings are correct. Suggested standardized settings are a frequency of 12.0MHz, Gain of 50 and Dynamic Range (DR) of 95. Ensure that that the time-gain is in the neutral position.
 3. Adjust settings if necessary, between patients. Ideally the image should be as large as possible, while allowing visualization of the skin surface as well as the bone. For each patient, the following settings should remain the same
 - i. Depth
 - ii. Gain
 - iii. Frequency
 4. Create a new exam
 - i. Enter in patient ID
 - ii. When the rectus femoris can be visualized appropriately, press “freeze” and then save picture.
 - iii. For the cross-sectional ultrasound measurement, ensure that there is copious gel and minimal compression of the skin.
 - iv. Label image with subject ID, location, scan no. etc. Suggest to record as: SubjectID_location at leg_timepoint of measurement_image number. E.g. ID01_1/2RL_1_3 (this shows subject 1, measured at 1/2 of right leg, first measurement, image 3.
 - v. Press “freeze” again to unfreeze pane, and repeat.
 5. Capture 3 images and save each image. Name each image appropriately.
 6. Export the DICOM images.
- D. Measuring the cross-sectional area
1. Using the appropriate software with DICOM format support (e.g. NIH ImageJ tool), draw the cross-sectional area by tracing the inner echoic edge of the rectus femoris cross sectional area.
 2. Record the cross-sectional area in cm²

Appendix Table 1. Functional status scale score by Pollack et al. 2009

Domains	Normal (Score = 1)	Mild Dysfunction (Score = 2)	Moderate Dysfunction (Score = 3)	Severe Dysfunction (Score = 4)	Very Severe Dysfunction (Score = 5)
Mental status	Normal sleep/wake periods; appropriate responsiveness	Sleepy but arousable to noise/ touch/ movement and/or periods of social non-responsiveness	Lethargic and/or irritable	Minimal arousal to stimuli (stupor)	Unresponsive, coma, and/or vegetative state
Sensory functioning	Intact hearing and vision and responsive to touch	Suspected hearing or vision loss	Not reactive to auditory stimuli or to visual stimuli	Not reactive to auditory stimuli and to visual stimuli	Abnormal responses to pain or touch
Communication	Appropriate non-crying vocalizations, interactive facial expressiveness, or gestures	Diminished vocalization, facial expression, and/or social responsiveness	Absence of attention getting behavior	No demonstration of discomfort	Absence of communication
Motor functioning	Coordinated body movements, normal muscle control, and awareness of action and reason	1 limb functionally impaired	≥2 limbs functionally impaired	Poor head control	Diffuse spasticity, paralysis, or decerebrate/decorticate posturing
Feeding	All food taken by mouth with age-appropriate help	Nothing by mouth or need for age-inappropriate help with feeding	Oral and tube feedings	Parenteral nutrition with oral or tube feedings	All parenteral nutrition
Respiratory status	Room air and no artificial support or aids	Oxygen treatment and/or suctioning	Tracheostomy	Continuous positive airway pressure treatment for all or part of the day and/ or mechanical ventilatory support for part of the day	Mechanical ventilator support for all of the day and night