P1108 Lactate/Pyruvate (L/P) Ratio

• Why are we collecting this sample?
• Cohort 1 only (≥ 6 to <18 years)
• Entry, weeks 4, 24, 96

• Testing lab is in Utah – ARUP
  – a nonprofit national reference lab, founded in 1984. It is an academic enterprise of the University of Utah and its Department of Pathology
  – processes an average of 45,000–50,000 specimens of blood, body fluid, and tissue biopsies/day

• www.aruplab.com
Participant Preparation

• Fasted and at complete rest

  – Fasted = no food or drink other than water for 8 to 10 hours before the test. Medications OK.
    • So best to schedule for morning visit
    • Staff may want to contact caregiver day before to remind them of fast
  – Complete rest = no exercise for several hours before the test

• Avoid any exercise of arm or hand before or during collection
Participant Preparation cont’d

• Draw specimen w/out use of a tourniquet or within 3 minutes of applying the tourniquet, but before releasing the tourniquet
  – medical literature is mixed on use of a tourniquet
  – prolonged application of a tourniquet is the most common cause of falsely elevated lactate
  – recording of fasting state and use of tourniquet will be included on L/P eCRF (LBW0160)
Your Preparation

• Ice Bath
• Put specimen collection tube and pyruvate collection tube (containing 2mL of 8% perchloric acid) on ice
• Both should be chilled prior to blood draw
• Pyruvate collection tube will be supplied to you (ARUP → Lab Center → your lab)
Specimen Collection

- Collect 2mL whole blood using a 2mL vacutainer (sodium or lithium heparin/ green top)
  - if drawing with a syringe, immediately transfer to a sodium or lithium heparin/ green top tube
- Mix thoroughly by inverting 10-15 times; place on ice
- Immediately add exactly 1mL whole blood to chilled pyruvate collection tube using calibrated pipette
- Mix specimen in perchloric acid by inverting tube 10-15 times
Specimen Preparation

- Place well-mixed sample on ice for 10 min
- Centrifuge sample for 10 min at 1500xg
  - Spin may be RT, but tubes and sample must be chilled.
- After spin, specimen will be separated into two layers: clear liquid on top of opaque layer of sediment. If this does not happen – repeat the spin
- Pipette approximately 2mL of the supernatant (clear) into ARUP standard transport tube (minimum 1 mL)
Specimen Preparation cont’d

• Standard Transport Tube will be supplied to you (ARUP → Lab Center → your lab)
• Freeze (-20°C) the supernatant immediately after separating from the sediment
• Discard opaque sediment layer & original collection tube in biohazard waste
To Note

- If less than 1mL of blood is added to the chilled pyruvate collection tube, pH of the supernatant will be too low for testing.
- If addition to perchloric acid is delayed, lactate concentration in whole blood increases by ~ 30% after 30 minutes, 50% after 1 hour, and 75% after 2 hours at RT.
- From participating lab feedback - the local lactate testing requires sodium fluoride / potassium oxalate (gray top NaFl-KOx) tubes so the anticoagulant for venous lactate is NOT the same as that for the L/P and therefore, precludes both measurements coming from the same blood draw.
Specimen Storage & Transport

• Store and Ship frozen to ARUP in “real-time”.
  – All samples collected w/in 1 week (7 days) may be “batched” and then must be shipped w/in 24 hrs
• Stability frozen is 1 month
• ARUP Requisition Forms will be available online through ARUP Connect system
• ARUP has personnel to accept shipments 24/7
• Reporting of Results – accounts will be set up with ARUP Connect
• Results should be available 2 days following receipt of sample
Suggestion

• Site staff should do practice runs of the lactate/pyruvate and “local” lactate visits prior to enrolling any participants

• At least 2 staff members functioning as a team, establish procedure they will follow to ensure time and temperature requirements of both lactate assays are met and allow for efficient completion of all study visit procedures
P1108 Lactate/Pyruvate Ratio

ARUP collection tubes #16567
P1108 Lactate /Pyruvate Ratio

ARUP Collection Tube (closeup) – this tube will be centrifuged
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ARUP Standard Transport Tube (is a 4mL Sarstedt tube)
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ARUP bag for sample transport
Critical – what is written on the specimen tubes, MUST match how the samples are identified on this sheet in the two boxes at top.
ARUP Connect

- Registered clients use this secure online resource to manage their referral testing
  - http://aruplab.com/training/online-tools
  - http://aruplab.com/training/connect

- the Lab Center will assist each site in getting registered and setting up their account
- Sites will not need to use this for ordering supplies (Lab Center will obtain needed supplies from ARUP for the sites)
- Sites will not be billed. Billing is handled by the IMPAAACT JHU budget/finance office
ARUP Connect cont’d

- **ARUP Order Status and Results**
  - view, print, and download test results and view the status of pending orders

- **ARUP Order Entry**
  - order lab tests via a secure internet connection

- **eExcept**
  - allows online access to view and respond to testing exceptions (likely to be specimen issues)
Lactate/Pyruvate Assay – Safety

• Perchloric acid is often used to denature and precipitate proteins in samples before various metabolites are measured in tissue, blood, and other body fluids
• stronger acid than sulfuric and nitric acid
• a strong acid is one that tends to lose the hydrogen ions from all of its molecules; fully dissociates
Lactate/Pyruvate Assay – Safety

- HClO4
- highly corrosive substance and causes severe burns on contact with the eyes, skin, and mucous membranes
Lactate/Pyruvate Assay – Safety

- perchloric acid Material Safety Data Sheet (MSDS) will be emailed to you when tubes are shipped to your site. It will also be posted to the P1108 protocol specific webpage (pswp).
- Please review and keep for your records
- Wear protective clothing as recommended