**INTEGRATE Lab Protocol**

**Purpose**

To determine the epidemiology of colistin-resistant CRE in long term acute care (INTEGRATE) through Sensititre assay and sequencing.

**Materials & Equipment**

* Blood agar plates
* Bead beating tubes
* Prefilled cryovials with TSB/15% glycerol
* Molecular grade water
* Sensititre plates
* Sensititre plate seal
* Demineralized water
* Mueller-Hinton broth
* 0.5 McFarland standard
* Disposable loops
* Multichannel pipette
* Nephelometer
* Incubators at 35C and 37C
* Vortex mixer
* Manual viewer

**Specimens Collected from Kindred Rancho Lab**

1. Clinical Cultures
   1. All clinical cultures designated as CRE will be collected based on routine ID and susceptibility testing at the Rancho Lab, including subjects with multiple CRE cultures.
   2. Isolates will be cultured on slants for shipment and include the following information on sample label:
      1. Data printout included with isolate
      2. Name / MRN / accession number
      3. Facility
      4. Anatomic source (e.g., blood, respiratory, urine, etc)
      5. Date of culture
2. Surveillance Cultures
   1. Any admission surveillance culture (rectal, perirectal, etc.) designated as CRE using routine ID and susceptibility testing will be collected, including duplicate or multiple CREs.
   2. Surveillance plate sweep will be conducted for patients in which the surveillance culture revealed growth on CRE selective media and can be sent along with admission surveillance sample culture.
   3. Isolates will be cultured on slants for shipment and include the following information on sample label:
      1. Data printout included with isolate
      2. Name / MRN / accession number
      3. Facility
      4. Anatomic source (e.g., blood, respiratory, urine, etc)
      5. Date of surveillance culture

**Documentation Procedure:** *See Infectious Disease LabVantage Training PowerPoint for detailed instructions*

1. Upon receipt to the lab, all isolates received from the Rancho lab will be recorded in LabVantage.
   1. Select ID\_INTEGRATE study, patient cohort, and general collection (duplicate samples checked).
   2. Enter the collection date of the sample from the patient, listed on the slant sample label.
   3. Generate a bacterial cell culture label for each slant to label the subculture plates, being sure to select the correct cultures. There should be a separate entry for each slant received.
      1. Clinical cultures are designated as “CC-1”, “CC-2”, etc.
      2. Surveillance cultures are designated as “SC-S” (sweep plate), “SC-1”, “SC-2”, etc.
   4. After initial sample accessioning, add the bacterial species for each isolate and generate bacterial cell isolate child samples and cell lysate samples from the bacterial culture parent sample for each slant (do not consume parent sample).
   5. Print labels for subculture plates, bead beating tubes, and cryovials.
2. Record all PHI (listed above) from slant sample label into secure BOX spreadsheet. Record the assigned subject ID and LabVantage barcode (S-#) into the spreadsheet, as well as the type of culture it is (SC or CC).

**UPenn Sample Processing Procedure**

* *Isolates will be delivered to the ARES lab on a weekly basis from the Rancho Lab. Upon receipt slants are to be logged in LabVantage (see above for details) and immediately refrigerated until ready for processing.*
* *From each slant, the following will be prepared: subculture on blood agar for Sensititre testing, a bead beating tube for sequencing, and a frozen bacterial stock to store at -80C.*

1. Subculture isolates for Sensititre assay to be conducted the following day
   1. Label a blood agar plate for each isolate with the corresponding LabVantage label.
   2. Select a few colonies from the slant, and streak for isolation on blood agar.
   3. Repeat for all slants.
   4. Incubate plates at 37C for 18-24 hours.

*Note: if Sensititre assay cannot be completed the following day, a new subculture is required.*

1. Prepare bead beating tubes for downstream DNA extraction and sequencing
   1. Label a bead beating tube for each isolate with the corresponding LabVantage label.
   2. Fill the bead beating tube with 500uL of molecular grade water.
   3. Scrape the slant to fill half of a 10uL loop with bacteria.
   4. Swirl the loop in the bead beating tube to transfer the bacteria.
   5. Repeat for all slants.
   6. Store bead beating tubes at -80C until ready for further processing by CHOP.
2. Prepare frozen bacterial stocks for long term storage
   1. Label a prefilled cryovial with TSB/15% glycerol for each isolate with the corresponding LabVantage label.
   2. Using a 10uL loop, scape the remaining bacteria on the slant.
   3. Swirl the loop in the cyrovial to transfer the bacteria.
   4. Repeat for all slants.
   5. Store cryovials at -80C.
3. Store slants at 4C until growth of subculture plate is confirmed the following day.

**SENSTITRE Assay Procedure**

1. Using a disposable loop, gather a small amount of bacteria from the agar plate (~two small colonies).
2. Emulsify in demineralized water and vortex to minimize large clumps.
3. Calibrate the nephelometer using the 0.5 McFarland standard.
4. Using the nephelometer, adjust the sample in demineralized water to 0.5 McFarland (1.5x108 CFU/mL) by adding more bacteria if necessary.
5. Transfer 10uL of the suspension into a tube of 11mL Mueller-Hinton broth.  Vortex well.
6. Steps 1 through 5 must be completed within 30 minutes.
7. Open the Sensisititre plate and check that the desiccant is orange in color.
8. Pour the broth into a sterile trough and using a multichannel pipette, pipette 50uL into each well.
9. Make a purity plate by placing a 10uL loop full of bacteria onto blood agar and streak for isolation.
10. Cover the plate with an adhesive seal.  Press firmly to ensure proper sealing.
11. Incubate plates at 35C for 18-24 hours.  Do not stack more than three high.

**The following day: Reading the Plates**

1. Do not remove the seal from the plates.  Check purity plate to confirm no contaminants in culture.
2. Place the plate on the manual viewer.  Growth will appear as turbidity or a “button” of cells deposited at the bottom of the well.
3. Record growth on the INTEGRATE SENSITITRE Assay Worksheet, copies of which are on BOX .  Record the MIC as the lowest concentration of cells that completely inhibits growth.
4. Record the MIC data in the 2nd tab of the INTEGRATE Isolate Master List spreadsheet on BOX.