**MSPACMAN/SPACEMAN Lab Protocol**

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| Purpose |

**MSPACMAN**: Microbiome sampling in pediatric post-acute care to understand multi-drug resistant organism risk, antibiotic effect and ventilator-associated infection

Pediatric samples are collected at CHOP by the CHOP team and dropped off in the MOTION lab for sample processing and biobanking. Intermittently, samples will be sent to the CHOP Sequencing Core.

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| Materials & Equipment |

* CRE CHROMagar – Hardy Diagnostics Cat# G323
* ESBL CHROMagar – Hardy Diagnostics Cat# G321
* MRSA CHROMagar – Hardy Diagnostics Cat# G307
* 1µL disposable loops
* 10µL disposable loops
* Sterile, disposable transfer pipettes
* Cryovials/E-swabs containing specimen
* Sterile cryovials containing TSB with 15% glycerol
* Fisherbrand cardboard freezer boxes
  + 8x8 for cryovials
  + 10x10 for bead-beating tubes
  + 10x10 for copan (dry) swabs

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| Procedures |

## **Specimen Receipt**

* + - 1. Samples will be collected and transferred into the appropriate container by the RT and/or RN and will be delivered on ice to the University of Pennsylvania School of Medicine; MOTION Laboratory. Samples will include those listed in table 1.

**Table 1**. Specimen types to be delivered to the MOTION lab.

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| **Sample Type** | **Container** | **Processing** |
| **Endotracheal Aspirate** | Cryovial #1 | For biobanking only – for downstream sequencing – tagged with a purple dot sticker |
| Cryovial #2 | For culture & biobanking (may have orange dots, indicating they might be pulled for sequencing if needed) |
| **Stool Swab** | E-swab | For culture |
| Copan Swab #1 | For biobanking only – for downstream sequencing – tagged with a red dot sticker |
| Copan Swab #2 | For biobanking only – freeze as is |

* + - 1. Upon receipt, store each of the specimens at 4°C until ready to be processed and accessioned into LabVantage.

## **Specimen Processing**

1. For those samples that require processing on CHROMagar, create child samples:
   1. Check the box next to each sample to be processed. **Note**: you can only process one sample type at a time (ex. Endotracheal Aspirate OR Stool Swab). Once selected, click “Create Child Samples.”
   2. Select the **EndoTrach2BacCult** or **StoSwab2BacCult** sample plan from the dropdown menu.
   3. **UNCHECK “CONSUME PARENT SAMPLE.”**
   4. **Check “Auto-confirm child samples.”**
   5. Increase the number of child samples to be created to 3.

A screenshot of a computer

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**Figure 2**. MSPACMAN Child Sample Plan

1. Click “**OK**” to complete child sample creation. Each child sample will now be linked to its parent and have its own sample identifier (S-000000-00000). Each parent sample will now correspond to three children samples each: with CRE, ESBL & MRSA Bacterial Culture labels.
2. Label one set of selective media plates with the Bacterial Culture labels:
   1. CRE CHROMagar
   2. ESBL CHROMagar
   3. MRSA CHROMagar
3. If frozen, allow for specimens to thaw completely. Vortex each for approximately 5 seconds.
4. Using a disposable transfer pipette, transfer a single drop of media onto the edge of a CHROMagar plate and tilt such that the drop runs down the middle of the agar. Using the urine streak method, streak across the plate with a disposable 1µL loop.
5. Date each plate and tape together (one stack per patient). Place the inoculated media into a **DARK** 37°C incubator for 24 hours.
6. Primary Specimen Storage
   1. Place each endotracheal cryovial specimen(s) into their filed location within the -80°C so that they can be referenced again should repeat testing need completed.
   2. Place each Stool Eswab specimen(s) into their filed location within the -80°C so that they can be referenced again should repeat testing need completed.
7. The following day, observe the results on each plate.
   1. If negative, dispose of the plate(s) in LabVantage and select the “disposed” option within the disposal status drop-down menu.
   2. If positive,
      1. **MRSA** – **Biobank morphologies based on Hardy Diagnostic’s colorimetric definition of positive growth (see below).**
         1. Create a child sample of the Bacterial Culture sample in LabVantage and label a cryovial (Use **BacCult2BactIso**). **Note**: The parent sample in this case will be consumed; uncheck “Consume Parent Sample.” Inoculate the vial with the MRSA isolate and freeze at -80°C, being sure that they are filed correctly in LabVantage.
         2. Select the child sample that has been created. Click “**Set** **Species**.” A free-form box will be presented where you can add morphologies. See Appendix A with morphology options.

A close-up of pink bubbles

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Non-susceptible *S. aureus* will produce pink colonies.

* + 1. **CRE** – **biobank ALL morphologies.**
       1. Create a child sample of the Bacterial Culture sample in LabVantage and label a cryovial (Use **BacCult2BactIso**). **Note**: The parent sample in this case will be consumed; leave “Consume Parent Sample” checked. Inoculate the vial(s) with the CRE isolate(s) and freeze at -80°C, being sure that they are filed correctly in LabVantage.
       2. Select the child sample that has been created. Click “**Set** **Species**.” A free-form box will be presented where you can add morphologies. See Appendix A with morphology options.

A close up of a blue and white liquid

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Non-susceptible *E. coli* produces colonies that are rose to magenta.

Non-susceptible *Klebsiella*, *Enterobacter* and *Serratia spp*. produce larger blue colonies, with or without a pink halo.

* + - 1. **~~Addendum (December 8~~~~th~~ ~~2022~~**~~) Culture positive growth (except growth of suspected E. coli (pink colony morphology) on cetrimide CHROMagar. In LabVantage, indicate growth results on cetrimide agar in colony morphology attribution as follows:~~
         1. ~~Positive growth: “pos cetrimide-~~*~~colony morphology~~*~~”~~
         2. ~~Negative growth: “neg cetrimide-colony morphology”~~

**The use of cetrimide was discontinued.**

* + - 1. **Addendum (January 3rd 2023)** Plate all positive growth onto MacConkey agar. In LabVantage, indicate growth results on MacConkey agar in colony morphology attribution as follows:
         1. Non-lactose fermenting growth: “NLF-colony morphology”
         2. Lactose fermenting growth: “LF-colony morphology”
    1. **ESBL** – **Biobank morphologies based on Hardy Diagnostics’ colorimetric definition of positive growth (listed above).**
       1. Create a child sample of the Bacterial Culture sample in LabVantage and label a cryovial (Use **BacCult2BactIso**). **Note**: The parent sample in this case will be consumed; leave “Consume Parent Sample” checked. Inoculate the vial(s) with the ESBL isolate(s) and freeze at -80°C, being sure that they are filed correctly in LabVantage.
       2. Select the child sample that has been created. Click “**Set** **Species**.” A free-form box will be presented where you can add morphologies. See Appendix A with morphology options.

Close-up of a petri dish with pink bacteria

Description automatically generatedA close-up of a petri dish

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*E. coli* will produce pink colonies.

*Klebsiella pneumoniae* will produce blue colonies.

*Proteus mirabilis* will produce yellow colonies.

* + - 1. **Addendum (January 3rd 2023)** Plate all positive growth onto MacConkey agar. In LabVantage, indicate growth results on MacConkey agar in colony morphology attribution as follows:
         1. Non-lactose fermenting growth: “NLF-colony morphology”
         2. Lactose fermenting growth: “LF-colony morphology”

1. On a quarterly basis, the research specialist is to transport the biobanked specimens for sequencing to PCMC. These samples will consist of both endotracheal aspirate cryovials and stool swabs. Stool specimens will contain a red dot sticker and respiratory specimens will contain a purple dot sticker.
2. Once complete, the lab will be notified that the extracted DNA is ready to be picked up and can be transported back to the ARES lab for long-term storage.

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| Appendix A: Colony Morphologies |

1. **Colony** **Morphology** – morphologies noted in LabVantage can be chosen from the selection below, but must be listed in this order:
   1. Color
      1. Blue
      2. Pink
      3. Yellow
      4. Purple
      5. Transparent
      6. Cream
   2. Surface
      1. Smooth
      2. Glistening
      3. Rough
      4. Dull
      5. Rugose
   3. Shape
      1. Punctiform
      2. Round
      3. Irregular
   4. Other Characteristics (if applicable)
      1. Swarming

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| Appendix B: Storage Notes |

MSPACMAN specimens are to be stored in the -80°C freezer. Boxes are created as needed and ***must*** adhere to the following naming scheme:

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| **Specimen/Sample Type** | **Box Naming Scheme** | **Containers** |
| Endotracheal samples | MSPACMAN Cryovials Box #\_ | Cryovials |
| Endotracheal samples | MSPACMAN Seq Cryovials Box #\_ | Cryovials with red dot |
| Stool swabs | MSPACMAN Copan Swabs Box #\_ | Copan swabs |
| Stool swabs | MSPACMAN Seq Copan Box #\_ | Copan swabs with purple dot |
| Bacterial stock in PBS glycerol | MSPACMAN Positive Child Samples Box #\_ | 2mL microtubes |
| Extracted DNA | TBD | 96-well plate |

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| Change Control |

Please note any changes made to the laboratory protocol following study launch by completing the table below. Changes must be approved by the study principal investigator and laboratory director. The SOP should be saved with the date of change saved as part of its file name.

|  |  |  |  |
| --- | --- | --- | --- |
| **Date of Change Implementation (MM/DD/YYYY)** | **Brief Description of Change** | **Change Completed By** | **Approved By** |
|  |  |  |  |