Instructor Guide: Timeline and Laboratory Stations Set-Up

Below you will find a timeline and details of what will be set-up for demonstration at each station.

The sub-culturing guidelines are designed for a single set of cultures at each station. If you anticipate a large class, you can adjust this by considering two set-ups per station.

Preparing for the Workshop

6 Months Prior to the Workshop

Start your general planning for the workshop (WS) at least 6 months out – see the separate Logistics Instructions document for the details on what you need to do for the non-wet WS parts.

3 Months Prior to the Workshop

Make sure you have all of the organisms needed, either purchased, lyophilized, already sub-cultured or frozen.

Make sure you have all of the equipment and supplies needed.

Identify the source of the culture media you will use, purchased or prepared in-house. Check expiration dates.

3 Weeks Prior to the Workshop

Order or prepare perishable media and reagents.

Follow the **sub-culturing schedule** for all of the organisms

- Use the **color-coded labels** to help you keep on schedule and to organize the plates and tubes. If you follow the labels you will have all the plates and tubes you will need for each Station.
- If the organisms have been frozen or lyophilized start sub-culturing 17 days out. This will help to make sure the organisms are healthy for the WS.

Prepare Gram stains from culture of each organism and the five Case Study unknowns

Print and laminate the **Station set-ups**.

No Earlier than 3 Days before the Workshop

Apply a defogging agent to the empty petri dish lids for swapping out the day of the workshop (optional method for preventing condensation).

Preparing for the Workshop cont.

Biochemical Tests Set-ups:

- Arginine decarboxylase — Using a 10 ul loop, touch a well-isolated colony and inoculate a tube of decarboxylase base. Mix well. A 10 ul loop should give you a heavy inoculum that is preferred with the decarboxylase test. Using the same loop, pick a second colony, and inoculate a tube of decarboxylase broth with Arginine. Again, make sure it is a heavy inoculum. Add sterile mineral oil to each tube to a level of 1 cm. Cap tightly and incubate at 35C without CO2.

- Motility — Using an inoculating needle, touch a well-isolate colony, and inoculate a tube of motility medium. Insert the needle vertically into the center of the medium to ¾ the depth of the agar in the tube. Try to keep the line of inoculum as vertical and clean as possible. Make sure caps are loose and incubate at 35C.

- Urease — Using a sterile stick or loop, touch a well-isolated colony, and inoculate a tube of the urea agar. Streak the surface of the slant. Do not stab the agar. Make sure caps are loose and incubate at 35C without CO2.

Day of the Workshop

In the laboratory – 2 Hours Prior to the Workshop

On the morning of the workshop, place all plates, tubes, and Gram stains indicated in each diagram at each station, except for the plates incubating at 42° which should kept at that temperature until just before the laboratory session.

Let all of the plates come to room temperature (about 1 – 2 hours).

Plate Defogging Instructions (2 options):

- Check to see if the plates are fogged up with condensation. If they are then wipe them out with a clean Kimwipe or tissue and add a de-fogging agent such as baby shampoo.

- Swap out the lids for the “defogged” lids that were prepared ahead of time.

Seal each plate with two pieces of cellophane tape at opposite edges to keep the lid from coming off during the laboratory.

Put out all of the job aids and talking points at each station.

Take out the 42° plates about 1 hour before you go into the lab and follow the de-fogging instructions.

Laboratory Demonstration – Stations Set-Up

The laboratory session consists of **six demonstration stations** at which the students can observe the organisms growing on plated or tubed culture media, rapid biochemical tests, and Gram stains. There are also **five unknown stations**, each based on a case study.

Use the **Station Diagrams** to organize each laboratory station. Tape down all diagrams on the benches where you will be placing the demonstration materials. This will help keep the station organized as the students pass through.

Each station should have at least 2 microscopes.

Because the rapid biochemical results are not stable, unless you are performing these tests as a demonstration during the laboratory session we suggest you use the color prints to show the reactions.

The students will be given a set of the tables to record their observations, along with the case study information for identification of the Unknowns.

Station 1 – *Bacillus*

Station 1 diagrams from Station Diagrams file

Catalase, if performing as a demonstration:

- All *Bacillus* spp. (+)
- Controls for demo: *S. aureus* (+) and *Streptococcus* spp. (-)

Station 2 – *Francisella*

- Station 2 diagrams from Station Diagrams file
- β-lactamase, for demo:
  - *F. tularensis* (+), *H. influenzae* (+) and
  - *Aggregatibacter* spp. (-);reaction depends on the strain used
  - Controls for demos: *P. mirabilis* (+) and *S. aureus* (-)
- Oxidase (for demo):
  - *F. tularensis* (-), *Aggregatibacter* spp. (+) and *H. influenzae* (-)
  - Controls for demos: *Ps. aeruginosa* (+) and *E. coli* (-)
- Catalase (for demo):
  - *F. tularensis* (+), *H. influenzae* (+) and *Aggregatibacter* spp. (+)
  - Controls for demos: *S. aureus* (+) and *Streptococcus* spp. (-)

Laboratory Demonstration – Stations Set-Up cont.

Station 3 – *Brucella*

- Station 3 diagrams from Station Diagrams file
- Oxidase (for demo):
  - *B. canis (+), O. ureolytica (+) and H. influenzae (-)*
  - *Controls: Ps. aeruginosa (+) and E. coli (-)*
- Catalase (for demo):
  - *B. canis* (+), *O. ureolytica* (+) and *H. influenzae* (+)
  - Controls if used: *S. aureus* (+) and *Streptococcus* spp. (-)

Station 4 – *Yersinia*

- Station 4 diagrams from Station Diagrams file
- Oxidase (for demo):
  - *Y. pestis* (-), *Y. pseudotuberculosis* (-), *S. sonnei* (-)
  - Controls for demo: *Ps. aeruginosa* (+) and *E. coli* (-)
- Indole (for demo):
  - *Y. pestis* (-), *Y. pseudotuberculosis* (-), *S. sonnei* (-)
  - Controls for demo: *E.coli* (+) and *P. mirabilis* (-)

Station 5a – *Burkholderia* mallei

- Station 5a diagrams from Station Diagrams file
- Oxidase (for demo):
  - *A. lwoffi* (-)
  - Controls for demo: *Ps. aeruginosa* (+) and *E. coli* (-)
- Indole (for demo):
  - *A. lwoffi* (-)
  - Controls for demo: *E.coli* (+) and *Ps. aeruginosa* (-)

Station 5b – *Burkholderia pseudomallei*

- Station 5b diagrams from Station Diagrams file
- Oxidase (for demo):
  - *B. thailandensis* (+), *Ps. st*utzeri (+)
  - Controls for demo: *Ps. aeruginosa* (+) and *E. coli* (-)
- Indole (for demo):
  - *B. thailandensis* (-), *Ps. st*utzeri (-)
  - Controls for demo: *E.coli* (+) and *Ps. aeruginosa* (-)

Unknowns – Stations Set-Up

Case A – *Francisella tularensis*

- Case A Station diagrams from Station Diagrams file
- Oxidase:
  - *Unknown* (-)
  - Controls for demo: *Ps. aeruginosa* (+) and *E. coli* (-)
- Catalase:
  - *Unknown (weak+)*
  - Controls for demo: *S. aureus (+) and Streptococcus* spp. (-)

Case B – *Brucella canis*

- Case B Station diagrams from Station Diagrams file
- Oxidase:
  - *Unknown* (+)
  - Controls for demo: *Ps. aeruginosa* (+) and *E. coli* (-)
- Catalase:
  - *Unknown* (+)
  - Controls for demo: *S. aureus* (+) and *E. coli* (-)

Case C – *Bacillus anthracis*

- Case C Station diagrams from Station Diagrams file
- Catalase:
  - *Unknown (+)*
  - Controls for demo*: S. aureus (+) and Streptococcus* spp. *(-)*

Case D – *Burkholderia thailandensis*

- Case D Station diagrams from Station Diagrams file
- Oxidase:
  - *Unknown* (+)
  - Controls for demo: *Ps. aeruginosa* (+) and *E. coli* (-)

Unknowns – Stations Set-Up cont.

Case E – *Yersinia pestis*

- Case E Station diagrams from Station Diagrams file
- Oxidase:
  - *Unknown* (-)
  - Controls: *Ps. aeruginosa* (+) and *E. coli* (-)
- Indole:
  - *Unknown* (-)
  - Controls for demo: *E.coli* (+) and *P. mirabilis* (-)
- Catalase:
  - *Unknown (+)*
  - Controls for demo*: S. aureus (+) and Streptococcus* spp. *(-)*