Appendix 3 – Detailed instructor protocols for all stages

Stage 1:
1.1 Instructor demonstrates parts of the microscope and how they function for entire lab. Students are asked to follow along with their own microscope. Students can each have a microscope, or be in groups of two to three, depending on lab organization.

1.2 Demonstration begins with 1) how to properly carry and transport the microscope and continues in the following order: 2) power & light source, 3) diaphragm & condenser and light intensity, 4) stage adjustment, coarse & fine adjustment knobs and finding proper contrast, 5) ocular lenses, 6) proper care and cleaning of the microscope, and last 7) proper storage of the microscope. Students are given lens paper for step 6 of this stage and instructed how to properly clean all lenses of the microscope.

Stage 2:
2.1 *Optional* Students are given prepared slides with the letter "e" or newsprint and given the task of placing them in the microscope and focusing with the low power lens. The instructor walks from group to group, giving students direction as needed. Once students obtain a clear and appropriately illuminated image, then they practice moving the slide around with the stage controls.

2.2 Instructor directs students to pick at least two prepared slides, from slides already set out, to view through the microscope. Students are encouraged to select slides from different labeled boxes in order to gain experience focusing on differently-sized specimens and/or samples with different stains. Instructor moves around the lab during this time and helps students when it is needed, as well as asking questions about what students see. Students draw quick sketches of what they see before moving to stage 3. The end of stage 2 requires students to put the microscope into 'storage' position with the slide stage at its lowest level and to put the prepared slides back into the correct storage box.

Stage 3:
3.1 Instructor begins stage 3 by demonstrating how to correctly complete a wet mount using either pond water or liquid culture. Emphasis is put on how much liquid to add as well as applying the coverslip at an angle to prevent bubble formation. Once demonstrated, students then perform their own wet mounts, again with the instructor walking around and viewing the student work. This is so that the instructor can observe and correct any mistakes before the students attempt to view their wet mounts under the microscope. After each student has made their wet mount, they are then at their own pace in viewing and sketching their observations. It is important to understand that the point of this step is to illustrate to the students why staining is such a useful tool, and thus, frustration in not being able to see anything is likely. It is the instructor's job to circulate the room and help abate the frustration by guiding the student in focus and contrast in order to be able to better see their specimens. A common error from students at this point is focusing on an air bubble.

3.2 The introduction of immersion oil also occurs during this stage after all students have focused their slides at 40X. The instructor should explain how the optics of light microscopes work, briefly, and how immersion oil is used to avoid refraction and resulting loss of light. The importance of using immersion oil ONLY on the 100X lens should also be emphasized. Proper care of the microscope when using immersion oil is also covered at the end of this stage.

Stage 4:
4.1 The transition into stage 4 begins with the instructor explaining how to perform a smear with heat fixing, and reiterating the purpose of both of these steps. A smear should have a dense area with many microbes (useful for initially finding the right plane of focus) and an area of low density (to view individual cells or small clusters). Care is taken to explain to students the proper amount of liquid to use

in the smear and why the smear shouldn't be boiled, as a common error of students is to heat before the water has evaporated. Students are taught to gently heat the slides while holding with their hand to evaporate all the water. Once it is completely dry, then the slide is transferred to a slide holder for heat fixation. The staining procedure is not covered in detail by the instructor, rather, after explaining the smear process, the instructor should circulate the room and answer questions individually with students who have started the smearing and staining steps on their own. This is at the students' individual paces, helping them out when needed.

4.2 The instructor should ask the students what differences they notice between the stained slide and the unstained wet mount. This is a good time for a brief, interactive discussion about staining to increase contrast, and its usefulness in microbiology.

Stage 5:

5.1 At the beginning of this stage, very brief details are covered are since this stage incorporates all of the previous steps. The instructor does quickly walk through the steps, emphasizing the destain step as critical to ensure the best possible results. Destain is dripped across the slanted slide until it runs clear, typically 10-20 seconds. Students again work at their own pace and the instructor circulates the room, helping students when it's needed. It is important to discuss with each student their individual results and what they mean, as well as what might have gone wrong and have the student explain how to correct it in the future.

Stage 6:

6.1 This stage begins with a review of all the steps learned in previous stages. The instructor leads a short discussion on how the Gram stain works and the purpose of each component of the staining procedure. Students are instructed on how to divide and label the microscope slide before staining and then are allowed to perform the entire staining process, from smear to immersion oil viewing without further instruction. The instructor should circulate the room, ensure that students are closing dye bottles, using separate droppers/pipettes for each stain and avoiding other common mistakes. Students' stains should be shown to the instructor under oil immersion at high magnification, while stating whether they believe the unknown isolate to be Gram negative or Gram positive. If their controls aren't clearly negative and positive, the student should discuss with the instructor what went wrong (see Table 1) and how to correct it in the future.