**Appendix 1**

Example Online Clinical Microbiology Unknown Case Study

**Instructor Note:** This is an example unknown. Additional questions to address theoretical concepts or additional laboratory applications can be added. For example, having the student identify the reagent used for each test before it is performed. Questions can be made all multiple choice to make grading easier for large classes.

(Note: **Bolded wording** is correct answer selection and/or feedback provided to the student upon submission of the assignment)

**Unknown Announcement: (Post in LMS details section):**

**IMPORTANT:**

1. Utilize the provided lectures, procedures, laboratory demonstration videos, assigned textbook readings, and organism identification flowcharts found within this course when needed. Examples include gross colony characteristics, quantitation guidelines for culture interpretation, and hemolysis pattern demonstration.

2. Take notes as you progress through the questions (similar to culture documentation in the clinical microbiology laboratory).

**Backtracking is prohibited (you will not be able to return to previous questions or change an answer once you have moved onto the next question)**

**Unknown**

1. A 47-year-old male underwent back surgery for spinal decompression and to repair a herniated disk. Four days after surgery the incision exhibited purulent, bloody drainage. Upon examination, the area was hot to the touch and inflamed. The physician aspirated the drainage and sent it to the clinical laboratory for a wound culture.

The following video is the blood agar, chocolate agar, and MacConkey agar plate. You can pause and rewind the video as many times as you like.

[insert unknown wound *Staphylococcus aureus and Escherichia coli* video]

Video link (provided public for you to view example):

https://echo360.org/media/ed3e7981-b3b7-4c81-8917-fea4416ef7e2/public

How many colony types do you see in this culture?

- a. One
- b. Two
- c. Three
- d. Four or more

2. There are two colony types growing in this wound culture. Each colony was subcultured to additional media for pure isolation. Examine the video of colony one. (3 points)

**Instructor note:** since backtracking to previous questions is prohibited the correct answer for the previous question is provided in the new question. This is to prevent students from being continuously penalized for previous answers.

[insert unknown *Staphylococcus aureus* video]

Record the colony morphology of this organism.

**Feedback:**

BAP: medium smooth, creamy (gold), beta-hemolytic

CHOC: same as BAP

MAC: No growth
Instructor Note: Students are instructed on what is expected to be documented regarding colony morphology in the lectures. Students are provided documents and demonstration videos to aid in determining quantitation, gross colony characteristics, and hemolysis patterns.

General Colony Morphology Grading Notes:
- No Growth should be documented for each plate not showing growth
- Quantitation of the growth if from primary culture (not subculture)
- If growth on BAP: size, color, and hemolysis are required
- If growth on MAC: lactose-fermenter or non-lactose-fermenter

3. Colony one is a smooth, creamy-gold, beta-hemolytic colony which does not grow on the MacConkey agar. The growth quantitation from the primary culture is many.

Interpret the Gram-stain of colony one from the blood agar plate.

This organism is a:

a. Gram-positive cocci in chains
b. **Gram-positive cocci in clusters**
c. Gram-negative rods
d. Gram-negative coccobacilli
e. Gram-negative diplococci

4. The organism is GPC in clusters.

What is the next step in the identification process for the organism?

a. Coagulase
b. **Catalase**
c. Bacitracin
d. Novobiocin
e. Microdase (modified oxidase)
f. PYR
g. Optochin
h. Bile esculin
5. The first step in the identification of the organism is the catalase test. Watch the following video of the catalase test. The catalase test is:

[insert catalase positive video]

Video link (provided public link for you to view example):
https://echo360.org/media/ca804d64-8c70-4ad1-a9f2-470b1ae30486/public

6. The catalase test is positive. The organism is a _______,

a. Negative
b. Positive

7. The organism is a beta-hemolytic *Staphylococcus* species. What is the next step in the identification process?

a. Coagulase
b. Bacitracin
c. Novobiocin
d. Microdase (modified oxidase)
e. PYR
f. Optochin
g. Bile esculin

8. The next step in the identification of the organism is the coagulase test. Watch the following video of the Staph latex coagulase test. The Staph latex coagulase test is:

[insert Staph latex positive video]

9. The Staph latex coagulase test is positive, there is agglutination was present. No further testing is needed the organism can be identified. Based on the test results what is the organism identified as:

a. Coagulase-negative *Staphylococcus* species not *saprophyticus*
b. *Micrococcus luteus*
c. *Staphylococcus aureus*
d. *Staphylococcus epidermidis*
e. *Staphylococcus saprophyticus*

10. Colony one is “Many *Staphylococcus aureus*”. In this surgical site wound culture this organism is considered:

a. Contaminant
b. Normal flora
c. Probable pathogen

**Instructor Note:** Antibiotic susceptibility testing can be included after this question if applicable to the course theory information. Examples include videos or pictures of disk diffusion or microdilution testing. Students can interpret the zone of inhibition (disk diffusion) or minimum inhibitory concentration (microdilution).

11. Colony one is a probable pathogen. There are two colony types growing in this wound culture. Each colony was subcultured to additional media for pure isolation. Examine the video of colony two. (3 points)

[insert unknown *Escherichia coli* video]

Video link (provided public for you to view example):
https://echo360.org/media/98f9a5c5-68e4-4117-8379-782e91b46013/public
Record the colony morphology of this organism.

Feedback:
BAP: large grey beta-hemolytic
CHOC: same as BAP
MAC: pink, lactose-fermenting

Instructor Note: Colony Morphology General Grading Notes:
- No Growth should be documented
- Quantitation if from primary culture (not subculture)
- If growth on BAP: size, color, hemolysis, and key characteristics are required, other documentation is optional
- If growth on MAC: must list as lactose-fermenter or non-lactose-fermenter

12. Colony two is large, grey, and beta-hemolytic on the blood agar plate. It appears as a pink colony on the MacConkey agar. Based on the colony morphology what is the expected Gram-stain of this isolate?
   a. Gram-positive cocci in clusters
   b. Gram-positive cocci in chains
   c. Gram-negative coccobacilli
   d. Gram-negative rods

13. This organism grows on the MacConkey agar so it is a GNR (a Gram stain is not necessary to perform as there is only one colony type on the BAP and MAC).
   It appears pink on the MacConkey agar. This means this GNR is a lactose:
   a. fermenter
   b. non-fermenter

14. This GNR isolate is pink on MAC which indicates it is a lactose-fermenter.
   What is the next step in the identification process for the organism?
   a. Coagulase
   b. Bacitracin
   c. GNR biochemical identification panel
   d. Novobiocin
   e. Microdase (modified oxidase)
   f. PYR
   g. Oxidase
   h. X and V factor requirements

15. The first step in the identification of the organism is the oxidase test. Watch the following video of the oxidase performed on this isolate. The oxidase test is:
[insert oxidase negative video]

16. The oxidase test is negative. Based on testing thus far the organism is a suspected:
   a. *Aeromonas* species
   b. *Campylobacter* species
   c. *Haemophilus* species
   d. *Enterobacteriaceae*
   e. *Pseudomonas* species
   f. *Staphylococcus* species
   g. *Streptococcus* species
17. Colony two is within the Enterobacteriaceae family based on colony morphology and oxidase test. What rapid test is the next step in the identification process?
   a. Coagulase
   b. Catalase
   c. Bacitracin
   d. Bile solubility
   e. Butyrate esterase
   f. Microdase (modified oxidase)
   g. PYR
   h. Oxidase
   i. **Spot indole**

18. The next step in the rapid identification of GNR isolates after the oxidase test is the spot indole test. Watch the following video of the spot indole performed on this isolate. The test is:

[insert spot indole positive video]

a. Negative
b. **Positive**

19. Colony 2 is a beta-hemolytic, lactose-fermenting, oxidase negative, indole positive GNR. No further testing is needed to identify this isolate. This organism can be identified as:
   a. **Escherichia coli**
   b. **Klebsiella pneumoniae**
   c. **Klebsiella oxytoca**
   d. **Proteus mirabilis**
   e. **Proteus vulgaris**
   f. **Salmonella** species
   g. **Shigella** species
   h. **Pseudomonas aeruginosa**
   i. **Acinetobacter baumanii**
   j. **Aeromonas** species

Feedback: Key identification characteristics of **Escherichia coli** include a beta-hemolytic, lactose-fermenting, oxidase negative, indole positive GNR. If the organism meets all of these criteria it can be identified without overnight testing (full GNR identification panel).

20. Colony two is “Many **Escherichia coli**”. In this surgical site wound culture this organism is considered:
   a. Contaminant
   b. Normal flora
   c. **Probable pathogen**

**Instructor Note:** Antibiotic susceptibility testing can be included after this question if applicable to the course theory information. Examples include videos or pictures of disk diffusion or microdilution testing. Students can interpret the zone of inhibition (disk diffusion) or minimum inhibitory concentration (microdilution).

21. This surgical site infection wound culture report is: Many **Staphylococcus aureus**
    Many **Escherichia coli**

Explain the clinical significance of isolating this organism from this culture including symptoms, pathophysiology, and epidemiology. (4 points)

**Feedback:** Both **Staphylococcus aureus** and **Escherichia coli** are considered pathogens from wound cultures. **Staphylococcus aureus** is the leading cause of skin and soft tissue infections and **Escherichia coli** is commonly isolated from wound infections. This patient had back surgery and S. aureus and GNR’s can lead to secondary infections in preexisting skin lesions such as a surgical incision.
### Appendix 2
Example Introduction to Clinical Microbiology Course Schedule with Corresponding Laboratory Demonstration Videos, Unknowns, and Case Studies.

| Weekly Topics                      | Course Assignments                                                                 | Laboratory Demonstration Videos (Supplement with Written Procedure)                                                                 | Online Unknowns and Case Studies                                                                 |
|-----------------------------------|------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| **Introduction to Infectious Disease** | Course Introduction  
Course participant introduction activity  
Lecture: Infection and Disease  
Lecture: Host-Pathogen Interactions  
Lecture: Innate Immunity & Adaptive Immunity | No demonstration videos for this module                                                                                      | No Lab for this Module                                                                         |
| **Introduction to the Microbiology Laboratory** | Lecture: Bacterial Culture Process Overview  
Lecture: Orientation & Safety in Lab  
Lecture: Microscope Use  
Lecture: Gram Stains  
Lab Unknowns: Gram Stains | 1. Microscope slide preparation from colonial growth for a gram-stain  
2. Microscope slide preparation from direct patient specimen for a gram-stain  
3. Gram stain procedure | Gram Stain Interpretation                                                                                                   |
| **Bacterial Culture Interpretation** | Lecture: Determination of Microbiological Isolate Clinical Significance Overview  
Lecture: Specimens and Culture Interpretation  
Lab: Introduction to Culture Interpretation | 1. Colony morphology interpretation  
2. MacConkey agar interpretation  
3. Alpha, Beta, & Gamma Hemolysis pattern interpretation  
4. Streaking for isolation technique | Colony Morphology Interpretation                                                                                             |
| **Antimicrobial Susceptibility Testing** | Lecture: Antibiotics and Resistance Mechanisms  
Lecture: Antimicrobial Susceptibility Testing Methods | 1. Preparation of a bacterial suspension equivalent to a McFarland turbidity standard  
2. Preparation of a lawn of growth for antimicrobial susceptibility testing  
3. Application of antibiotic disks to agar for Kirby-Bauer disk diffusion  
4. Kirby-Bauer disk diffusion interpretation | Kirby-Bauer Disk Diffusion Setup and Interpretation  
E Test Interpretation  
Microdilution Interpretation                                                                 |
| **Gram-positive bacteria** | Lecture: Staphylococcus & Micrococcus  
Lecture: Streptococaceae (Streptococcus & Enterococcus)  
Lecture: Gram-Positive Rods  
Lab Unknowns: Gram-Positive | 1. Colony morphology interpretation videos  
   a. *Staphylococcus aureus*  
   b. *Staphylococcus epidermidis*  
   c. *Staphylococcus saprophyticus*  
   d. *Streptococcus agalactiae*  
   e. *Streptococcus pyogenes*  
   f. *S. pneumoniae*  
   g. *Viridans Streptococcus* group  
   h. *Enterococcus*  
2. Bacitracin disk susceptibility  
3. Bacticard™ Strep Test  
4. Bile esculin hydrolysis | *Staphylococcus* species Identification Unknown  
*Streptococcus* species Identification Unknown  
*Enterococcus* species Identification Unknown  
*Listeria monocytogenes* Identification Unknown                                                                 |
| Lecture: Gram-Negative Rods | 1. Colony morphology interpretation videos |
|-----------------------------|------------------------------------------|
| Lecture: Gram-Negative Rods | a. *Acinetobacter baumanii* |
| Lecture: Gram-Negative Rods | b. *Aeromonas* species |
| Assignment: GNR Concept-Map/Flowchart | c. *Campylobacter jejuni* |
| Lab Unknowns: GNR and GNCB | d. *Escherichia coli* |
| | e. *Haemophilus influenzae* |
| | f. *Klebsiella* species |
| | g. *Proteus mirabilis* |
| | h. *Pseudomonas aeruginosa* |
| Exam 1: covers content from modules 1-5 | 2. MacConkey agar interpretation |
| Lecture: Gram-Negative Diplococci | 3. Oxidase test |
| Lab Unknowns: GNDC | 4. Spot indole test |
| | 5. *Haemophilus* Quad Plate Set & Interpretation |

### Gram-negative bacteria

| Lecture: Gram-Negative Rods | 1. Colony morphology interpretation videos |
|-----------------------------|------------------------------------------|
| Lecture: Gram-Negative Rods | a. *Acinetobacter baumanii* |
| Lecture: Gram-Negative Rods | b. *Aeromonas* species |
| Lecture: Gram-Negative Coccobacilli | c. *Campylobacter jejuni* |
| Assignment: GNR Concept-Map/Flowchart | d. *Escherichia coli* |
| Lab Unknowns: GNR and GNCB | e. *Haemophilus influenzae* |
| | f. *Klebsiella* species |
| | g. *Proteus mirabilis* |
| | h. *Pseudomonas aeruginosa* |

### Exam

| Lecture: Gram-Negative Diplococci | 1. Colony morphology interpretation videos |
|-----------------------------|------------------------------------------|
| Exam 1: covers content from modules 1-5 | a. *Moraxella catarrhalis* |
| Lecture: Gram-Negative Diplococci | b. *Neisseria gonorrhoeae* |
| Lab Unknowns: GNDC | c. *Campylobacter jejuni* |
| | d. *Escherichia coli* |
| | e. *Haemophilus influenzae* |
| | f. *Klebsiella* species |
| | g. *Proteus mirabilis* |
| | h. *Pseudomonas aeruginosa* |

### Miscellaneous bacteria

| Lecture: Mycobacteria | 1. Colony morphology interpretation videos |
|-----------------------|------------------------------------------|
| Lecture: Anaerobes | a. *Moraxella catarrhalis* |
| Lecture: Chlamydia, Mycoplasma, Spirochetes, Rickettsia | b. *Neisseria gonorrhoeae* |
| Current event topic: refer to Module 14 | c. *Campylobacter jejuni* |
| | d. *Escherichia coli* |
| | e. *Haemophilus influenzae* |
| | f. *Klebsiella* species |
| | g. *Proteus mirabilis* |
| | h. *Pseudomonas aeruginosa* |

### Anaerobes

| Lecture: Anaerobes | No demonstration videos for this module |

### Mycobacteria

| Lecture: Mycobacteria | No Lab for this Module |

### Current event topic

| Lecture: Mycobacteria | No demonstration videos for this module |

### Anaerobes

| Lecture: Anaerobes | No Lab for this Module |
| Mycology Parasitology | Lecture: Mycology  
Lecture: Parasitology  
Lab Unknowns: Mycology | 1. *Candida albicans* colony morphology interpretation videos  
2. Germ tube setup & interpretation  
3. Rapid Trehalose setup & interpretation | *Candida albicans* Identification Unknown  
Parasitology specimen Identification Unknowns |
|---|---|---|---|
| Viruses | Lecture: Viruses  
Lecture: HIV and Opportunistic infections | No demonstration videos for this module | No Lab for this Module |
| Body Systems: Bloodstream | Lecture: Bloodstream infections  
Lecture: Sterile Body Fluid Infections  
Lab Cases: Sterile Body Sites | No demonstration videos for this module | Meningitis Case Study (Cerebral Spinal Fluid Culture): *Neisseria meningitidis*  
Bacteremia Case Study (Positive Blood Cultures; 3 of 3 sets): *Streptococcus pneumoniae*  
Gonococcal Arthritis Case Study (Synovial Fluid Culture): *Neisseria gonorrhoeae*  
Pneumonia Case Study (Pleural Fluid Culture): *Klebsiella pneumoniae* |
| Exam 2: Urinary Tract | Exam 2: covers content from modules 6-10  
Lecture: Urinary tract infections  
Lecture: Skin/Tissue/Wound Infections  
Lab Cases: Sterile Body Sites | 1. Urine culture setup  
2. Urine culture interpretation with single pathogen  
3. Urine culture interpretation with multiple colony types | Urinary Tract Infection Case Study (Urine Culture): *Staphylococcus saprophyticus*  
Urinary Tract Infection Case Study (Urine Culture): *Streptococcus agalactiae*  
Urinary Tract Infection Case Study (Urine Culture): *Proteus mirabilis*  
Contaminated Urine Culture: >100,000 cfu/ml  
Mixed Gram-positive flora  
Wound Infection Case Study (Intra-abdominal Wound Culture): *Enterococcus* species  
Wound Infection Case Study (Surgical Site Lower Back Wound Culture): *Staphylococcus aureus* and *Escherichia coli* |
| Body Systems: Respiratory Tract | Lecture: Sexually transmitted infections  
Lecture: Respiratory tract infections  
Lab Cases: Gastrointestinal infections  
Lab Cases: Sites with Normal Flora | 1. Sputum culture interpretation  
2. Hektoen Enteric Agar interpretation  
3. Stool culture interpretation | Gastroenteritis Case Study (Stool Culture): *Salmonella* species  
Gastroenteritis Case Study: Negative Stool Culture; suspect viral or parasitic pathogen  
Group A Streptococcus Screen (Throat Culture): *Streptococcus pyogenes*  
Pneumonia Case Study (Sputum Culture): *Pseudomonas aeruginosa* with Normal Respiratory Flora |
| Current Event | Current Event Preparation and Submission  
Current Event Presentations Viewing | No demonstration videos for this module | No Lab for this Module |
| Discussion Board |
|------------------|
| Final comprehensive exam |
Appendix 3
Learning Management System (LMS) Recommended Features.

1. Features Necessary to Create Unknown Assignment:
   a. Ability to create sequential questioning within assignment (quiz, exercise, unknown, etc.) function
   b. Embed pictures within questions
   c. Embed videos within questions
   d. Display one question at a time
   e. Prohibit backtracking (lock question after answering)
   f. Provide immediate pre-built feedback upon submission of unknown including:
      i. Correct answer(s)
      ii. Incorrect answer(s)
      iii. Instructor generated information
   g. Multiple Question Types
      i. Short answer
      ii. Fill-in-blank
      iii. Matching

2. Instructor Grading:
   a. Ability to provide customized feedback for each individual student by question and overall assignment

3. Student Features:
   a. Ability to review correct and incorrect answers immediately upon submission and on-demand thereafter
   b. Ability to communicate with instructor within assignment after submission
   c. Functionality to complete assignment multiple times for additional practice (if allowed by instructor)
## Survey feedback regarding online laboratory unknowns.

| Online Human Pathogenic Microbiology Course                                                                 | Medical Laboratory Science Professional Program Clinical Microbiology Course                                                                 |
|-----------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| I was a little concerned about taking a course with a lab online, but it ended up being one of my favorite and most challenging aspects of the course. I liked that they really required you to know the material and weren't something that you could just Google. | I would have missed out if we didn’t have the online unknowns, they gave me the ability to practice identifications when the bench was slow and didn’t have many cultures. |
| I really enjoyed learning how to read slides and the process to setting up plates! I feel I learned a lot from the lab unknowns. | The online unknowns are my favorite part of the laboratory practicum courses. They help prepare me for my clinical rotation and study for exams. |
| The videos provided practical lab skills and good technique. Very useful.                                   | I really liked doing the online unknowns. It felt like I was right there in the lab and working through the workups. I learned a lot doing them. |
| I personally struggled at times with the laboratory unknowns, but I really appreciate them because it really sets us up as if we are a microbiology tech and goes through the steps we would need to take in a clinical setting, which again is setting us up for success. | I enjoyed the case studies/unknowns the most because establishing that contextual connection to the material empowered me to learn more and ask deeper questions. That was very gratifying for me as I truly love micro! |