Reading Hospital: School of Health Sciences
MLS Program: 2021 – 2022
Microbiology – Bacteriology

Instructor:

Ethan Kentzel ethan.kentzel@towerhealth.org O – 484.628.7898

Course Description:

Clinical Microbiology covers the theoretic principles of bacteriology and antimicrobial susceptibilities. Virology, parasitology, and mycology will have individual syllabus and objectives that are separate from the bacteriology component of clinical microbiology. This course aims to provide the principles and application of microbiology testing and identification of bacteria in the clinical laboratory based off ASCLS Entry Level Curriculum, ASCP BOC Content, and ASCP Lab Final Task Skills. The course covers many topics: gram positive cocci, gram positive rods, gram negative rods, gram negative cocci, mycobacterium, acid fast bacillus, aerobes, anaerobes, and antimicrobial susceptibility testing. Additional subtopics will be biochemical testing to identify and differentiate staphylococcus from streptococcus, members of Enterobacteriaceae from non-Enterobacteriaceae, fastidious organisms, the types of agar/media used to grow bacteria, and correlation of anatomical body locations with likely pathogens with clinical signs and symptoms.

Mode of Instruction:

The didactic curriculum consists of a series of in person lectures throughout the entire. The estimated weekly hours of lecture vary between two (2) to four (4) hours. Some didactic instruction and laboratory testing may overlap with other class materials. Additional assignments may be given throughout the course. If any unforeseen events occur to disrupt in person didactic lectures the sessions will move to an online style and format. *This syllabus is subject to change and you will be notified in writing by email and provided an updated copy*.

General Course Objectives:

At the conclusion of each unit of instruction, the learner should be able to demonstrate understanding of the specified unit objectives with 77% or greater accuracy on a written exam unless otherwise specified.

Required materials:

Bailey and Scott's Diagnostic Microbiology. 15th edition. Elsevier

Attendance:

Students are expected to attend each class session and to follow attendance policies established by the RHSHS: Medical Laboratory Science program. It is the sole responsibility of the student to arrange the completion of missed work. Please contact the instructor if you are expected to be late or missing from an assigned lecture.

Testing, Grading, and Evaluation:

There is seven (7) exam for this course and one includes virology (separate course – 1 exam). Additional assignments may be given as needed to support, assess, and achieve learning objectives. The exam(s) will be scheduled by the instructor after the completion of a unit/module. The exam dates may change, and you will be promptly notified in writing. Non-

graded case studies and supplemental learning may be assigned at instructor's discretion to assess comprehension and retention of course materials. The course requirements and evaluations are based upon achievement of the course objectives. All assignments must be completed and submitted by the due date. The percent weight for the determination of final course grade are as follows:

Grading System:			
A	= 94 – 100	F	<77
A-	= 90 – 93.9		
B+	= 87 - 89.9		
B	= 83 - 86.9	WP	= Withdrawal Passing
B-	= 80 - 82.9	WF	= Withdrawal Failing
C+	=77 - 79.9	I	= Incomplete

Course Content/Itinerary:

Lecture	Topic on Exam	Chapter
Lecture 1	Intro to Bacteriology: Taxonomy and ID	1
Lecture 2	Bacterial Genetics, Metabolism, and Structure	2
Lecture 3	Specimen Management and Bioterrorism	5 and 79
Lecture 4	Contemporary methods for Bacterial Identification and agar plates	7
Lecture 5	Antimicrobial Testing	10 and 11
Lecture 6	QC in the Micro Lab	77
Lecture 7	Staph and micrococcus	13
Lecture 8	Strep and Enterococcus	14
Lecture 9	Non-Branching, Catalase + GPR	15, 16, 17, 18
Lecture 12	Enterobacteriaceae (Ox – GNR)	19 → 25 30; 33
Lecture 13	Vibrio, Aeromonas, Helicobacter, & Campylobacter (Ox + GNR)	
Lecture 14	Pseudomonas & (GNR non-fermentors)	
Lecture 15	Diarrheal Diseases	
Lecture 16	Ox + GNCB: Moraxella + Neisseria	27 → 32 34 →37; 39
Lecture 17	Fastidious GNR: HACKEK OX	
Lecture 18	Fastidious GNR: Zoonotic and other GNR	
Lecture 19	Fastidious GNR + GNCB: Hemophilus	
Lecture 20	GNR with special media	
Lecture 21	Mycobacteria, Mycoplasma, and Ureaplasma	42 and 44
Lecture 22	Anaerobes	40 and 41
Lecture 23	Spirochetes	45
Lecture 24	Intracellular pathogens: Chlamydia, Rickettsia, etc.	43

The specific unit objectives for clinical bacteriology within clinical microbiology. Upon completion of each unit and the course, the students are expected to meet the following course objectives:

I. Fundamental understanding of laboratory setting:

(1) Quality Control
  (a) Identify and control sources of error from pre-analytical, analytical, post analytical phases of testing.

II. Media, Agar, colony morphology, and organism ID:

1) Describe the following terms:
   a) Enriched
   b) Differential
   c) Selective
   d) Non-selective
2) Compare and contrast selective vs non-selective media
3) Compare and contrast enriched vs non-enriched media
4) Compare and contrast fastidious vs non-fastidious organism
5) Compare and contrast solid vs broth media
6) Compare and contrast which bacteria grow on which agar
   a) Gram positive organisms
      i) PEA and CNA
   b) Gram negative organisms
      i) MAC and EMB
7) Compare and contrast useful differential bacterial patterns on which agar
   a) Hemolysis patterns on SBA
   b) Lactose fermentation on MAC and EMB
   c) H_2S production on XLD and HE plates
8) Compare and contrast when to use and what will grow in broths:
   a) GN broth
   b) Chopped meat
   c) Thioglycolate
   d) Selenite
9) Compare and contrast when to use and what these biochemicals are useful for bacterial ID:
   a) Catalase
   b) Coagulase / Staphyloslide
   c) Indole
   d) Oxidase
   e) MR/VP
   f) Citrate
   g) PYR
   h) LAP
   i) H_2S
   j) Urease
   k) ONPG
   l) Amino acid metabolism
   m) Nitrate reduction
   n) Carbohydrate fermentation and oxidation

i) TSI
ii) LIA

o) Rapid testing and APIs
p) Automated methods of bacterial ID

III. Gram Positive Cocci:

1) Differentiate Staphylococci and Streptococcus species based on their catalase reaction.
2) Explain the slide coagulase and tube coagulase tests and interpret their reactions for Staphylococcus species.
3) Describe the difference between hospital acquired MRSA and community acquired MRSA.
4) Identify the test that detects the mecA gene and how it can provide a presumptive identification of oxacillin resistance.
5) Explain the formation of biofilms in regard to infections with coagulase negative Staph.
6) Differentiate Staphylococci and Micrococci, in regards to, Lysostaphin and their susceptibility to bacitracin and furazolidone.
7) Explain the importance of Staphylococcus saprophyticus and how it is distinguished from other Coagulase Negative Staph.
8) Explain the difference between Enterococcus faecium, Enterococcus casseliflavus, and Enterococcus gallinarum; including but not limited to colony morphology, motility, and vancomycin resistance.
9) Recognize the reactions of LAP, Esculin, and PYR for all Streptococci species.
10) Predict specific infections caused by Gram positive cocci given their microscopic and morphologic appearance for the most commonly isolated organisms; including but not limited to Staph aureus, Group A Strep, Group B Strep, Strep Pneumoniae, and Enterococci.
11) Explain the term “inducible resistance” in Staphylococcus and determine the method used to test for this.
12) Explain the importance of screening maternity patients for Group B Strep and describe the 2 clinical patterns.
13) Determine the colony morphology, optochin result, and microscopic appearance of Strep. pneumoniae.
14) Evaluate the significance of finding the following organisms in a blood culture: Strep bovis, Abiotrophia, Staph aureus and Coagulase Negative Staph.
15) Devise a mean of using a vancomycin susceptibility disk for differentiating gram positive organisms from gram negative organisms and interpret expected results.
16) Define the hippurate hydrolysis and CAMP tests and explain why these are key tests in the identity of beta-hemolytic Streptococci.
17) Interpret the beta-hemolytic Streptococci when given specific bacitracin susceptibility results.
18) Identify the organism based on its ability to grow at bile esculin, 6.5% sodium chloride.

19) Name the Lancefield group to which a particular species of Streptococci belong.
20) Explain the phenomenon of nutritionally variant streptococci and determine their growth requirements.
21) Propose an explanation for a blood culture that does not grow on plates despite detection of an organism on the gram stain.
22) Assess the importance (pathogen vs. normal flora) of the various Gram Positive Cocci discussed based on the body site in which it is found.
23) Explain the colony morphology and microscopic appearance of Rhodococcus equi.
24) Explain the susceptibility pattern of a VRE and dictate why isolation is important.
25) Predict the susceptibility of Vancomycin in most Streptococci species.
26) Explain the colony morphology, microscopic appearance, and susceptibility pattern of Pediococcus.
27) Predict the tests needed to differentiate Aerococcus urinae, Leuconostoc, and Viridans Streptococcus.

IV. Gram Positive Rods:

1) Describe the natural habitats for Nocardia and Streptomyces and their transmission to man.
2) Evaluate the microscopic appearance (Gram stain and acid fast stains), macroscopic (colony) appearance, and other characteristics of Nocardia and Streptomyces for similarities and differences
3) Name the three most commonly isolated species of Nocardia.
4) Explain the use of casein, tyrosine, and xanthine agars for identification of Nocardia and Streptomyces and use the reactions obtained with these agars to aid in differentiating among the species.
5) Compare Nocardia and Streptomyces in regard to pathogenicity and biochemical characteristics.
6) Explain the relationship of the cell wall component mycolic acid and its relationship to the acid fast property.
7) Compare and contrast GPR by:
   a) Aerobic vs anaerobic growth conditions
   b) Biochemicals
8) Compare and contrast:
   a) Nocardia
   b) Actinomyces
   c) Bacillus
   d) Corynebacterium
   e) Clostridium

V. Enterobacteriaceae, Vibrio, Aeromonas, Helicobacter, Campylobacter, and Bacterial Agents of Diarrhea

1) Describe the characteristics which all of the Enterobacteriaceae have in common.
2) Predict the motility of a given member of the Enterobacteriaceae.
3) List the 3 types of antigens found on Enterobacteriaceae and describe whether they are heat labile or heat stable.

4) Judge the significance of a given member of the Enterobacteriaceae as it relates to its ability to cause disease vs. its presence as normal flora.
5) Differentiate the various genera and species of select Enterobacteriaceae when given the results of the following biochemical reactions: VP, Citrate, Indole, Phenylalanine, Urea, H_2S, Gelatin, Arginine, Lysine, and Ornithine
6) Predict the appearance of key Enterobacteriaceae on MacConkey, HE, and XLD agars.
7) Explain the microscopic morphology of Yersinia and describe the transmission of *Yersinia pestis* to humans.
8) Describe the disease caused by *Yersinia enterocolitica* and the reason that it can be implicated in contaminated blood units.
9) Predict the motility of *Yersinia enterocolitica* at a given temperature.
10) Describe the microscopic morphology of Vibrio and the diseases caused by various Vibrio species.
11) Describe the types of infections caused by Aeromonas and how it is transmitted to humans.
12) List two ways in which enteric pathogens cause diarrheal disease and how the two ways can be differentiated by clinical symptoms and findings.
13) Have a basic understanding of Salmonella nomenclature (traditional vs. recent).
14) Describe how people become infected with Salmonella and Shigella.
15) Compare Salmonella and Shigella regarding the number of organisms required for infection.
16) Resolve a discrepancy in which an isolate of Salmonella might not type with O antisera.
17) List the species names and O antigens for the four serogroups of Shigella.
18) Associate classic gastroenteritis terms and descriptions (rice water stool, dysentery, etc.) with the organisms that cause them.
19) Be familiar with the genus and species of bacteria that can cause diarrheal illness, how they are transmitted, and the disease state they produce.
20) List the specific serogroup of *E. coli* which can be a cause of hemorrhagic diarrhea and can lead to hemolytic uremic syndrome. Describe the way in which this organism is acquired and the specific reaction used to screen for this organism.
21) Describe the various strains of *E. coli* that can be involved in gastroenteritis.
22) Describe the effect that antibiotic treatment has on Salmonella, enterohemorrhagic *E. coli*, and *S. aureus* induced gastroenteritis.
23) Describe the growth requirements of Campylobacter.
24) Explain the role of antibiotic usage in the diarrheal illness caused by *Clostridium difficile* and the difficulties this organism presents in the hospital environment.
25) Describe the disease process and symptoms caused by *Clostridium botulinum* intoxication.
26) Predict the most probable identification of an enteric pathogen given a hypothetical case study that includes clinical presentation, appearance of colonies isolated, and/or biochemical reactions.

27) Recommend specific media, temperature, and atmospheric requirements to isolate pathogens from stool.

VI. Gram Negative Cocci:

1) List the 2 primary species of Neisseria which cause infection in humans.
2) Explain the type of eye infections caused by Neisseria gonorrhoeae in newborns and describe the way in which these infections are prevented.
3) Judge the adequacy of a specimen submitted for culture of Neisseria gonorrhoeae to include the transport and environmental conditions.
4) Describe the microscopic morphology of Neisseria species.
5) Distinguish the various species of Neisseria in regard to their growth on Martin Lewis agar and reactions against glucose, maltose, lactose, sucrose, and ONPG.
6) Describe the use of the term PPNG.
7) Evaluate morphological characteristics and biochemical tests necessary to identify the gram negative cocci.
8) Name the disease with a high mortality rate caused when Neisseria meningitidis becomes fulminant and spreads rapidly throughout the body.
9) Compare Neisseria cinnerea and Neisseria gonorrhoeae in regard to biochemical reactions.
10) Explain the microscopic morphology of Moraxella catarrhalis and list the main reactions used to identify this organism.
11) Describe the relationship of gram negative cocci to normal flora in the body and their reactions.
12) Explain how the diagnosis of Neisseria gonorrhoeae differs in males and females.

VII. Fastidious Gram Negative Rods:

1) Recommend the appropriate growth requirements including media, atmosphere and/or temperature required of the various fastidious gram negative bacilli discussed.
2) Describe the microscopic morphology of Haemophilus, including H. ducreyi.
3) Explain the phenomenon of satellitism.
4) Name and describe the disease processes caused by Bordetella pertussis, Francisella tularensis and Haemophilus ducreyi.
5) Evaluate a possible case of bacterial vaginosis given findings of cell and bacterial types found in a vaginal smear.
6) Recommend 2 methods of detecting infections with Bordetella pertussis and the media used to isolate this organism.
7) List the names of the 2 diseases caused by Legionella pneumophila.
8) Describe the source of infection by Legionella.
9) Name the substance which is required for growth of Legionella and the agar used to isolate this organism.
10) Name the 3 methods, often ordered as a triad, that are used to diagnosis infections with Legionella.

11) Differentiate the various species of Haemophilus based on their X and V requirements, explain the methods used to determine their requirements, and list the substances considered X and V factors.
12) Propose an identification of a Haemophilus species given a reaction of the porphyrin test.
13) Compare and contrast the various species of Brucella in regard to their animal host and CO_2 requirements.
14) Judge the adequacy of safety procedures when working with possible isolates of Brucella and Franciscella.
15) Describe the method of transmission of Pasteurella multocida to humans and list some of the main characteristics of this organism.
16) Describe the relationship of the fastidious gram negative bacilli to normal flora.
17) Propose a possible identification of a fastidious gram negative bacilli when given a disease state scenario and indicate its most common environmental source.
18) Describe the unique morphology (colony and microscopic) of the fastidious gram negative bacilli.
19) Select the most probable organism when given a description of the colony, oxidase reaction, site of isolation, presence as normal flora, and microscopic morphology to include Actinobacillus, Achromobacter, Capnocytophaga, Cardiobacterium and
20) Name the organism that causes rat-bite fever and Haverhill fever and describe its microscopic appearance.
21) List the organism which causes granuloma inguinale.

VIII. ***Non-Enterobacteriaceae GNR [GLUCOSE OXIDIZERS]***

1) Contrast the differences in formulation of TSI agar with OF medium for the detection of glucose fermentation.
2) Analyze 3 biochemical reactions that distinguish the glucose nonfermenting organisms from the Enterobacteriaceae.
3) State the most commonly isolated non-fermenting gram negative rod.
4) Select the most probable gram negative, glucose nonfermenting bacilli when given a specific type of infection
5) Describe the fermentative/oxidative capabilities of Pseudomonas aeruginosa.
6) Separate Pseudomonas aeruginosa from Pseudomonas putida and Pseudomonas fluorescens using two key biochemical tests.
7) Distinguish between the key characteristics of Stenotrophomonas maltophilia and Burkholderia cepacia.
8) Discuss the pigments produced by Pseudomonas aeruginosa.
9) Analyze key characteristics to differentiate between the types of disease processes caused by Burkholderia mallei and Burkholderia pseudomallei.
10) Compare the gram reaction, morphology, oxidase, catalase, and motility reactions for the Acinetobacters versus the Enterobacteriaceae..
11) Analyze the biochemical tests most useful in identifying Pseudomonas aeruginosa.
12) State the significance in knowing a gram negative rod has resistance to the compounds polymyxin B/colistin.

13) Evaluate the results of a set of biochemical reactions and suggest the most probable organism.

IX. Mycobacteria

1) Differentiate the Mycobacteria from other bacteria based on one main characteristic.
2) Categorize a particular isolate into one of the 4 commonly recognized groups of Mycobacteria, according to its pigmentation and growth characteristics.
3) Explain the disease states caused by the most common Mycobacteria.
4) Explain the reason for a special digestion/decontamination and concentration procedure of specimens for culture of Mycobacteria.
5) List a molecular method used commonly to identify some of the most commonly isolated Mycobacteria.
6) Recommend 2 conventional tests to identify Mycobacterium tuberculosis.
7) Explain the person to person transmission of Mycobacterium tuberculosis.
8) Describe the 2 most common stains used to detect acid fast bacilli and compare their sensitivity.
9) Describe the type of patients in which Mycobacterium avium complex is usually isolated.
10) Compare and contrast the types of media used to isolate Mycobacteria to include their advantages and disadvantages.
11) Given a hypothetical case study, evaluate the site of isolation and results of a set of biochemical reactions and suggest the most probable Mycobacteria.
12) Describe the environmental source of the various species of Mycobacteria.
13) When given various pigment and temperature reactions of an isolate, categorize the isolate by its chromogenic pattern and propose a possible species.

X. Anaerobes

1) List the clinical symptoms indicating an anaerobic infection and list predisposing factors leading to anaerobic infections.
2) Evaluate a given specimen type and its transport to the laboratory for its suitability for anaerobic culture.
3) Analyze the effects oxygen has on anaerobic organisms and explain what the effects might be if a proper atmosphere is not attained and maintained.
4) List the components of a proper anaerobic atmosphere.
5) Categorize the given types of anaerobic media, as to whether they are used as non-selective or selective agars, and mention what organisms would be inhibited or recovered to include: BBE agar, egg yolk agar, CCFA, LKV agar, and CDC ANA.
6) Compile a short list of the most common anaerobes according to body site.
7) Compare/contrast the advantages and disadvantages of the various types of holding and incubation methods to include Gas-pack jars, Anoxomat, anaerobic biobags and an anaerobic glove box.
8) Name the two basic components necessary in all anaerobic media for expected recovery of all clinically significant anaerobes.

9) List the 3 special potency antimicrobial disks used for preliminary identification and discuss their use.
10) Explain the Nagler test and lipase/lecithinase reactions and explain their use.
11) Prepare a short list of clues that suggest a patient has an anaerobic infection, to include the relationship and evidence of infection.
12) List the methods which can be used for antimicrobial susceptibility testing on anaerobes.
13) Evaluate the types of infections produced along with any distinctive microscopic and colonial morphology for the most commonly isolated anaerobic organisms, including but not limited to: Actinomyces, Bacteroides fragilis, Bifidobacterium, Peptostreptococcus, Clostridium perfringens, Clostridium tetani, Fusobacterium nucleatum, Clostridium difficile, Clostridium septicum, Bilophila, Clostridium botulinum, Prevotella, Porphyromonas, Eubacterium, and Veillonella.
14) Discuss basic anaerobic bacterial characteristics to include smear evaluation, gram results by genus, and colony morphology.
15) List the members of the Bacteroides fragilis group
16) Define the terms exogenous and endogenous anaerobic infections.
17) Compare and contrast obligate anaerobe, faculatative anaerobe, aerotolerant anerobe, microaerophile, capnophile as to levels of pertinent gases, such as oxygen and carbon dioxide.

XI. Antimicrobial susceptibilities:

1) Support the need for doing or not doing susceptibilities on an organism when given a hypothetical situation.
2) Explain the ways organisms become resistant to antibiotics.
3) Compare the site of action of the major classes of antibiotics to include the beta lactams, fluoroquinolones, macrolides and aminoglycosides.
4) List at least 2 antibiotics generally used to treat urinary tract infections.
5) Categorize a particular antibiotic as a Penicillin derivative, cephalosporin derivative, aminoglycoside, macrolide, or quinolone.
6) Describe the use of beta-lactam inhibitors and list at least two currently used beta-lactam inhibitors.
7) Given a hypothetical patient's clinical presentation and infective agent, suggest a possible antibiotic to be used.
8) Explain the clinical use of the Serum Bactericidal Test (aka: Schlichter test).
9) Compare and contrast the most common methods of susceptibility testing to include disk diffusion, microdilution, E test, and automated susceptibilities in regard to turn-around-time, advantages, and disadvantages.
10) Explain the MBC test and its clinical usefulness.
11) Support the need to not perform susceptibility testing on respiratory isolates of Haemophilus which are beta lactamase negative.
12) Support the need to do gentamicin and streptomycin synergy testing for isolates of Enterococcus from blood cultures.

13) Correlate the results of a hypothetical oxacillin agar screen, PBP2 results, and cefoxitin testing.
14) Explain the use and importance of the vancomycin agar screen for Staphylococci.
15) Support the testing of Staphylococci against cefoxitin rather than testing against oxacillin.
16) Describe the way in which organisms become resistant to antibiotics.
17) Explain the terms indifferent, additive, synergistic and antagonistic.
18) Propose an antibiotic which might be used to treat infections with organisms that produce an ESBL or KPC resistance mechanism.

XII. ***Spirochetes***

1) Compare and contrast the microscopic morphology of the genuses Treponema, Borrelia, Leptospira, and Spirillum.
2) Name the diseases caused by the following: T. pallidum biotype pallidum, T. pallidum biotype pertenue, T. pallidum biotype carateum, and T. pallidum biotype endemicus.
3) Discuss the clinical manifestations of syphilis.
4) Explain the tests used to diagnose syphilis to include screening and subsequent confirmation.
5) Describe the role of Borrelia in tickborne & relapsing fevers and explain why fevers come and go during the course of the disease.
6) List the cause of Lyme disease and describe how it is transmitted to man.
7) Evaluate the results of an ELISA test and a Lyme Western Blot test for the probability that the patient has active Lyme disease.
8) List the cause of Leptospirosis and Weil Disease and describe how it is transmitted to humans.
9) Describe the disease caused by *Spirillum minus* and the mode of transmission from reservoir to human.

XIII. Mycoplasma and Intracellular bacterial pathogens:

1) Compare and contrast the Mycoplasma, Chlamydia, and Rickettsia in regard to whether they are free-living or require cell culture and whether they contain DNA, RNA or both.
2) Describe the characteristic of Mycoplasma that distinguishes them from other bacteria and makes them resistant to cell-wall antibiotics.
3) Describe the colony appearance of Mycoplasma and problems in detecting this organism on gram stain.
4) Name the most common species of Mycoplasma and describe the associated disease state.
5) Identify the genus and species of all Mycoplasmataceae that are associated with urogenital tract infections.
6) Sketch the growth cycle of Chlamydia including elementary bodies and reticulate bodies.
7) Predict the most probable infecting organism (Chlamydia trachomatis, Chlamydia psittaci, or Chlamydia pneumoniae) when given a description of a disease process.

8) Recommend the best method to detect Chlamydia trachomatis based on the body site and the reason for testing.
9) Identify reservoirs, vectors, and diseases associated with the following: R. rickettsia, R. typhi, R. prowazekii, O. tsutsugamushi, and R. akari.
10) Identify reservoirs, vectors, and diseases associated with the following: E. chaffeensis and A. phagocytophilia.
11) Explain the detection of Ehrlichia in Wright stained smears.
12) Identify reservoirs, vectors, and diseases associated with the following: B. henselae, B. quintana, B. bacillaformis, and A. felis.
13) Recommend the best method to isolate Bartonella from blood cultures.
14) List the cause of Q fever and describe its pathogenicity and transmission to humans