HW #4 Culture and SensitivityName **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

MCB 2010C – Fall 2016 Valencia College – Dr. Gessner

**Identifying Microorganisms in the Medical Micro Lab and Determining Which Antibiotic to Use**

Ideally, when a patient has an infection, before the physician prescribes an antibiotic using a “shot-gun” approach, a culture and sensitivity (C&S) is recommended to identify the etiology (cause) of the infection and to determine the best antibiotic to treat the infection. In our lab, and in this homework assignment, you will:

1. Be given an unknown facultative anaerobic gram negative bacillus to identify as a lab group.
2. Determine what antibiotics would best treat a patient with an *E. coli* infection and a *Staphylococcus aureus* infection.
3. Learn about rapid multi-test systems used to identify microbes in today’s medical microbiology labs.
4. Learn about automated computerized systems used to perform the entire C & S procedure

**BACKGROUND**

Methods of Collecting Specimens from Infected Sites of the Human Body:

Specimens are typically obtained with some type of **collection apparatus**\* (swab, syringe, etc.) using **aseptic protocol**; they are typically **stored in a transport medium** (to preserve the specimens)and are **often refrigerated** until delivered to the lab so that no changes can occur in the sample before it gets to the lab.

* **Throat cultures**: Swab the pharyngeal area around the tonsils, not the roof of the mouth
* **Blood cultures**: Blood is normally sterile and should not have a typical flora
* **CSF cultures**: spinal tap (lumbar puncture) between 3rd and 4th lumbar vertebrae
* **Urine cultures**: catheterization or clean catch midstream (antiseptic solution and water or antiseptic towelette used to clean the urethral opening)
* **Urethral cultures**: avoid urination 4 hours prior to culturing to test for gonorrhea and Chlamydia, use swab or applicator stick
* **Vaginal and cervical cultures**: use swab or applicator stick
* **Tissue fluids**: sterile needle aspiration
* **Saliva**: more commonly taken for dental diagnosis, patient expectorates into a container
* **Sputum**: obtained by coughing or catheterization (need to avoid contamination with saliva)
* **Skin**: swabbed or scrapings taken using a scalpel
* **Feces** can also be cultured

Because many signs and symptoms of disease can be due to multiple infectious

 etiologies, we use the following steps to scientifically identify the

 name of the organism causing each patient’s infection: inoculation, incubation, isolation, inspection and identification (to the genus and species name).

 A. Inoculation

 - The specimen is placed in or on a sterile medium to allow bacterial

 growth

 - The sample of cells you take from the collection device\* and put on

 media to culture (grow) them is called an *inoculum*

 - ISOLATION STREAK PLATING: the objective is to obtain a *pure*

 *culture* (only one single species isolated in or on growth media)

separating your normal flora from the suspected pathogen

 - mixed cultures contain 2 or more known bacteria

 - contaminated cultures contain some unknown bacteria

 in the culture

1. Incubation

 - Individual bacteria are too small to be seen with the naked eye, so they

 are placed in incubators to promote binary fission.

 - Incubators are set to the optimum temperature and contain the

 appropriate atmospheric gases for bacterial growth to occur

1. Isolation

 - Each bacterium placed on growth media reproduces by binary fission so that eventually (usually within 24-48 hours) you can see a massive pile of cloned bacteria, which you are able to be see with the naked eye = *a colony*

 - **Obtaining isolated colonies IS THE GOAL** in identifying the etiologies

 of infectious disease and identifying the best antibiotic to use

 - Theoretically, it is assumed that each isolated bacterial colony arose from

 one bacterial cell by binary fission

 - The type of media used here can help later in identifying various species

 of bacteria (selective, differential and enriched media)

 - Isolated colonies are then also used to identify the best antibiotic

 to treat the patient using the Kirby Bauer technique or tube dilution

1. Inspection

 - Macroscopic inspection of the colonies (pigments and colony

 characteristics)

 - Microscopic examination of slides made of stained smears from colonies can give important information to the physician such as Gram stain reaction, size, shape and arrangement of cells. Further stains may allow more information about the cells to be obtained:

 acid fast, spores, flagella (motility), capsules

E. Identification

- Various biochemical tests (examples: API 20, Enterotube) are run on isolated bacteria so that identification to the genus and species name is possible. (Bergey’s Manual of Systematic Bacteriology as the benchmark used in most bacterial identification schemes); in modern medical microbiology labs, a C&S can be done by various methods as you will discover through the following homework questions.

 - Once completed, used cultures are disposed of appropriately in biohazard

 containers and are autoclaved or incinerated to sterilize all equipment contaminated with the pathogenic microorganism.

1. Just as you did in our lab, you are given a pure culture from which a gram stain produces gram positive cocci. Both a catalase test and a coagulase test performed on this unknown microbe test positive. **Name the genus and species of the isolated bacterium** (remember to write it as a scientific name, like your scientific name: Homo sapiens).
2. Using the following link, answer the questions about culturing microbes from a wound:

<http://www.youtube.com/watch?v=53I0H5HlbAA>

* 1. After assessing the wound, what do you use to irrigate the wound?
	2. Should you use the same gloves that you used to assess and clean the wound, when taking a culture of the wound?

1. View the following link and answer the following questions.

[*http://www.youtube.com/watch?v=Hl6vItGt4qQ*](http://www.youtube.com/watch?v=Hl6vItGt4qQ)

After a five minute incubation period, the Quick Vue In Line ® Strep A test can be read.

1. A positive test result is seen when a blue line appears next to the letter \_\_\_\_\_ and a pink line appears next to the letter \_\_\_\_\_.
2. This rapid diagnostic test is looking to see if a patient is infected with group A strep; how is the sample obtained to perform this test?
3. When looking at microbiological tests to determine whether a patient does or does not have an infectious disease, **test specificity** and **test sensitivity** are important factors to look at when determining the usefulness of each test. Watch this YouTube video about test sensitivity and specificity to answer the following questions.

 <http://www.youtube.com/watch?v=ICKaSKoDPmI>

* 1. Do diagnostic tests which have a tendency to miss patients, who actually have a disease tested for, have many false positive or false negative results?
	2. Do tests that detect many false positive results (people testing positive for a disease, although they do not have the disease) have low specificity or low sensitivity?
1. Watch the video about the OraQuick® Rapid screening test for HIV 1 and 2 and answer the questions:

 <http://www.youtube.com/watch?v=5FBWORY91J4>

* 1. How long does it take to get results from the OraQuick® Rapid HIV Test?

* 1. What does it mean if no reddish-purple line appears next to the triangle labeled C?
1. Watch the YouTube video about the Enterotube system used to identify the etiology of a patient’s infection:

 [http://www.youtube.com/watch?v=wWj5LvLSHD8&list=PLnc\_S0Gfnp6f\_K- lgT0vTtFJCFScM8tNa](http://www.youtube.com/watch?v=wWj5LvLSHD8&list=PLnc_S0Gfnp6f_K-%09lgT0vTtFJCFScM8tNa)

* 1. The Enterotube system is a miniaturized multitest rapid identification test to identify enteric bacteria. What are enteric bacteria?
	2. What is the name of the reagent needed to read the indole test of the Enterotube? (Do not write Indole reagent!☺)
1. There are various ways of testing pathogens for antibiotic sensitivity. In your lab, you performed the Kirby Bauer agar diffusion method, but there are other techniques that can be used as well. Watch the following YouTube video to learn about using the Etest system for antibiotic susceptibility:

 <http://www.youtube.com/watch?v=K0nrFj8lFfA>

* 1. Both the Kirby Bauer and the Etest system use the same media to grow the pathogenic microbe. What is the name of that media?
1. In large advanced microbiology laboratories, automated computerized systems are used to determine the C & S of a patient’s infection. Watch the following link to answer the following questions.

<http://www.youtube.com/watch?v=lz486jTHQ1E&list=UU71xcbm_hG6nvaS3N9_FeJQ&index=8&feature=plcp>

* 1. How long does it take to find the C & S of a gram negative organism, using the MicroScan Walkaway 96Plus®?
	2. What is the maximum number of patient panels that can be analyzed simultaneously, using the MicroScan® Walkaway 96Plus® system?

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**Diagnosing diseases other than bacteria**

9. Watch the following video to watch an innovative way to diagnose malaria, a protozoal disease.

[*https://www.bing.com/videos/search?q=diagnosing+malaria&&view=detail&mid=8E9FDA99535F5C07C3BD8E9FDA99535F5C07C3BD&FORM=VRDGAR*](https://www.bing.com/videos/search?q=diagnosing+malaria&&view=detail&mid=8E9FDA99535F5C07C3BD8E9FDA99535F5C07C3BD&FORM=VRDGAR)

a. What body fluid is needed to diagnose malaria?

b. In the video, we see that a smartphone app has been developed which can recognize the malaria protozoan in patients with the disease. What power of magnification does the smartphone app create to allow the diagnosis of malaria?

**TREATING INFECTIOUS DISEASES**

Read Chapter 12 in your textbook to answer the following questions about treating infectious diseases:

10. Antibiotics generally work by targeting different cell parts or molecules found in bacterial cells. For each of the following antibiotics give the bacterial cell target:

 a. Penicillin:

 b. Tetracycline:

 c. Vancomycin:

 d. Erythromycin:

 e. Sulfasoxazole:

 f. Ciprofloxacin:

 g. Polymixin B:

 h. Streptomycin:

11. T F (Circle one) Many anti-fungal medications can harm human tissues since both fungi and humans contain eukaryotic cells.

12. Name three protozoal infections treatable with Flagyl (metronidazole).

13. Watch the following video about treating patients with the flu:

<https://www.bing.com/videos/search?q=you+tube+antiviral+medications&&view=detail&mid=F51392AEF1FC965F72CDF51392AEF1FC965F72CD&FORM=VRDGAR>

a. Name four (4) high-risk groups of people who need to be treated when they get infected by the flu virus.

b. When do you need to start treatment for someone who has been exposed to a person who has the flu?

c. How long does it take for the flu vaccine to provide good protection from the flu?

14. Watch the following video and answer the questions about HIV treatment:

<https://www.bing.com/videos/search?q=you+tube+treating+hiv&&view=detail&mid=585C4CF595350E813885585C4CF595350E813885&FORM=VRDGAR>

 HIV viruses are called retroviruses because they contain RNA which has to be converted back to DNA to enable the virus to reproduce. HIV viruses work to take over your CD4 T lymphocyte cells, which are a part of your immune system. ARV’s, anti-retrovirus medications exist to help treat patients with an HIV infection, but they are not a cure.

a. If ARV’s don’t cure people with HIV, why are they still important to give to HIV patients?

 b. There are 6 basic groups of ARV’s. Why do we need to give medications from 2-3 different ARV groups in a “cocktail” instead of just one when treating the disease?

 GRADE = \_\_\_\_\_\_\_\_/25