REVIEW: Bio 139 Lab Practical #1
All labs from beginning of the semester are included on this exam.

Use Lab Review questions #1-77 (except #30-31),
review sheet for Lab Quiz #1 **plus** this

General guidelines for preparation: Especially regarding the many biochemical tests, focus your effort on understanding each test, and how it relates to other tests. You should be able to summarize what type of metabolism or biochemical reaction is tested, what the significance of the test result is, know any important products or special conditions provided. If the results of one test allow you to predict the results of another test, know this. Know how each test is read (some are simply + or -, but others are more complex). Remember that very often, fermentations produce acid which is detected by pH indicators that turn yellow at low pH (this is NOT ALWAYS true, but it will help). Aerobic metabolism, and amino acid catabolism, raise pH. **For every biochemical test you will be given an uninoculated control medium.** This means you won't need to memorize every color change; for many tests, identifying the positive result is obvious if you compare the media to what it looked like before. You will **not** be asked to name any of the detection reagents added to biochemical tests after growth (i.e., Kovac's, ferric chloride, alpha naphthol, etc.)

KOH test: + KOH = viscosity/slime produced = Gram – bacteria because the cells lyse and spill DNA;
--KOH = Gram + bacteria (thick cell wall keeps DNA inside)

Spectrophotometry: To use spectrophotometer: insert sterile media blank and set absorbance to 0 (transmittance to 100%). Read absorbance of sample (see scale in lab book; absorbance is on the bottom, reads "backward" to nearest 0.01). **NO** growth curve calculations, but be able to calculate serial dilutions & CFU. Remember that colony counts are only valid between about 30 & 300 per plate (fewer: statistically inaccurate; higher: too many to count TMTC). With pour plate method, only a few colonies actually grow on the agar surface; the others are embedded and appear much smaller. Spectrophotometry reads *all* cells, living, sick, or dead; pour plate method (serial dilution & colony count) measures only viable bacteria.

Lysozyme: antibacterial enzyme. Mechanism of action: hydrolysis of glycosidic bond between NAM & NAG of peptidoglycan. Gram + bacteria are more sensitive, especially *Micrococcus* species (which form **yellow** colonies). Natural sources: egg white, tears, nasal secretions. Lysozyme in egg white inhibits the growth of Micrococcus out to a significant dilution.

BCP-sugar broths. Type of metabolism tested: carbohydrate fermentation. What is actually detected? pH and H2 gas. BCP = pH indicator (brom cresol purple); **yellow** at acid pH, purple at neutral. **One** sugar included in each broth (glucose, lactose, or sucrose). If the bacteria can ferment it, acid products of fermentation are produced & media turns yellow. If **hydrogen** gas is produced as well, it will be trapped in the Durham tube. CO2 gas is water soluble and does not collect in a Durham tube (see hot loop test). Glucose is the prototype food source: all fermentative bacteria will use glucose. Sucrose & lactose are disaccharides, must be cleaved into monosaccharides. Not all bacteria, and not all fermenters, can do this, so glucose fermenters may or may not ferment sucrose/lactose. **Obligate aerobes cannot ferment.** (they absolutely require oxygen; they only produce energy by aerobic respiration; remember fermentation does not require oxygen.) **Indifferent/aerotolerant bacteria *only* ferment.** They do not use oxygen even when it is available, because they do not respire aerobically, they only do (glycolysis followed by) fermentation.

Triple Sugar Iron (TSI): Type of metabolism tested: carbohydrate fermentation. What is actually detected? pH, gas, sulfide. Agar slant. Aerobic surface, (relatively) anaerobic butt. Tests for sugar

fermentation; gas production; H2S production (black precipitate). Glucose, sucrose, lactose ALL present; see lecture notes re: why 10X more sucrose & lactose (to drive fermentation even on the slant surface). pH indicator: yellow = acid; red/brick red = alkaline. Carbohydrate fermentation produces acids; aerobic respiration raises the pH. Results expressed as slant/butt A = acid; K = alkaline. A/A = ferments glucose, AND either sucrose or lactose or both. K/A = ferments glucose only. K/K = no fermentation. Gas? It produces cracks in the agar, or blows it up toward the lid. Hydrogen sulfide? Black in middle of agar.

Catalase. *Directly* tests for activity of the enzyme catalase, which breaks down H2O2 (hydrogen peroxide) into water & oxygen. Drop H2O2 onto cells, look for bubbles. What are the bubbles? (oxygen gas). Catalase + bacteria can make iron porphyrin groups (a kind of prosthetic group also found in cytochromes). Catalase + test *indirectly* indicates bacteria have cytochromes = have electron transport chain = respire aerobically. **Catalase – bacteria only ferment (indifferent/aerotolerant). Oxidase test:** directly tests for a *specific* cytochrome, cytochrome c. Some catalase positive bacteria (esp. *Pseudomonas)* will be oxidase +, most will be oxidase – (have cytochromes but not c). **Cannot be oxidase +, catalase --.** To do the oxidase test, scrape a colony into oxidase reagent (soaked in filter paper). Look for a color change to dark red = +

IMViC: Important for distinguishing genera of the **Enterobacteriaceae** family. **Indole:** directly tests for indole. *Indirectly* indicates tryptophan metabolism (into indole & pyruvate), or tryptophanase activity. Add (Kovac's) reagent directly to the broth; bright red/pink ring at surface = + test (indole present). **Methyl red (MR)**: directly tests for pH change. pH indicator is methyl red, red at very acid pH = + test. Type of metabolism tested for: mixed acid fermentation of carbohydrate. Mixed acid fermentation is a pathway that produces stable acid end products which accumulate enough to overcome the **large amount of buffer** present in this media (stable acids much stronger than that required to affect BCP-glucose). Can be BCP-glucose + and MR – but not other way around (BCPglucose indicates fermentation; MR indicates a specific fermentation pathway). **Voges-Proskauer (VP):** directly tests for alcohols such as acetoin & butanediol (note: pH **neutral)**. Type of metabolism tested for: butanediol (alcohol) fermentation of carbohydrate. **MR & VP can't BOTH be positive;** they are different fermentation pathways, the bacteria will only be using one. **Citrate test:** directly tests for pH change. Metabolism tested for: utilization of citrate as sole carbon & energy source (no carbohydrate present), activity of enzyme citrase. Products of citrate metabolism are alkaline, media turns blue.

Urea agar: type of metabolism tested for: amino acid catabolism, breakdown of urea. Directly tests for pH increase due to ammonia production. Urease breaks down urea into NH3 & CO2. + test: hot pink.

Lysine Iron Agar (LIA): type of metabolism tested for: lysine (an amino acid) utilization, by either decarboxylation (anaerobic, butt; produces CO2) or deamination (aerobic, slant; produces NH3). Either type of lysine catabolism increases the pH, turning the slant brick red, or the butt purple/dark purple. Important: the media also contains glucose. If the bacteria ferment, they will acidify the butt and turn it yellow. If the bacteria ferment and ALSO can decarboxylate lysine, the butt will first turn yellow, and then the pH will rise back to neutral as the bacteria turn to lysine as a food source. Ultimately, the butt will appear purple (unchanged), but this indicates + lysine decarboxylation. Test also asks: H2S production?

Ornithine decarboxylase: type of metabolism tested for: ornithine decarboxylation (amino acid catabolism). Directly detects pH change. Alkaline = dark purple/blue. Decarboxylation requires anaerobic environment: cover with mineral oil. Products of ornithine decarboxylation: putrecine & CO2.

Phenylalanine deaminase: type of metabolism: deamination of phenylalanine (amino acid catabolism). Directly detects phenylpyruvic acid (PPA), one of the two products (other is ammonia). Detection reagent turns PPA green +. Yellow is negative.

Hot Loop Test: type of metabolism: heterofermentation (carbohydrate fermentation pathways with more than one final product, in particular, CO2). Directly detects dissolved CO2 gas. Especially useful for identifying catalase – species. CO2 is water soluble, did NOT collect in Durham tubes of BCPsugar tests. Here, tightly closed lid traps CO2 in the media. Hot loop drives gas out of solution, you see foam (+ test).

Litmus milk test: skim milk has carbohydrate (lactose) & protein (casein). Pink = acid = lactose fermentation. Blue = alkaline; with watery top = casein proteolysis (amino acid catabolism for aerobic ATP production). White = colorless reaction / reduction of litmus (bacteria use litmus as terminal electron acceptor). May be pink at surface where oxygen in the air oxidizes the litmus. Solids/ppt = curd formation. {*We'll learn this later in the semester*: Pink with solid chunk: milk protein precipitated due to denaturation from acid production. Soft, flowing curd: specific proteolysis of casein into polypeptides (NOT amino acid catabolism). Liquid is called **whey**.}

Skim milk agar: type of metabolism: exoenzyme production for nutrient acquisition. Exoenzymes act outside of the cell membrane (e.g., in the periplasmic space) allowing bacteria to use large polymers as food sources without transporting those large polymers into the cell. Skim milk agar directly detects casease/caseinase activity (a protease that degrades casein into free amino acids), evidenced by zone of clearing the agar around colony. Hold up to the light to see. **Starch agar:** type of metabolism: exoenzyme production for nutrient acquisition. Directly detects degradation of starch into glucose (evidence of amylase activity). Iodine stains starch dark brown; look for clearing around the "colony" where the iodine does not stain. **Fat agar:** type of metabolism: exoenzyme production for nutrient acquisition. Detects hydrolysis of fat into fatty acids & glycerol, action of lipase. See zone of clearing when held up to light. **Gelatin:** exoenzyme. Degradation of gelatin (protein) into amino acids. What is observed: gelatin liquefies, even when chilled.

Nitrate broth. Type of metabolism tested: ability of bacteria to reduce nitrate to nitrite or N2 gas by using nitrate as a terminal electron acceptor. Directly detects nitrite (turns red after reagents are added); also, Durham tube directly traps nitrogen gas. Can be positive for nitrite but not have N2 gas.

Esculin test. Type of metabolism: a very specific reaction to hydrolyze esculin into glucose + esculetin. Product tested for: esculetin; turns agar slant dark brown.

O-F test: "oxidative-fermentation" or false fermentation. Type of metabolism: partial oxidation of glucose to weak acids (requires oxygen; NOT a fermentation). What is detected: pH change to mildly acidic, seen as a YELLOW color at the surface of the media. Note that true fermenters will turn the whole thing yellow. ***Pseudomonas*** species are O-F positive, but cannot ferment glucose, so would have a negative BCP glucose test.