Microbiology Practical Exam

Day #1 SHORT VERSION

Day #1:

1st swab: Set up Antibiotic Disk Testing on Mueller Hinton Plate (Lab #25)

2nd swab: Set up Nut, Starch, EMB, PEA

Day #2 SHORT VERSION

Day #2:

1. Set up OF-G & TSI tubes (Lab #13)
2. Make Negative Stain (Lab #4) & dry overnight in bin
3. Make Gram Stain (Labs #5 &3) - Leave on slide warmer overnight
4. Read Antibiotic Disks (Lab #25)
5. Read Starch Plate (Lab #13) - DON"T dump iodine in sink. Put in biohazard bag.
6. Read PEA - 24 hour time limit
   - Compare growth to Nut & EMB: Inhibited/Selective? Differential?
7. Read Nut & EMB if time, or reinc
   1. Compare growth to each other & PEA: Inhibited/Selective? Differential?
8. LEAVE ROUGH DRAFT!!

Day #3 SHORT VERSION

Day #3:

1. Read ALL plates & tubes that were set up on Day #1 or 2. If not enough time, refrigerate.
   - Record observations & interpretations.
   - Compare plate growth to each other: Inhibited/Selective? Differential? (Lab #12)
   - Record colony morphology on nutrient (Lab #0)
   - Read TSI & OF-G. (Lab #13)
2. Catalase test with H2O2 (Lab #19)
3. If time, stain gram stain slide (Lab #5)
4. If time, read Negative Stain & Gram Stain
   - SHOW ME AN APPROPRIATE AREA of the slide - not too thick or thin to see shape & arrangement
5. LEAVE ROUGH DRAFT!!

Day # 4 & 5 SHORT VERSION

Day #4 & 5:

- Finish any work from Day #1-3 that has not been completed.
- LEAVE ROUGH DRAFT!!
- ALL PLATES & TUBES & SLIDES should be on the numbered cart

Must: SAFETY: Flames -hair, sleeves, clean tables, hand sanitize

Day 1: Enough needles & loops'

Lids have water droplets in them?? "Shake" the lids out if needed.

Use 1st Swab: for sensitivity testing (needs to dry 5 min before putting disks on)

1. Read Lab #25 for set-up
2. Use MH (or large Nutrient if so instructed) - CHECK FOR CONTAM

Use 2nd swab (only need ONE swab for this part) to:

1. Place **SMALL** INNOCULUM (Just TOUCH unless other specified) on plates in following order: Nut, Starch, EMB, PEA.
2. Then streak/zig-zag with loop (streak for isolation if large plate used)

Gone next day or want to get ahead? Set up next day now? Refrig to read?

Incubation: Put plates in numbered slot in incubator & original unknown tubes in numbered area of the CLASS test tube rack.

Read Ahead to Prepare for tomorrow, 2nd Day of Practical, Be ready to:

1. Set up OF-G
2. Make Neg Stain (Lab #4) & Gram Stain SMEAR (Lab #5 & 3) IF TIME
3. Read plates set up today – be ready!

Day 2

1. Set up the following: Gone yesterday? Work at dif table.
   A. OF-G & TSI (From plate w/best growth; Nut? MH –but not near disks?)
2. Make slides-gram stain & negative smear. Use FROSTED slide & pencil. For **GRAM**:**WAX circle goes on UNDERSIDE. Leave overnight on slide warmer.
3. Read Antibiotic Disk Plate– Read zone sizes. If "2" zones, comment and then measure inner complete inhibition. Plate goes onto cart in "numbered" spot.
4. Special Considerations:
   A. If contaminated/scant growth: ask me. Incubated upside down? Careful.
   B. Need to redo? Ask 1st.
   C. Date & record observations-comment on unusual observations (ie-2 zones inhibition, yellow contaminant not on streak, relatively tiny growth-break through??...) IF think CONTAMINATED H2O, oil, stick, pipet – tell me.
   D. Need more needles, wax pencils? Other supplies on table.
5. Starch – Iodine in beaker. Don't dump I2 into sink – ONLY plate to put into "Bag".
6. Read other plates (PEA, EMB, Nut) that were set up yesterday – NOTE Time Limits!
   A. Record OBSERVATIONS (relative ease of growth -/+/++/etc, color, - to see if any plates appear to be selecting and inhibiting. Selective?? Differential??)
   B. Add BASIC interpretations (GN, A/A, LF, oxidizer, etc. as appropriate)
   C. NOTE: Time frame for reading given – reinc Nut & EMB. Put PEA in cart spot.
   D. ***Mechanisms not needed on rough draft-but could start Final Draft & be done ahead of reading.
7. Place rough draft of lab report in report bin-have date for set up, 24 hour, etc.
8. Put plates that are done incubating on numbered cart – even if haven't set up gram stain yet. **DON'T toss them!!** (Put tubes that are done in the numbered rack in the hood.)
9. Prepare for tomorrow: Suggest reading info for reading tests & making smears

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Day 3, 4, 5

1. Highlights on rough draft-meaning. Missing rough drafts?
2. Early out? Short day? Record observations (SEE procedures) & leave interpretation till tomorrow if needed.
3. CONTAM pipet, stick, oil…? Tell me.
4. Special considerations:
   A. Date & record observations-comment on unusual observations (ie-2 zones inhibition, yellow contaminant not on streak, relatively tiny growth-break through??...)
5. Read tests: **ALL plates that have incubated 48 hours** should be on cart end of period.
   A. Finish Nutrient – form, margin, elevation, size in mm, color. Put in hood.
   B. Finish EMB – compare ease of growth & size to Nutrient. Color? Basic interp? Put in hood.
   C. Finish Starch – Add iodine, read, place plate in hood, NOT cart so doesn't spill.
   D. OF- G: **Compare to unused tubes**. Color & basic interp. Put tubes in rack on cart.
6. Catalase:H2O2 in old petri lid. Use stick; in lysol beaker when done. Observe & Interp.
7. Slides – Stain if time today. (Should have made smears yesterday)
   A. Slide warmers. Frosted ends-PENCIL. Put "sliders" in disinfectant beaker. Gram iodine in **dropper bottle** (not beaker). **Leave iodine on table**, NOT in bin.
   B. Redo until satisfied
   C. **SHOW ME APPROPRIATE AREA OF SLIDE**, – not too thick/thin to tell shape & arrange, in focus for both the gram stain and the negative stain, "WAITING LIST"
   D. When done (or negatives while drying, grams waiting to read), place slides on top of your pile of "done" plates.
8. Rough Draft – LEAVE in bin. **Use phone to take pic of rough draft if you want a copy.**
9. Final Report Specific & detailed interpretations (based on previously recorded observations) & mechanisms – HOW/WHY/CAUSE of ALL TYPES results

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Final Report Info

For each test include:

1. OBSERVATIONS; what you see
2. INTERPRETATIONS; what do the observations tell you about the organism?
   A. GP
   B. Breaks down sucrose
   C. Etc.
3. MECHANISMS;
   A. Since this is required no matter what your results are, mechanisms for the final report can be started before all tests are completed.
   B. Why do cells stain as GP vs. GN? How does each step of the gram stain contribute to the identification of those differences between GP & GN cells?
   C. Why is there a color change if sucrose is used? What is in the media and what does it react with?
   D. Etc.
   E. USE YOUR OWN WORDS-not quote directly from book or manual
   F. Include mechanism discussion even if your organism is “negative”. If neg, tell what pos looks like and vice versa.
4. Unusual results; contradictory with another test, result not clear, etc.