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| <b>Day 1</b>   | Streak for isolation on different media at different aerobic conditions                                   | 1 Blood Agar – CO <sub>2</sub><br>1 TSA – O <sub>2</sub>   | Causative organism must be separated from contaminate for identification. CO <sub>2</sub> enhances growth of <i>Streptococcus</i> . Type of hemolysis can help with isolation.                             | Record CS number & clues                                |
|                | Gram Stain  | Use fresh Gram controls  | Gives tentative idea of the type of organisms present. Helps eliminate possible organisms.   | Record Gram Stain results                               |
|                | Research organisms causing similar symptoms & normal flora of collection site                             | Use text, lab website, library, etc... cite sources  | Narrow down possibilities to eliminate unnecessary tests. Start filling out Diagnosis Information Sheet.   | Record research findings                                |
| <b>Day 2</b>   | Visually examine plates for 2 isolated organisms, check with instructor/IA                                | Distinguish different organisms using colony morphology & hemolysis.   | Colony morphology & hemolysis will help narrow down possibilities.   | Record observations                                     |
|                | If isolation is unsuccessful, show plate to instructor/IA. May need to re-streak.                         | If isolation is unsuccessful, patiently return to Day 1 & re-streak for isolation.                                       | The instructor/IA have more experience observing these organisms; they may see differences that are not obvious.   | Record observations & results                           |
|                | If isolation is successful, streak isolates onto separate plates to obtain pure cultures.                 | Prick the center of a well isolated colony & re-streak on maintenance plates. TSA for staphs & enterics, BHI for streps. | Verification of pure isolation is necessary. Pure culture plates are needed to work from.  | Very carefully keep track of each organism.             |
|                | If isolation is successful, Gram stain the 2 distinct isolates.   | There should be one Gram (-) & one Gram (+) organism.  | Gram staining isolates helps ensure the isolation & cultivation of pure cultures.  | Record observations & results                           |
| <b>Day 3</b>   | If both organisms are separated with pure cultures growing on stock plates, proceed to selective tests.   | Use information from the unknown lab, research, & Gram stains to determine appropriate selective tests to conduct.       | Conducting inappropriate tests represents unclear thinking & unnecessary expenditure of money & personnel time. Tests done incorrectly will give misleading results causing confusion & misidentification. | Construct a blank table of tests to record observations |
|                | If both organisms are not separated with pure cultures growing on stock plates, re-streak until they are. | Streak original broth culture for isolation. Isolate both organisms & re-streak on maintenance plates for pure culture.  | Contaminated cultures not purely isolated will give you at best, ambiguous results & at worst, wrong results.  | Don't get your organisms mixed up!                      |
| <b>Day 4/5</b> | Read test results, repeat tests with ambiguous or conflicting results.                                    | Make sure to follow test procedures exactly.   | Use micro website to look up test procedures & to determine what positive & negative tests look like.  | Record results in test table                            |
| <b>Day 6/7</b> | Complete antibiotic sensitivity testing on causative organism.  | Use MHA plates for staphs & enterics, BHI for streps.  | Identify antibiotics the causative organism is sensitive & resistant to.   | Record & interpret results                              |