Flow chart Aerobic Gram positive organisms

1. Gram stain morphology
   - Rods
   - Cocci

2. Rods
   - Regular rods
   - Irregular, coryneform

3. Irregular, coryneform
   - Beta hemolytic on Day 2
   - Catalase negative
     - Listeria
     - Bacillus
     - Lactobacillus

4. Regular rods
   - Beta hemolytic on Day 2
   - Catalase negative

5. Catalase +
   - Coagulase +
   - Coag negative staphylococcus or Micrococcus
     - Yes
     - No

6. Catalase negative
   - Beta hemolytic
     - Yes
     - No

7. Coag negative staphylococcus or Micrococcus
   - Yes
   - No

8. Coag Neg
   - Staphylococcus aureus
   - See Strep chart

9. Stop here OK

10. Arcanobacterium

11. Corynebacterium

12. S. pneumoniae other viridans strep, or Enterococcus
Flow chart for streptococci on Blood Agar Plate (BAP)

- Gram positive cocci pairs & chains
- Catalase negative

Beta hemolytic

- Regular size colony
- Tiny colony < 0.5 mm

Alpha or non hemolytic

- Regular size colony
- Tiny colony < 0.5 mm

Yes
- Group B Strep or rarely Enterococcus (PYR +)
- Caramel, burnt sugar, or butterscotch smell
- Can have grp A, C, G, or F antigen
- “S. anginosus (“S. milleri”) group

No
- Other beta strep
- PYR +
- Enterococcus

Yes
- S. pyogenes

Grp. B = CAMP + & PYR Neg

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Beta hemolytic Streptococci

Major pathogens: Streptococcus pyogenes (group A) & S. agalactiae (group B)

In abscesses and body fluids also S. anginosus group (“S. milleri”)

Gram + cocci chains

Catalase negative

PYR negative & CAMP +

or

PYR positive

or

Group B positive

S. agalactiae (group B)

Tiny colonies <0.5 mm
Caramel smell
“S. milleri” group

If none of the above:

B-strep not A or B

Group A positive

S. pyogenes (group A)

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**Alpha streptococci vs Streptococcus pneumoniae on sheep blood agar**

Alpha hemolytic (green color) and do not grow on MacConkey; catalase negative

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**Streptococcus pneumoniae:**
- Colonies more mucoid, may have concave centers.
- Dissolve in 10 min with bile (sodium desoxycholate)
- Zone of inhibition around optochin (P) disk

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**Viridans streptococci:**
- Pathogens only in blood cultures.
- Colonies smaller & more dry
- Will not dissolve with bile
- No zone around P disk

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**Optochin Sensitive**

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**Staphylococci**

**Major pathogen:** *Staphylococcus aureus*

**Others:** *S. lugdunensis* in abscesses and serious wounds; coagulase-negative staph in multiple blood cultures

- **Gram + cocci clusters**
- **Catalase positive**

**Slide coagulase**

- **Positive**
  - Typical hemolytic colony
  - *Staphylococcus aureus*

- **Negative**
  - Perform tube coagulase test

**PYR**

- **Positive**
  - Perform tube coagulase test
- **Negative**
  - If PYR and coagulase negative:
    - Coagulate negative staphylococci

**If tube coagulase positive:**

- **Staphylococcus aureus**

**If PYR positive:**

- **Staphylococcus lugdunensis**

**Positive but clumpy**

Colony more white; non-hemolytic on Day 1

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Flow diagram for Gram Neg Rods on BAP & MacConkey (NOT for stool isolates)

- **Lactose + (fermenter) on Mac**
  - Yes
    - **Indole +**
      - No
        - Colony mucoid
          - Odor of fresh bread
            - No
              - Enterobacter, Providencia, Citrobacter, Others
            - Yes
              - **Klebsiella**
                - Rare Enterobacter
      - Yes
        - **Non-mucoid**
          - E. coli
            - Rare Citrobacter or other Enteric GNR
          - **Indole +**
            - Yes
              - **Oxidase +**
                - Colony metallic
                  - Odor of grapes
                    - Yes
                      - B. cepacia, B. pseudomallei, Pasteurella, Others
                    - No
                      - P. aerug
                        - No
                          - **Swarming on BAP**
                        - Yes
                          - **Indole +**
                            - Yes
                              - **Proteus sp.**
                            - No
                              - E. coli
          - No
            - **Oxidase -**
              - **Swarming on BAP**

- No
Flow chart for identification of stool pathogens (enteric fecal pathogens)

Major pathogens: Salmonella, Shigella, Vibrio, Campylobacter, Enteropathogenic E.coli
Other pathogens: Aeromonas

Campy agar after 48 h
- Oxidase +, moist, beige colonies
  - Campylobacter

Lac neg on Mac
- Oxidase Neg
  - Possible pathogenic E. coli
    - Need additional tests

Lac + on Mac
- Oxidase +
  - Green
    - Possible Vibrio parahemolyticus
  - Growth on TCBS
    - None
      - Yellow colonies on HEK
        - Oxidase +
          - Possible Aeromonas
    - Possible pathogenic E. coli
      - Need additional tests

Oxidase Neg
- Swarming on BAP
  - Proteus
- Possible Salmonella, Shigella or Yersinia enterocolitica or pathogenic E. coli
  - Need additional tests

Salm on HEK agar
- Perform serotyping

TSI = S. typhi, Shigella, & Salm
Pseudomonas aeruginosa
Major pathogen in any site

- Rough colonies on BAP
- Non-lactose fermenters on MacConkey
- Usually beta hemolytic
- Grape-like smell
- Oxidase positive

Gram – rods (In blood culture) longer & thinner than enteric rods

Colonies can be metallic

Pigment usually green but can be purple or blue

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**Haemophilus influenzae**

Chocolate agar for isolation; does not grow on BAP without V factor added

- Gram negative rod with coccoid and filamentous forms on Gram stain from plate
- Grows well on chocolate agar
- Smells like mice or mild bleach
- Requires X (hemin) and V (NAD)
- Grows on BAP next to streak of Staph aureus
- Grows on Mueller-Hinton with XV factor disk
- Gram negative coccobacilli in original specimen
- Satellite around various colonies on original BAP culture plates
- X disk only
- V disk only

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