Lab 6 Goals and Objectives:

Exercise 9: Aseptic Technique: Check results
Exercise 10: Pure Culture Technique: Check results
   Red = S. marcescens / Small yellow = M. luteus / Cream = E. coli

Exercise 18: Motility Determination
   Each pair makes wet mount slides (supplement pg 51) for:
   Bacillus subtilis (broth) DO THIS LAST AND OBSERVE QUICKLY!
Each pair makes 2 Motility Test Media tubes: one for P. vulgaris and one for S. aureus

Exercise 27: Effect of Oxygen on Growth
   Team up with other pair at your bench and split the work (group of 4):
   One pair use (broth): Staphylococcus aureus Bacillus subtilis
   Enterococcus faecalis Escherichia coli
   Clostridium sporogenes Clostridium rubrum
For your pair’s set of 3 organisms, make (per pair):
   2 Anaerobic Agar plates (all 3 on one, line inoculations)
      label 1 ANAEROBIC (put in jar), 1 AEROBIC (put in bin)

(From the Media List in the Supplemental packet)
Motility Test Medium
   Inoculation method: vertical single stab with straight needle
   Contains: nutrient medium with low (0.5%) agar concentration (semisolid) and TTC which changes from colorless to dark pink in the presence of bacterial growth (enhances visualization)
   Discriminates motility (presence of flagella), ability to “swim” through media
   Results: organism growing only in line of inoculation = non-motile
   organism appears as haze beyond line of inoculation = motile

Figure 18.4

Amy Warenda Czura, Ph.D.
Obligate aerobes: 20% $O_2$
Obligate anaerobes: $O_2$ toxic
Facultative: w/ or w/o, better with $O_2$
Microaerophiles: 5-10% $O_2$
Aerotolerant: ignore $O_2$

**Fluid Thioglycolate Medium (FTM)**
Inoculation method: loop transfer, careful mixing, screw cap must be loose
Contains: rich medium with very low agar content (viscous)
Sodium thioglycollate (removes oxygen)
Resazurin oxygen indicator (pink when oxidized: $O_2$ present)
Discriminates oxygen requirements: obligate aerobes, obligate anaerobes, facultative anaerobes, microaerophiles, aerotolerant
Results:
- growth only at top = obligate aerobe
- growth only at bottom = obligate anaerobe
- even growth throughout = aerotolerant
- heavy growth at top, lighter growth at bottom = facultative
- growth only in middle = microaerophile

**Brewer's Anaerobic Agar**
Inoculation method: surface streak with loop, must be incubated in Brewer's anaerobic jar (water + gas pack = $H_2 + CO_2$, $H_2$ combines with $O_2$ creating $H_2O$, sealed jar is oxygen free). Inoculate in conjunction with a second plate in aerobic 20% oxygen atmosphere to determine $O_2$ needs.
Contains: Nutrient agar with sodium thioglycolate and resazurin
Discriminates oxygen requirements if read in conjunction with normal aerobic incubated plate: obligate aerobes, obligate anaerobes, facultative anaerobes, aerotolerant
Results:
- growth on aerobic agar only = obligate aerobe
- growth on anaerobic agar only = obligate anaerobe
- even growth on both = aerotolerant
- heavy growth on aerobic, lighter growth on anaerobic = facultative

**Lab 6 Goals and Objectives:**

**Exercise 9: Aseptic Technique:** Check results
**Exercise 10: Pure Culture Technique:** Check results
- Red = *S. marcescens* / Small yellow = *M. luteus* / Cream = *E. coli*

**Exercise 18: Motility Determination**
Each pair makes wet mount slides (supplement pg 51) for:
- *Bacillus subtilis* (broth) **DO THIS LAST AND OBSERVE QUICKLY!**
- Each pair makes 2 Motility Test Media tubes: one for *P. vulgaris* and one for *S. aureus*

**Exercise 27: Effect of Oxygen on Growth**
Team up with other pair at your bench and split the work (group of 4):
- One pair use (broth): *B. subtilis* 
- Other pair use (broth): *S. aureus* 
- *Enterococcus faecalis* 
- *Clostridium sporogenes* 
- *Clostridium rubrum*
For your pair’s set of 3 organisms, make (per pair):
- 2 Anaerobic Agar plates (all 3 on one, line inoculations)
  - label 1 ANAEROBIC (put in jar), 1 AEROBIC (put in bin)