**Haemophilus**

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**Haemophilus species**  
**Gram Stain**  
- Tiny, faintly staining pleomorphic gram negative coccobacilli or filaments  
  - Safranin counterstain for >2 minutes  
  - Substitute carbolfuschin for safranin

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**Haemophilus species**  
**Epidemiology**  
- Normal flora of upper respiratory tract  
- Exceptions  
  - *Haemophilus ducreyi*  
    - Always pathogenic

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**Haemophilus species**  
**Virulence factors**  
- Capsule – most significant role  
  - Most invasive infections caused by encapsulated strains  
  - Antiphagocytic

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**Haemophilus species**  
**Epidemiology**  
- *Haemophilus influenzae* type B and *Haemophilus ducreyi*  
  - Person to person transmission plays key role  
  - Exogenous sources  
- Other Haemophilus species and strains  
  - Endogenous sources

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**Clinical Significance**  
*Haemophilus influenzae*  
- Normal flora of respiratory tract  
- 6 antigen types (a-f) based on capsular polysaccharides  
- Type b causes most human disease  
- Un-encapsulated (untypable) strains can cause disease
**Clinical Significance**

*Haemophilus influenzae*

- Respiratory tract infections
- Meningitis
- Otitis media
- Conjunctivitis (pinkeye)
  
  *(H. influenzae biogroup aegypticus)*

**Clinical Significance**

*Haemophilus ducreyi*

- Causative agent of Chancroid / Soft Chancre (STD)
- Specimen: base of lesion

**Clinical Significance**

*Other Haemophilus species*

- Usually nonpathogenic (NRF)
- May cause:
  - Bacteremia
  - Endocarditis
  - Respiratory tract infections
  - Abscesses

**Specimen Handling**

*Haemophilus species*

- Avoid drying & temperature extremes
- Setup culture immediately
  - Chocolate agar
  - Or BAP with Staph streak
  - Incubate 5-10% CO₂ in moist environment

**Specimen Handling**

*Haemophilus ducreyi*

- Specimen should be collected from base of the lesion
- Specimen should be inoculated directly to enriched media
- Incubate 5-10% CO₂ in moist environment for up to 5 days
**Haemophilus species**

**Direct Detection**
- Gram stain
  - Tiny gram negative coccobacilli
- Serological testing
  - Latex agglutination (most sensitive)
  - EIA (greater specificity)

**Haemophilus ducreyi**

**Direct detection**
- Gram stain: GNCB, arranged as "school of fish" or "railroad tracks"

**Haemophilus species**

**Growth Characteristics**
- Fastidious
- Facultative
- Colony morphology
  - Chocolate agar

**Haemophilus species**

**Growth Characteristics**
- Colony morphology
  - Sheep BAP & MAC = no growth
  - Sheep BAP with Staph streak
    - Satellitism
    - Horse BAP = evaluate hemolysis

**Haemophilus species**

**Growth Requirements**
- Requires blood factors for growth
  - X factor (Hemin)
    - Heat stable
    - Present in RBCs
  - V factor (NAD)
    - Heat labile
    - Produced by some bacteria and yeast
    - Present in RBCs, yeast, potatoes

**Haemophilus species**

**Identification**
- Determine X and V factor requirements
- Methods:
  - Conventional disks
  - Quad plate
- Determine hemolysis on horse BAP
**Who am I?**

**Haemophilus species**

**Identification**
- Determine Porphyrin production
  - Does organism have its own enzymes to produce hemin (X factor)?

**Haemophilus species**

**Identification**
- Rapid ID kits
- Typing of capsular antigens
  - Coagglutination test
  - Molecular methods

**Antibiotic Therapy**
- Ampicillin is drug of choice (historically)
- Beta-lactamase production
- Susceptibility testing done
  - Disk diffusion (Haemophilus test media)
  - Broth dilution
  - E-test
- NCCLS document for Haemophilus

**Haemophilus species**

**Summary**
- Epidemiology
- Clinical significance
- Specimen handling and processing
- Growth characteristics
- Identification