# **Staphylococcus, Micrococcus, and Rothia (Gram-Positive Cocci, Catalase-Positive)**

**Characteristics:** Gram-positive cocci in clusters or tetrads. They are **catalase positive** (bubbling with H₂O₂).

**COAGULASE POSITIVE**

1. Staphylococci

* facultative anaerobes;
* Coagulase positive
* DNase positive
* Beta haemolytic
* Ferments manitol

1. *Micrococcus* is

* strictly aerobic
* often pigmented yellow colonies
* have larger cocci often tetrads
* Coagulase positive
* Modified oxidase test > Microdase. Staphylococcus is negative.
* Bacitracin-sensitive (Staphylococcus is resistant)

1. *Rothia* **mucilaginosa** (formerly *Stomatococcus*)

* weakly catalase positive.
* Gumdrop colonies
* API

**COAGULASE NEGATIVE**

* CoNS (e.g. *S. epidermidis*, *S. saprophyticus*)
* **Novobiocin Susceptibility:** Among CoNS: *S. saprophyticus* (which causes UTIs) is **novobiocin-resistant**, vs *S. epidermidis* (and most other CoNS) are **novobiocin sensitive**.

# **Streptococcus and Enterococcus (Gram-Positive Cocci, Catalase-Negative)**

**Characteristics:**

* Gram-positive cocci in chains or pairs.
* **catalase negative**.
* Non motile
* Mostly facultative anaerobes (some prefer CO₂).
* Carbohydrates fermented into lactic
* produce lactic aminopetidase.
* Differentiated by haemolytic pattern and Lancefield group
* Alternative genera: Aerococcus, Gemella, Abiotrophia, Granulicatella, Rothia etc.

**Hemolysis-Based Identification:** Hemolysis is assessed first, then specific tests:

* **β-hemolytic streptococci:**
  + Could be *Streptococcus pyogenes* (GAS), *S. agalactiae* (GBS), or others (Groups C, G, etc.).
  + **S. pyogenes** 
    - **bacitracin-sensitive.** (any zone of inhibition) whereas most other β-hemolytic streps (including GBS) are resistant. A positive bacitracin test presumptively identifies Group A strep.
    - does not grow t at 10deg, 45deg, 6.5% NaCl, pH 9.6, 40% bile.
    - **PYR positive**
  + For GBS
    - **CAMP Test:** Used on β-hemolytic isolates that are bacitracin-resistant to identify *S. agalactiae*. *GBS* is **CAMP-positive** – it enhances hemolysis of *S. aureus beta-lysin* in a streaked perpendicular pattern (arrowhead zone of enhanced β-hemolysis).
    - **PYR Negative** *S. agalactiae* unlikely GAS and Enterococcus
    - **Hippurate positive hydrolysis:** *S. agalactiae* hydrolyzes hippurate (deep purple color with ninhydrin), distinguishing it from other β-hemolytic streps (hippurate-negative).
* **α-hemolytic streptococci:** Mainly *Streptococcus pneumoniae* and “viridans group” streptococci.
  + **Step pneumo** 
    - **Optochin (P disk) optochin-sensitive** > zone of inhibition 14mm with ethylhydrocupreine HCl), whereas viridans streptococci are optochin-resistant.
    - **Bile Solubility:** *S. pneumoniae* colonies **lyse in bile salts** > clear solution in sodium deoxycholate test), distinguishing them from bile-insoluble viridans strains.
    - **colony morphology** draughtsman colonies – concave center
    - **PYR-negative**
  + **Viridans streptococci** 
    - a heterogeneous group of α or non-hemolytic species
    - 5 groups: S. mutans, S. salivarius, S. anginosus, S. mitis, S. bovis
    - **optochin-resistant**
    - **bile insoluble**.
    - **PYR-negative** and generally identified by exclusion
    - definitive species ID often requires biochemical panels or MALDI.
    - S. mitis group S. pseudopneumoniae looks like pneumo but i) no capsule ii) not bile soluble

S. bovis:

DNA cluster 1: S. equinus (now includes S. bovis)

DNA cluster 2: S. gallolyticus subsp gallolyticus, pasteurianus and macedonicus

DNA cluster 3: S. infantarius subsp. infantarius, coli

DNA cluster 4: S. alactolyticus

alpha or no haemolysis

Lancefield D, but unlike Enterococci are PYR neg and arginine neg

grow on bile aesculin, cannot grow in 6.5% NaCl

* **Non-hemolytic (γ) cocci:** Could be Enterococcus or Group D Streptococcus (e.g. *S. bovis* group).
  + **Bile-Esculin Test:** Enterococci and Group D strep can hydrolyze esculin in presence of bile. **Bileesculin positive** result (orange > black of medium) indicates Group D antigen organisms.
  + **6.5% NaCl Broth: Growth in 6.5% NaCl** (turbidity) is characteristic of *Enterococcus* species, whereas *Streptococcus bovis* group (non-enterococcal Group D strep) will **not grow in high salt**.
  + **PYR Test:** Enterococci are **PYR positive** (pink), while Group D streps (e.g. *S. bovis*) are PYR negative (orange).
  + These tests together differentiate Enterococcus (bile-esculin+, salt+, PYR+) from *S. bovis* group (bile-esculin+, salt–, PYR–).

**Summary of Key Differentiation Tests for Streptococci:**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Species/Group | Haemolysis | Bacitracin (A disk) | CAMP | PYR | 6.5% NaCl | Bile esculin | Optochin |  |
| **Group A**  **Strep (*S. pyogenes*)** | β (clear) | Sensitive (any zone) | – | **+** | – |  | – | Small colonies; causes strep throat, etc. |
| **Group B**  **Strep (*S. agalactiae*)** | β (narrow) | Resistant | **+** | – | – |  | – | Larger milky colonies; neonatal sepsis. |
| **Enterococcus**  **(*E. faecalis*, etc.)** | usually γ (or α) | Resistant | – | **+** | **+** | **+** | – | Can be weakly hemolytic; UTIs, endocarditis. |
| **Group D**  **Strep (*S. bovis* group)** | γ | Resistant | – | – | – | + | – | Bile-esculin +, associated with colon lesions. |
| **Strep pneumoniae** | α (green) | – | – | – | – |  | **Sensitive** to optochin; **bile soluble** | Lancetshaped  diplococci;  pneumonia, meningitis. |
| **Viridans Strep** | α or γ | – | – | – | – |  | Resistant to optochin; not bile soluble | Normal oral flora; cause endocarditis. |

*Notes:* “–” indicates not typically tested or not applicable for that organism.

* Enterococci (especially *E. faecalis*) can sometimes show weak β-hemolysis.
* **PYR** is positive for *S. pyogenes* and Enterococcus.
* **CAMP** is specifically for *S. agalactiae*.
* **Enterococcus** can be presumptively identified by bile-esculin positivity and growth in salt
* **S. pneumoniae** by optochin sensitivity

Lancefield grouping:

|  |  |
| --- | --- |
| Group |  |
| A | pyogenes,  dysgalactiae subsp. equisimilis |
| B | agalactiae,  [halichoeri, porcinus] |
| C | dysgalactiae  equi |
| D | Enterococcus  bovis |
| F |  |
| G | canis,  some Enterococcus crossreact |
| R,S,T | suis |
| ungroupable | suis, |
| non | pneumoniae, viridans, |

Strep anginosus has variable grouping

* Strep intermedius is non groupable
* Strep anginosus is A, C, F, G (F most common)
* Strep constellatus subsp. constellatus C, F, G
* Strep constellatus subsp. pharyngis: C

# **Anaerobic Cocci (Gram-Positive Anaerobic Cocci)**

**Characteristics:**

* formerly grouped as “**Peptostreptococcus**”
* related genera such as *Peptoniphilus, Finegoldia, Parvimonas*, etc.
* Tiny Gram-positive coccal cells (sometimes in chains or pairs) and grow only under anaerobic conditions.
* Catalase **negative**
* **Generally y haemolysis apart from Fingeoldia which are beta haemolytic.**
* These organisms are part of normal flora of the mouth, gut, and genitourinary tract
* can cause abscesses and polymicrobial infections when displaced.
* Often labs do not speciate all anaerobic cocci unless needed; they may report “anaerobic Gram-positive cocci” as a group.

**Identification Notes:**

* **Colonial Morphology:** On anaerobic blood agar, colonies may be small and opaque. Some (like *Finegoldia magna*) produce a foul odor.
* **SPS Disk Test:** *Peptostreptococcus anaerobius* is classically **sensitive to SPS** (sodium polyanethol sulfonate, 5 μg disk), whereas most other anaerobic cocci are resistant.
* **Indole Production:** Some anaerobic cocci (e.g. *Peptoniphilus asaccharolyticus*) are **indole positive** (pink with Kovac’s reagent) which can aid identification in a panel of tests.
* API or MALDI-TOF for identification