



دانشگاه اصفهان

دانشکده علوم و فناوری های زیستی، گروه زیست شناسی سلولی مولکولی و میکروبیولوژی، آزمایشگاه میکروبیولوژی



# آزمایشگاه باکتری شناسی ۱

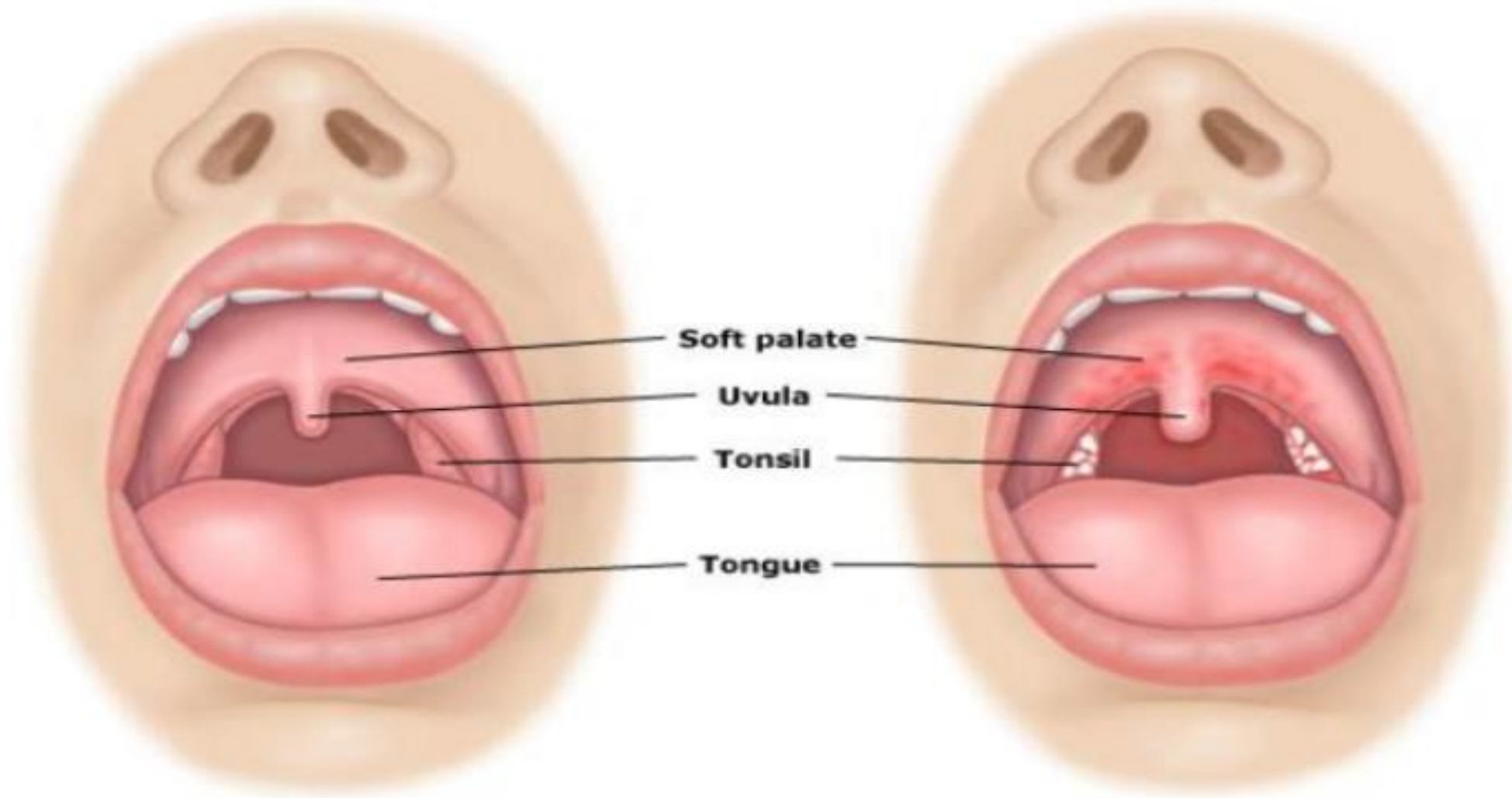
- ۱- نمونه گیری از گلو به منظور جداسازی استرپتوکوکوس ساکن در گلو
- ۲- رنگ آمیزی گرم و آشنایی با خصوصیات میکروسکوپی و ماکروسکوپی استرپتوکوکوسی گروه A، گروه B، استرپتوکوکوس ویریدانس



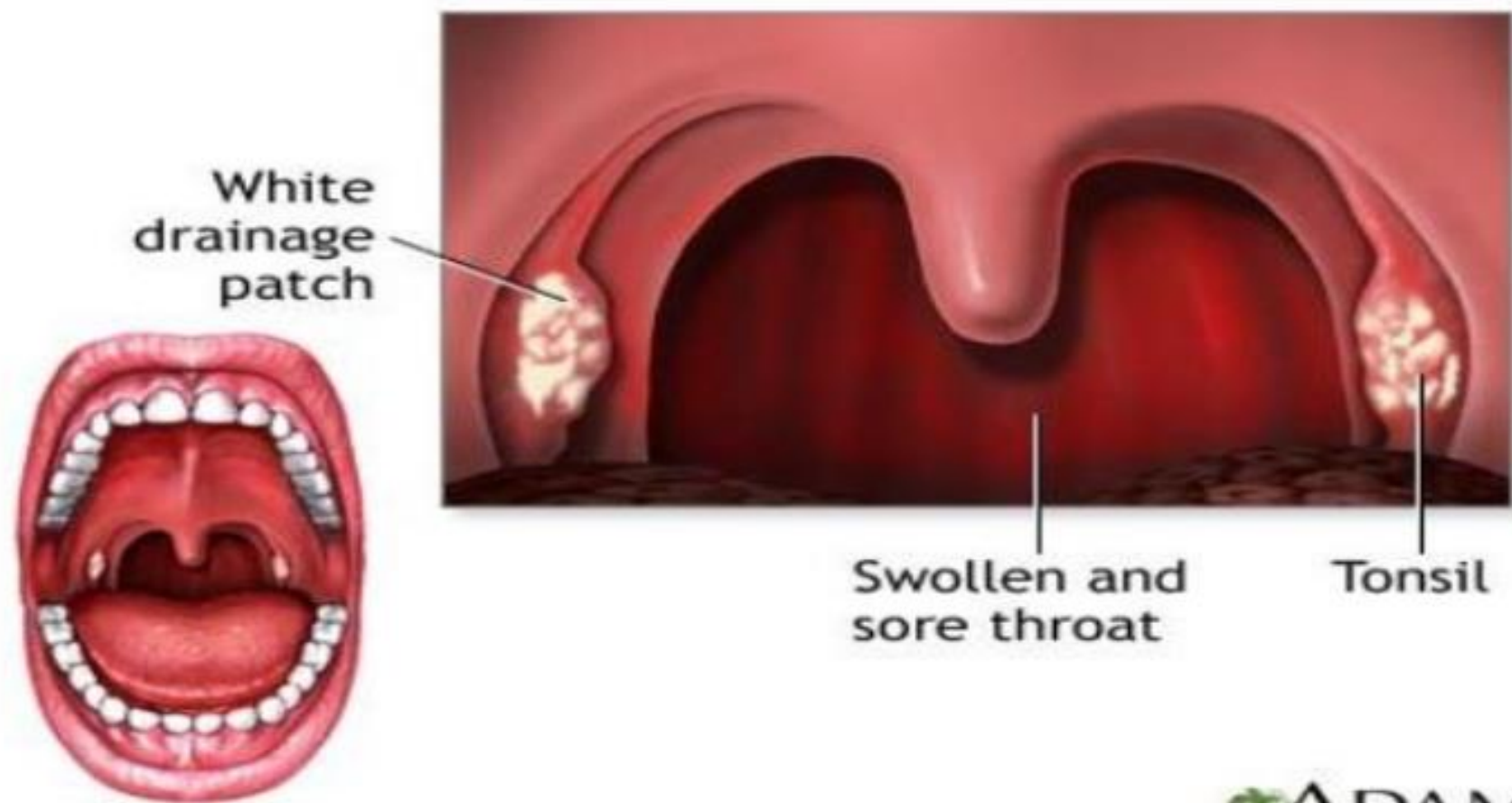
## Group A Streptococcus infection manifest with Throat Pain

Normal

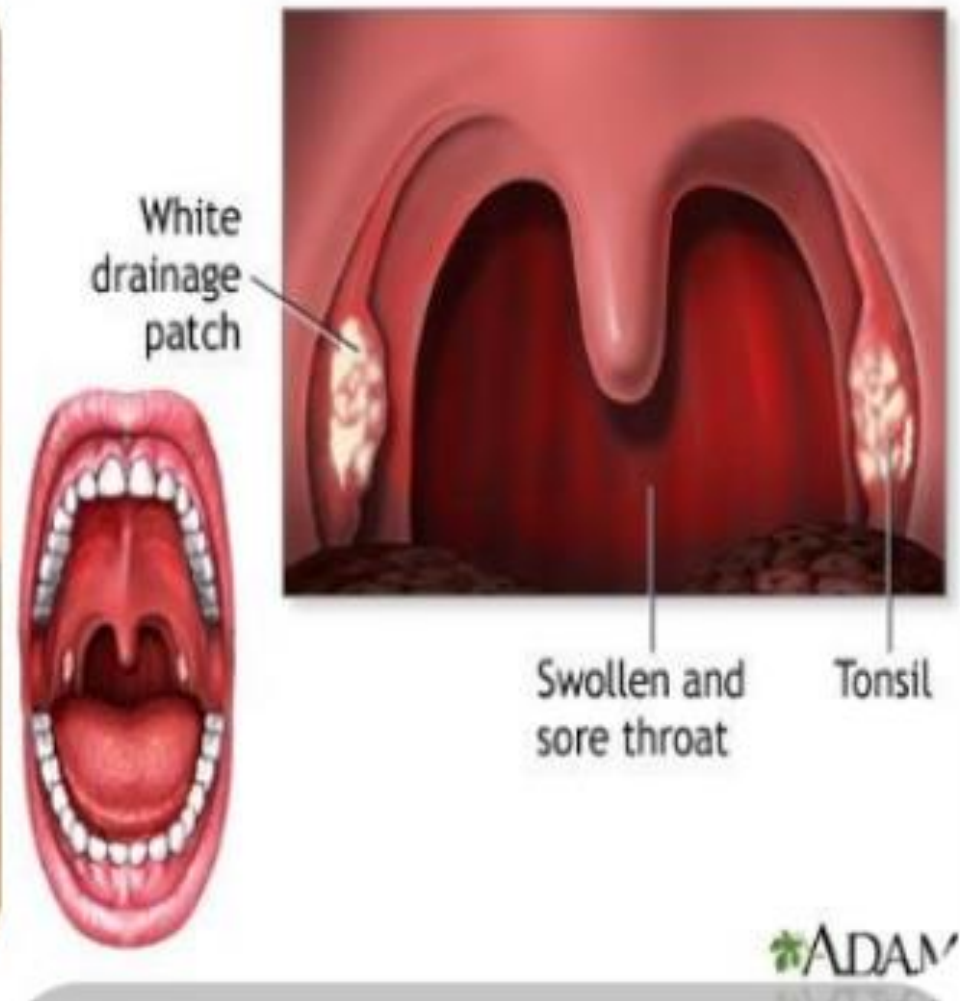
Strep



# Infection of Tonsils



# Collecting a appropriate specimen is highly essential





# LAB DIAGNOSIS

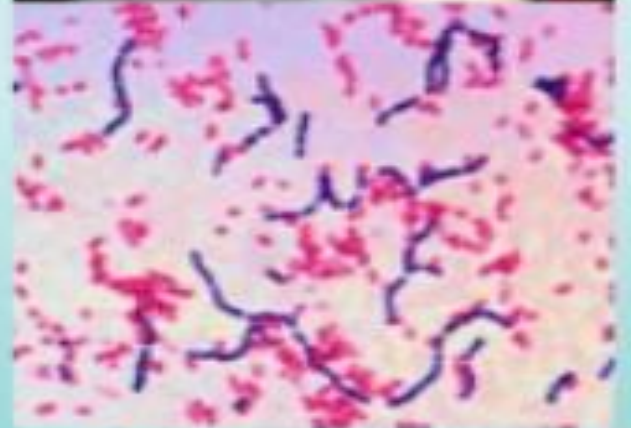
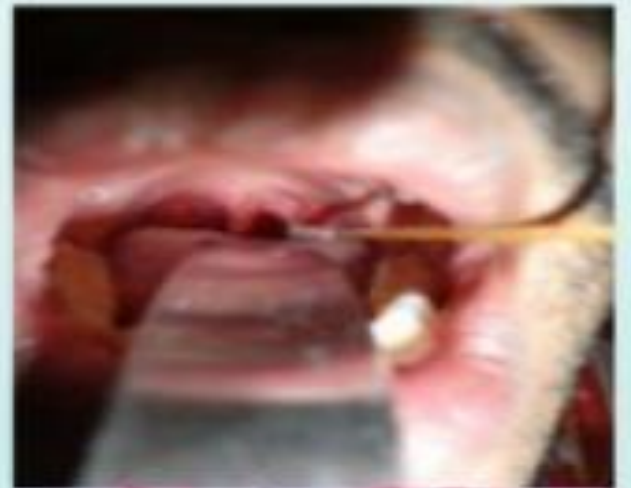
- ❖ In acute infections, diagnosis is established by culture
- ❖ In non-suppurative complications are diagnosed by demo. Of antibodies

## 1) SPECIMEN

- Throat swab, pus swab or exudates are collected
- In RF & GN serum is collected for serology

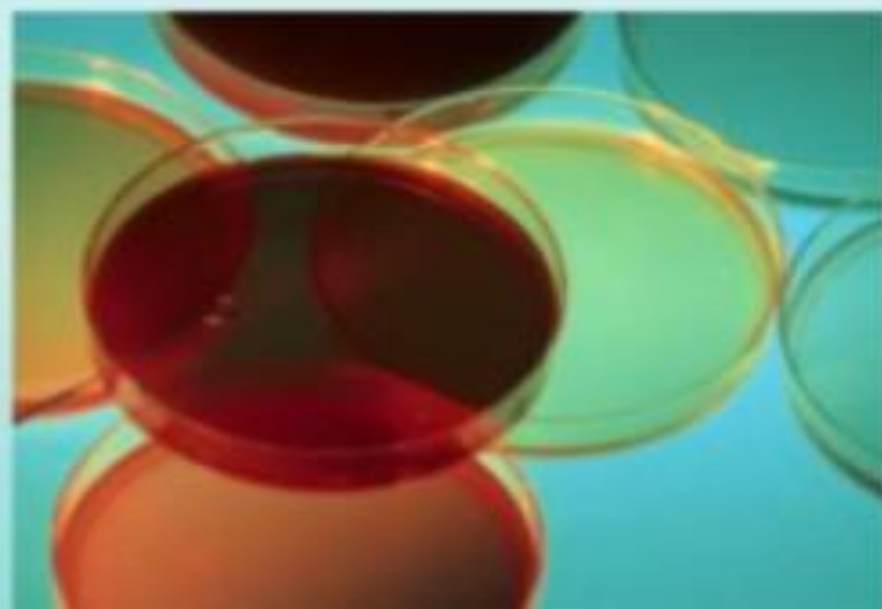
## 2) MICROSCOPY

- Observing gram stained films from pus
- Presence of gram positive cocci in chains indicate the infection
- Smears are of no value in infections of throat or genitalia. Y???



### 3) CULTURE

- ❑ Swabs collected from affected site are plated immediately or send to lab in PIKE'S MEDIUM
- ❑ ( Blood agar containing 1 in 10 lakh crystal violet & 1 in 16,000 sodium azide)
- ❑ The specimen is plated on blood agar & incubated @ 37°C anaerobically or under 5-10% CO<sub>2</sub>





# میتود قطع نوار (کت استریک) Cut Streak method

■ ایجاد شرایط میکروفیلیک در یک محیط غیر هوای

■ اجرای کلچر گلو در Blood Agar Plate

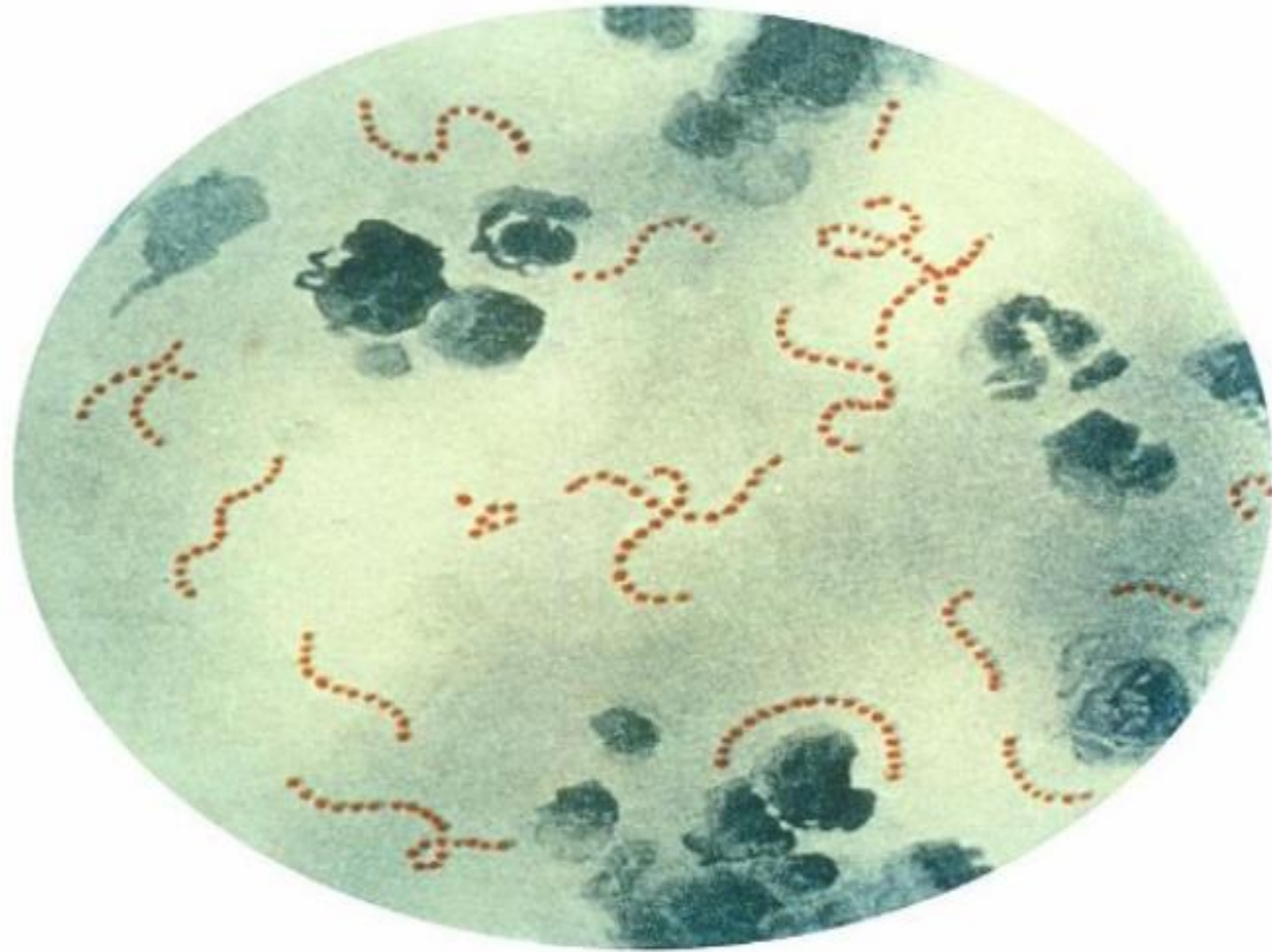
■ نشان دادن هیمولایزین های اوکسیجن دار ناثابت که توسط گروه A استرپتوکوک بیتا ایجاد میشود.

- Provides microaerophilic conditions in an aerobic environment
- Performed on throat cultures plated on BAP
- Demonstrates the presence of oxygen labile hemolysins produced by BSGA



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# Streptococci as seen in a Specimen



# Introduction

- Gram positive cocci
- Arrangement : chains or pairs
- Part of normal flora
- Causes pathogenic infections also

# DIAGNOSIS

1. Microscopy
2. Culture – Bacitracin Test (Taxo-A)
3. Antigen detection tests – Enzyme-linked immunosorbent assay (ELISA) or agglutination tests
4. Antibody detection
  - ASO titer – for respiratory disease
  - antiDNAse & antihyaluronidase – for skin infections

# 1. HEMOLYSINS

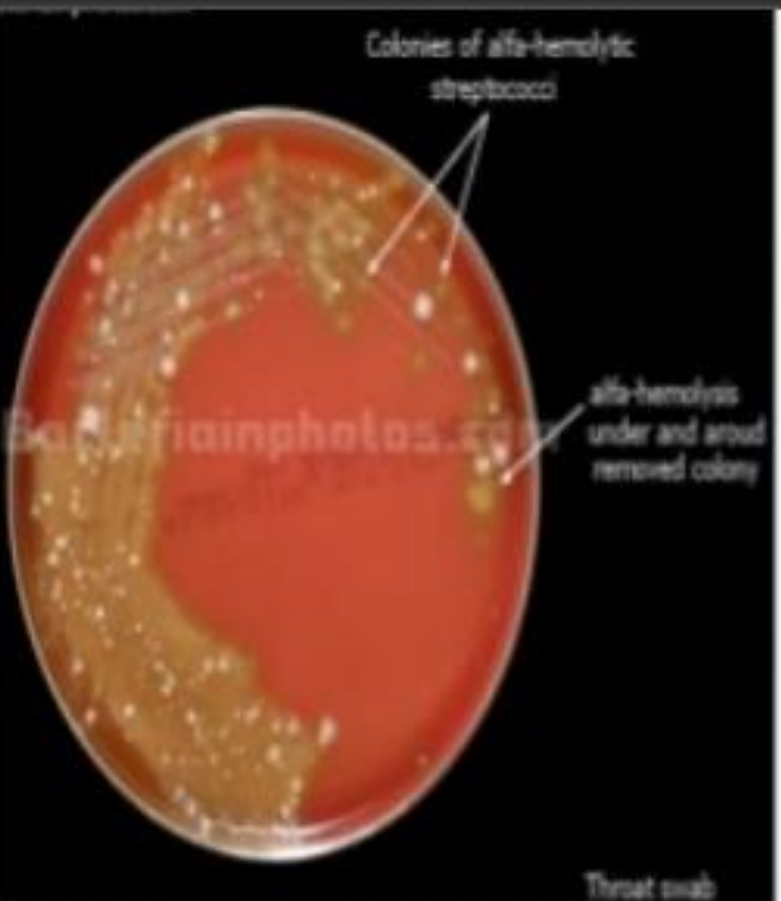
2 types ————— **STREPTOLYSIN O AND S**

## ☐ **STREPTOLYSIN O**

- ❖ Oxygen labile & heat labile
- ❖ Inactive in oxidized form but reactivated on treating with mild reducing agents
- ❖ Lethal on i.v injection and is cardiotoxic & leucotoxic activity
- ❖ Its antigenic and antistreptolysin O appears in sera following its infection

### ❖ ASO TITRE

- Earlier done by hemolysis inhibition test
- Now done by serological method of latex agglutination
- ASO titer > 200 units is significant & suggests a recent or recurrent infection with streptococci



### ALPHA HEMOLYSIS

- Greenish discoloration with partial hemolysis
- Small zone of lysis (1-2mm) with indefinite margins
- Unlysed RBCs seen under microscope



### BETA HEMOLYSIS

- Sharply defined, clear, colourless zone of hemolysis
- 2-4 mm wide
- RBCs lysed completely



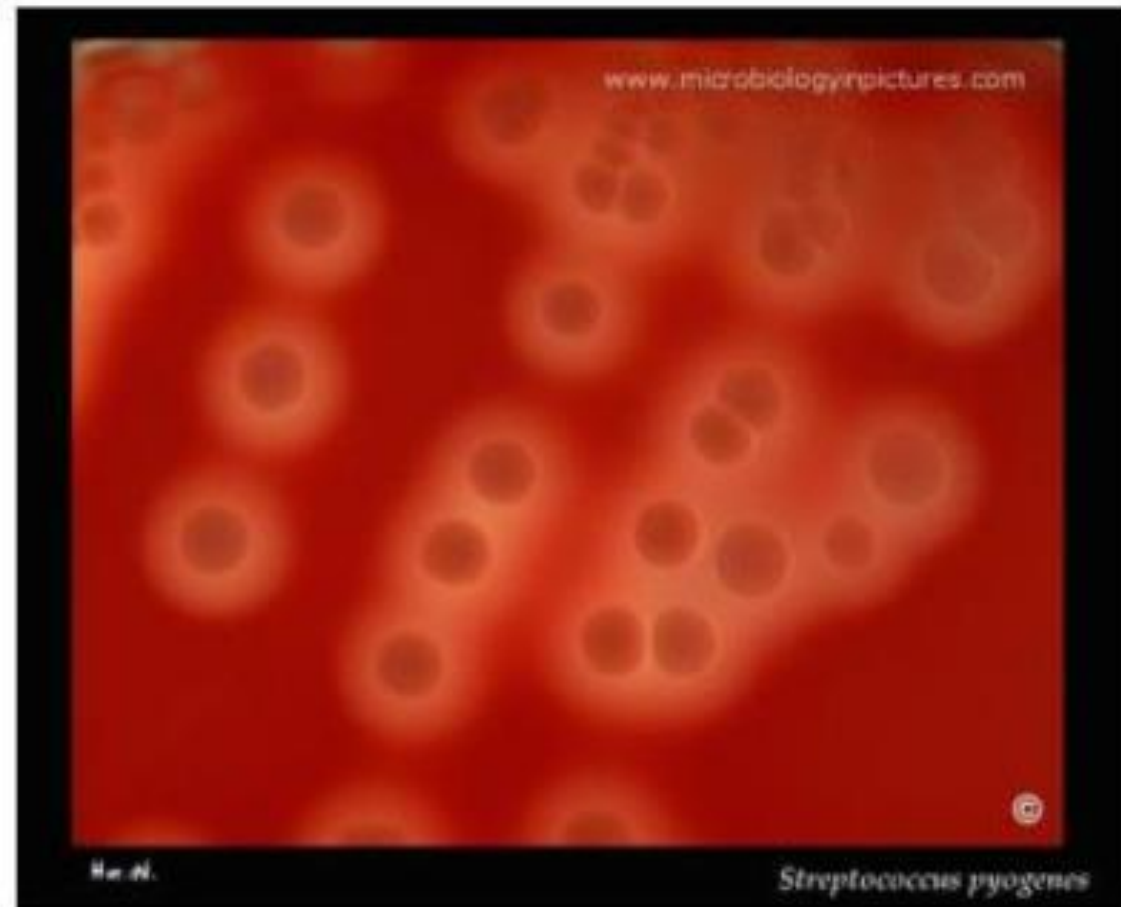
### GAMMA HEMOLYSIS

- No hemolysis

# Hemolysis

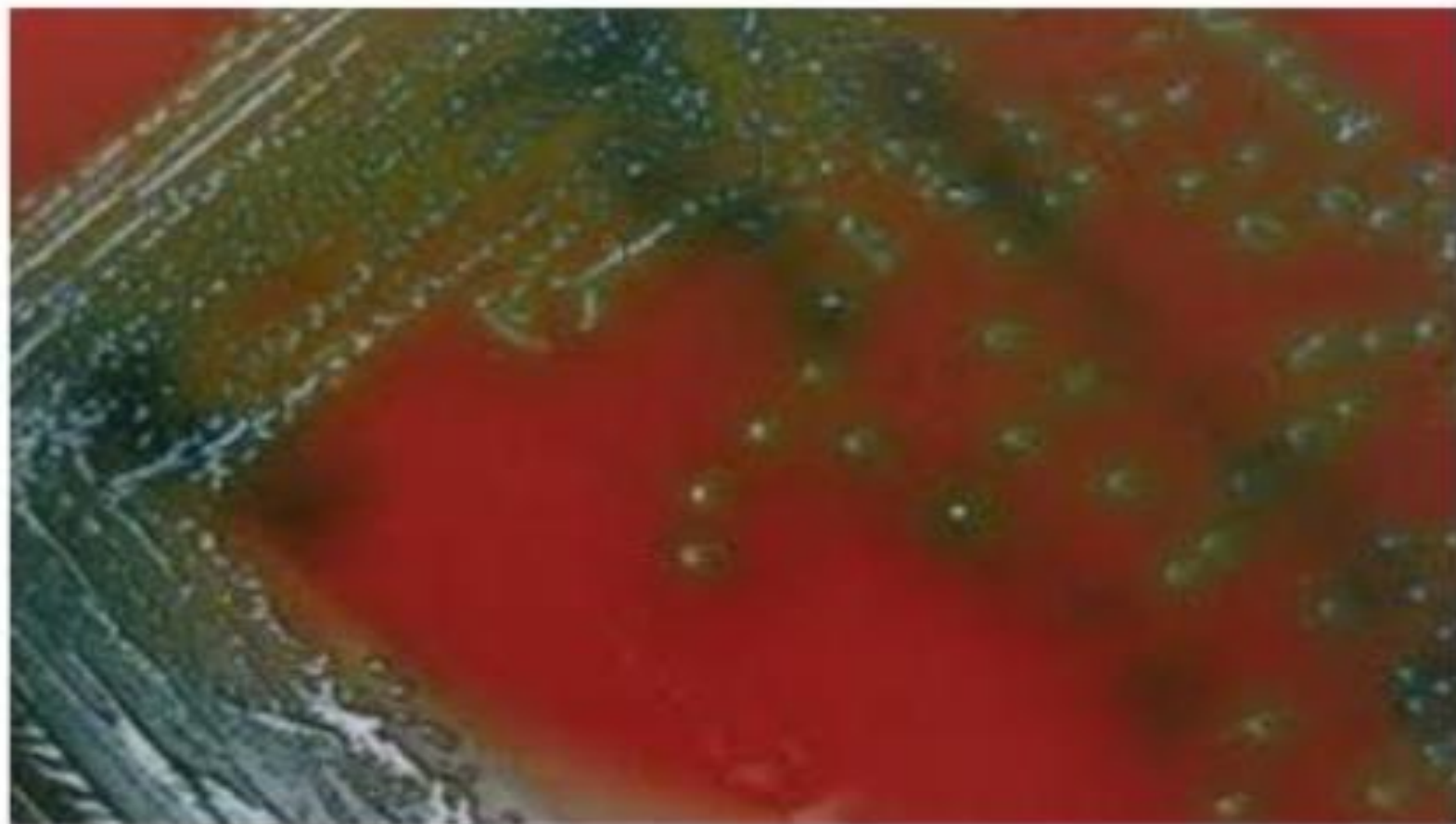


# Beta hemolysis





# Alpha hemolysis



# Biochemical reactions

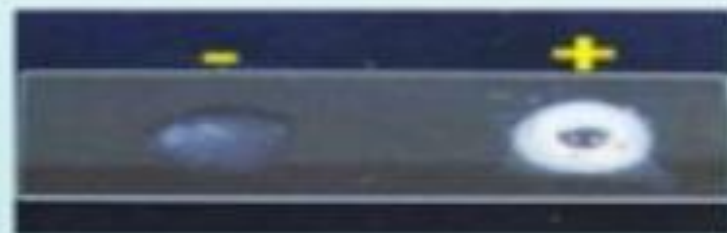
Ferments several sugars producing acid but no gas

How to differentiate strep. from pneumococci?

They are catalase negative

Not soluble in 10% bile

Failure to ferment ribose differentiate *S.pyogenes* from other streptococci



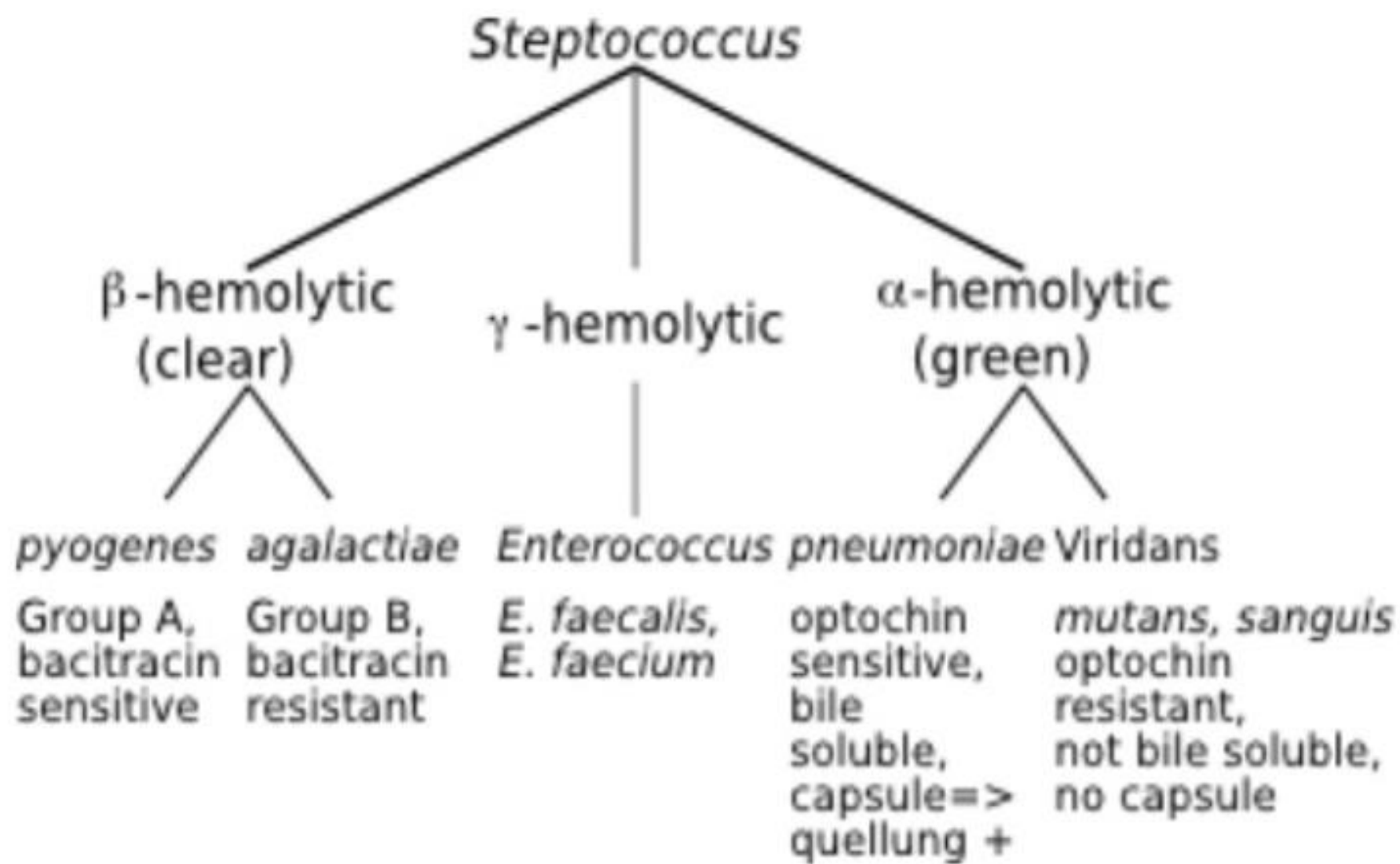
# CULTURAL CHARACTERISTICS

- ❖ Aerobes as well as facultative anaerobes
- ❖ Grown best at 37°C ( 22-42°C )
- ❖ Grows in media rich in fermentable carbohydrates or enriched media with blood or serum
- ❖ **ON BLOOD AGAR**
  - Shows beta hemolysis
  - Virulent strains form matt colonies
  - Avirulent strains form glossy colonies
  - Some strains with capsule form mucoid colonies
- ❖ **ON LIQUID MEDIA**
  - Growth occur as a granular turbidity with a powdery deposit in glucose or serum broth

# Classification of Streptococci

- Brown`s classification
- Lancefield grouping
- Griffith typing

# Brown`s classification



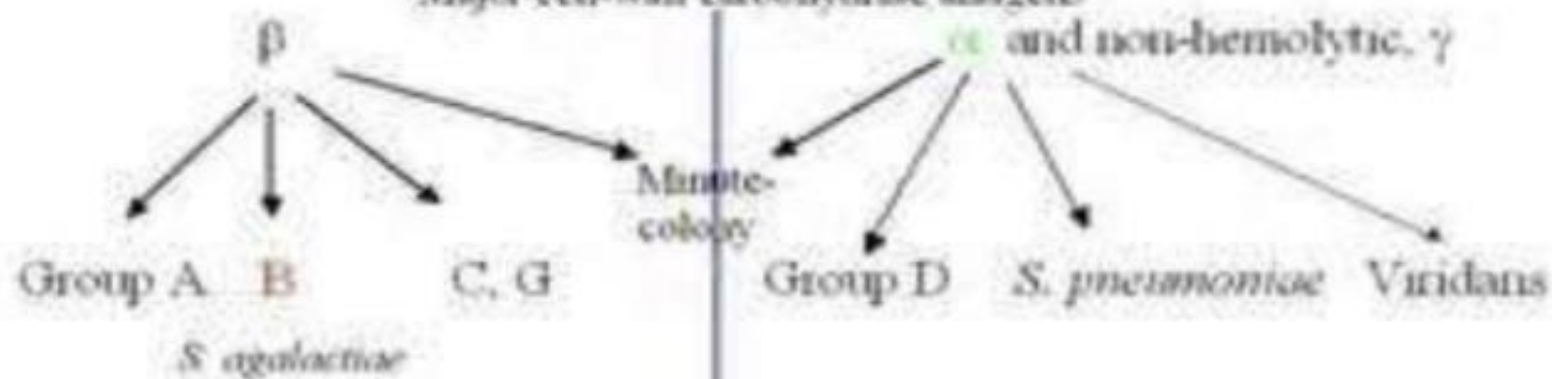
# Lancefield grouping

## *Streptococcus* classification

Hemolysis on Agar plates containing Sheep Blood

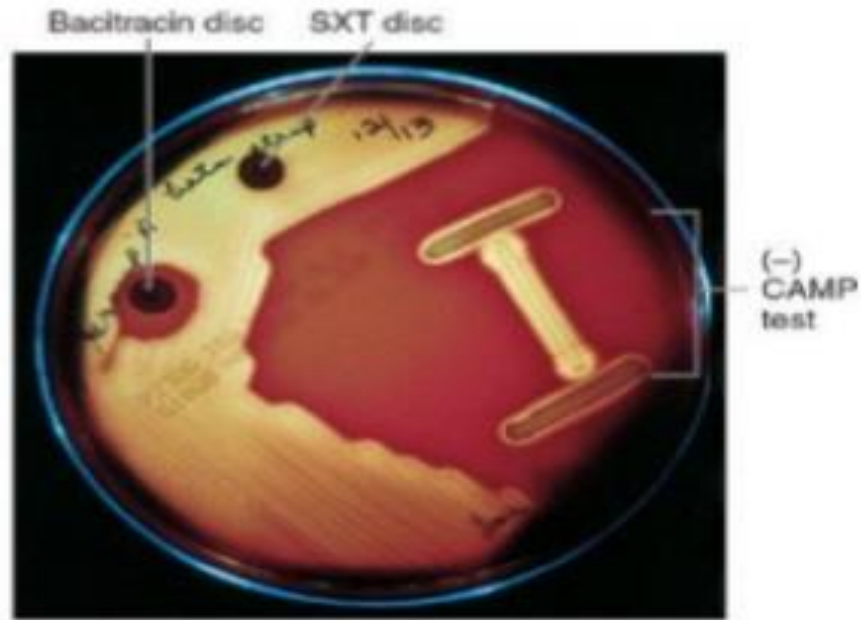
Lancefield Groups (A, B... T)

Major cell-wall carbohydrate antigens



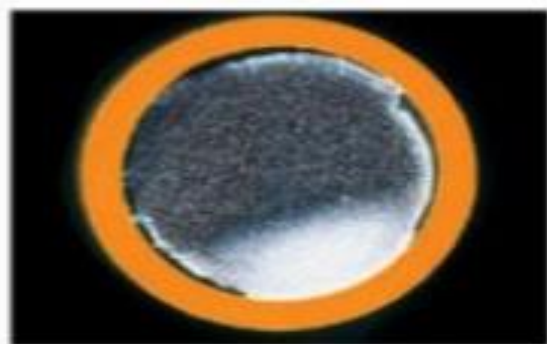
# Streptococcal tests

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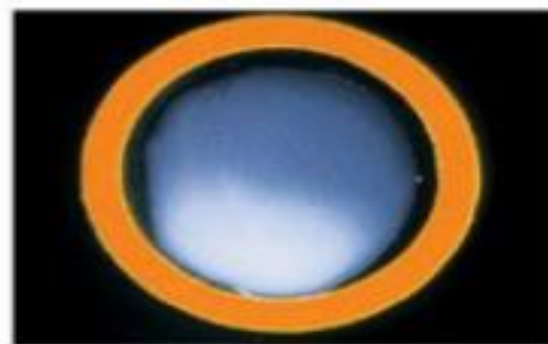


(a)

**Bacitracin disc test- only**  
Streptococcus pyogenes is sensitive to minute bacitracin conc.  
Group A streptococci are negative for SXT sensitivity and the CAMP test

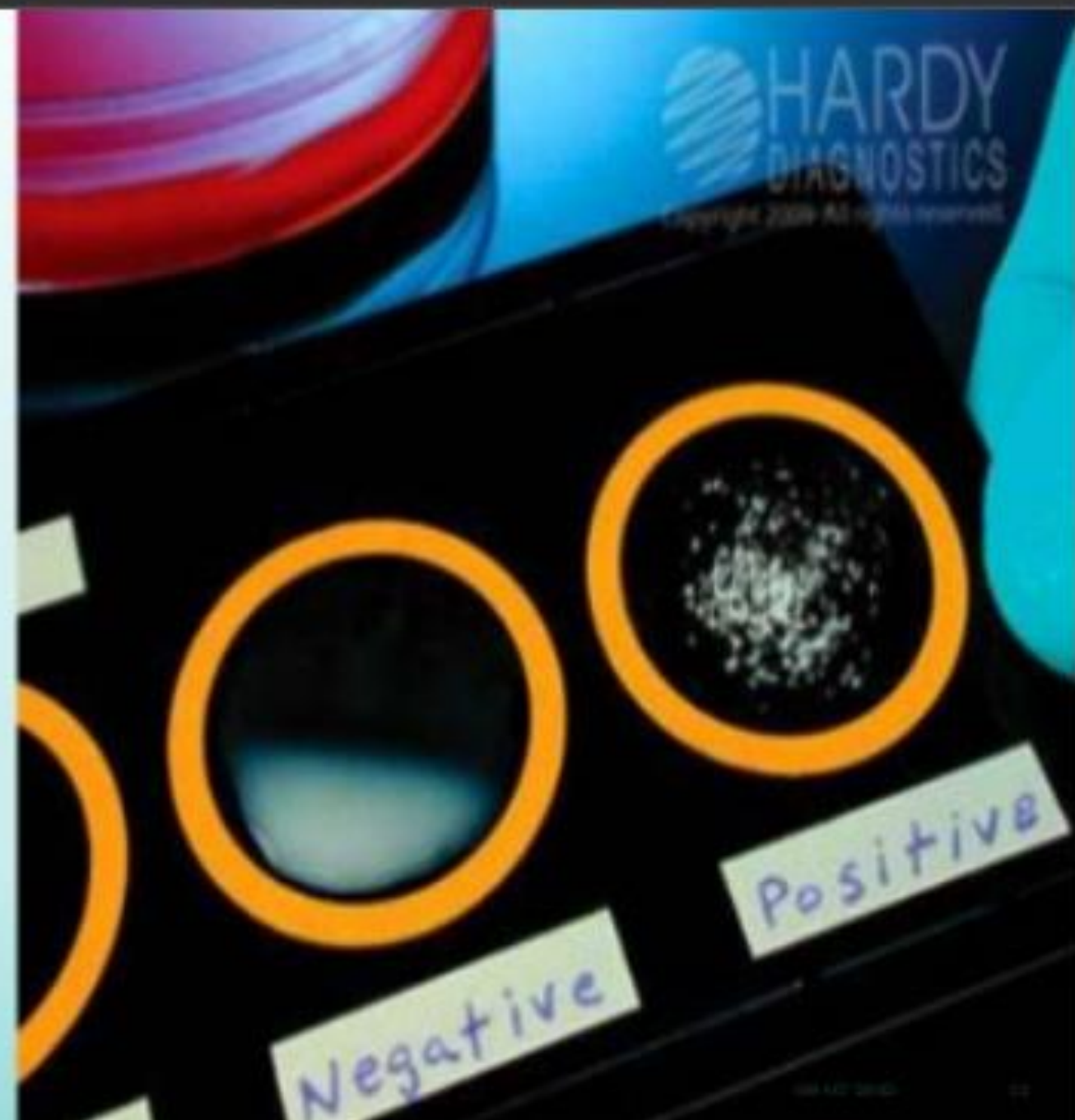


Positive reaction



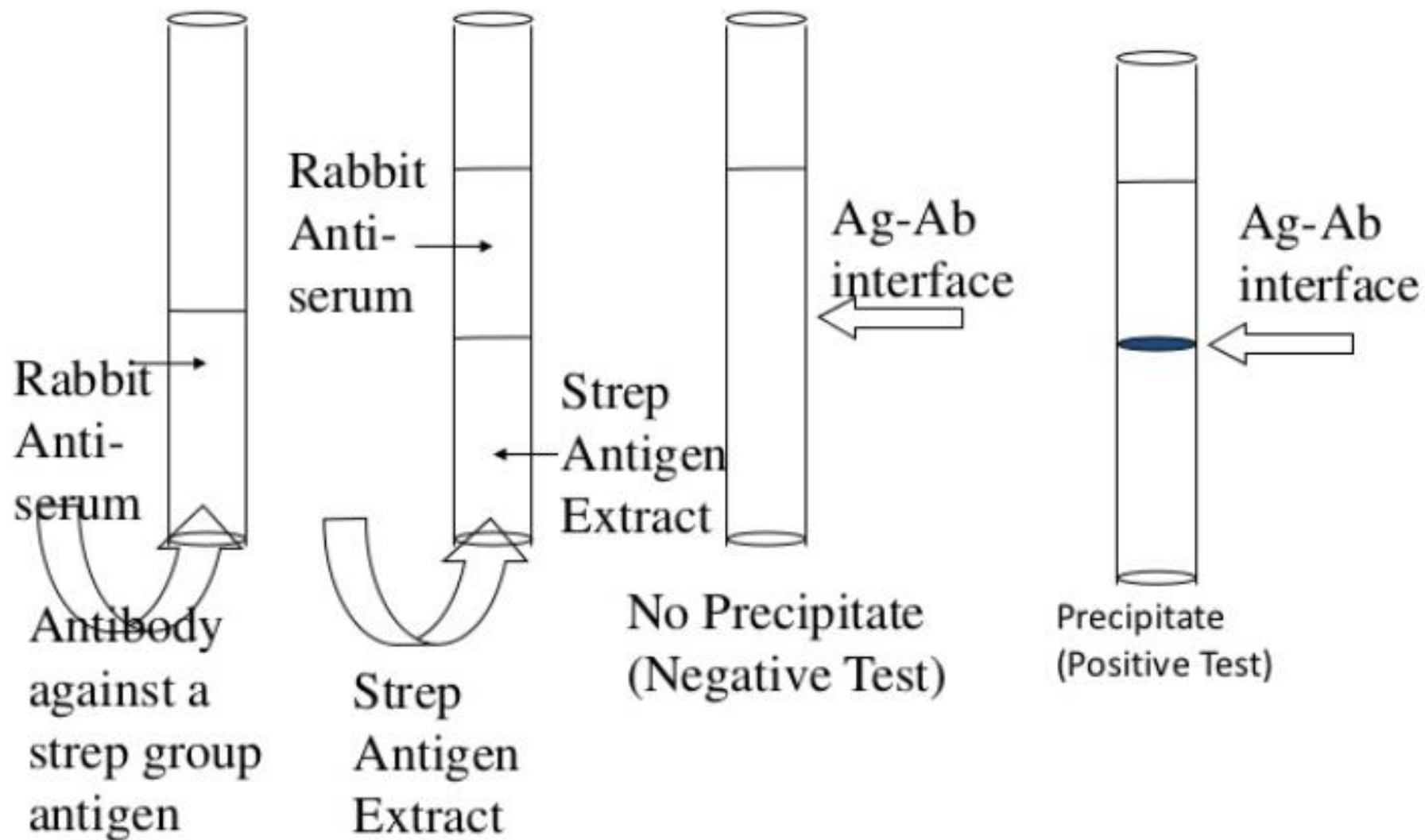
Negative reaction

Rapid, direct **test kit for diagnosis of group A infections**, throat swab introduced to latex beads and monoclonal antibodies  
Positive-the C-carbohydrate on group A streptococci causes clumping  
Negative-milky smooth reaction

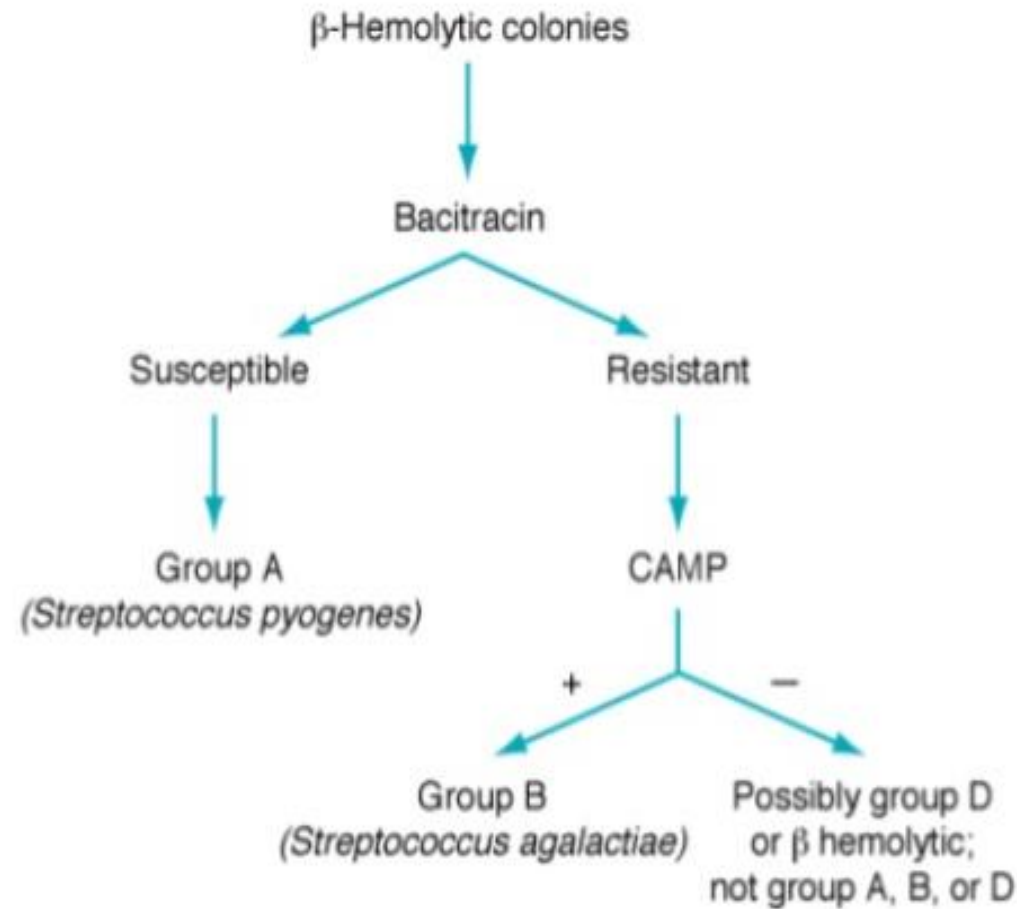




# Lancefield Capillary Precipitation



# Identification Scheme



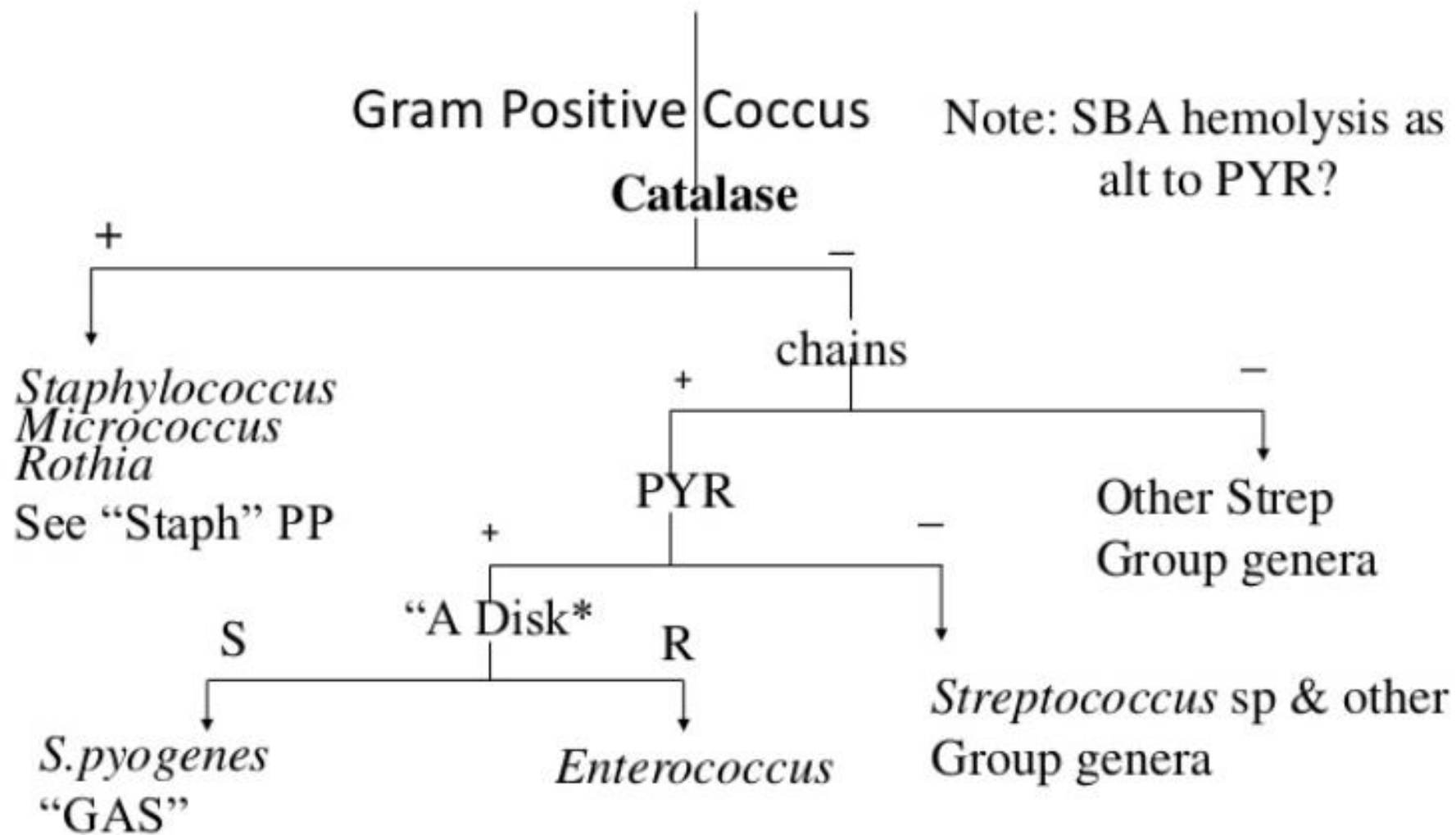
**Schema to differentiate Group A and B  
from other β-hemolytic streptococci**

# Diagnostic Laboratory Tests

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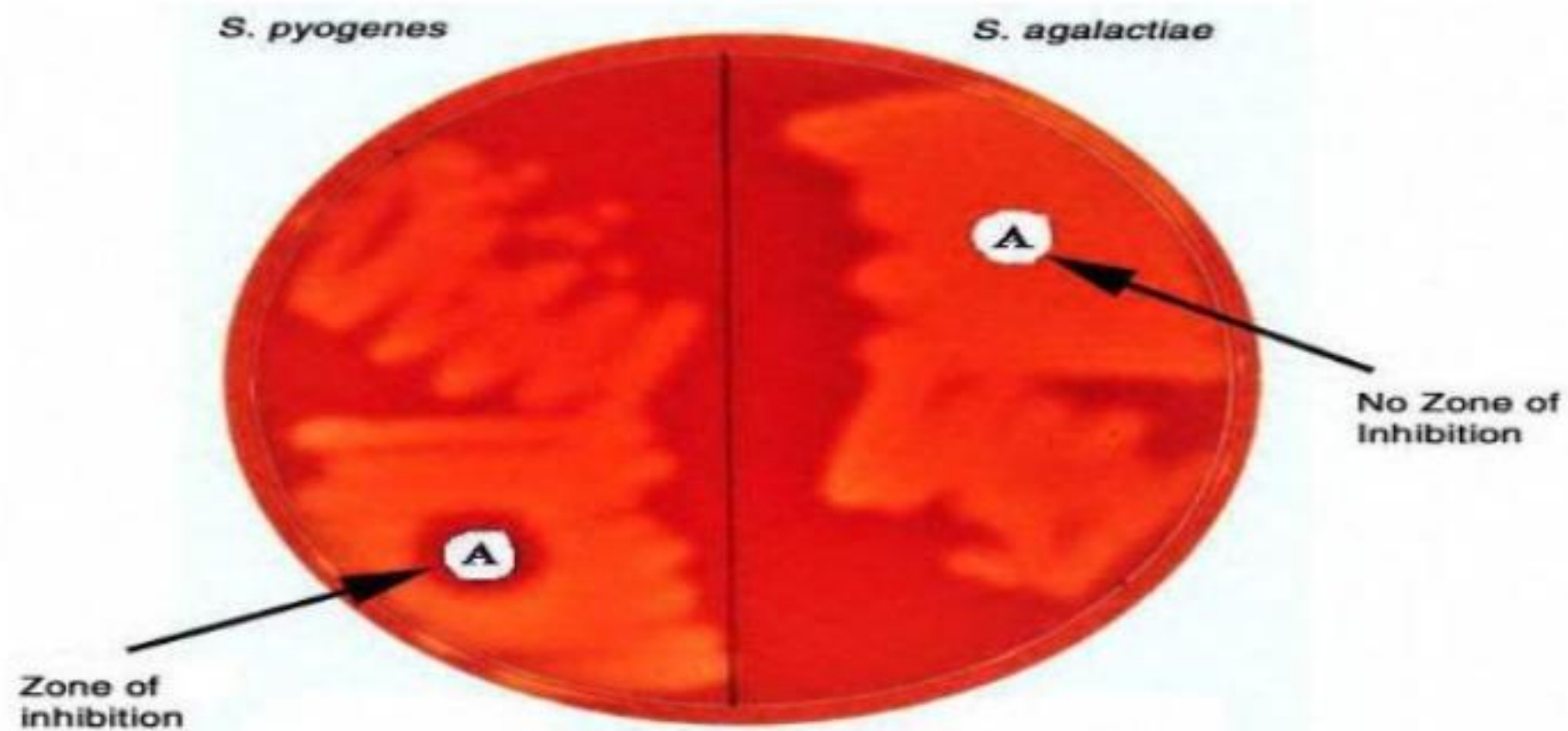
- **CAMP factor** positive
- **Hippurase** positive

# Preliminary Grouping of Gram Positive Cocci



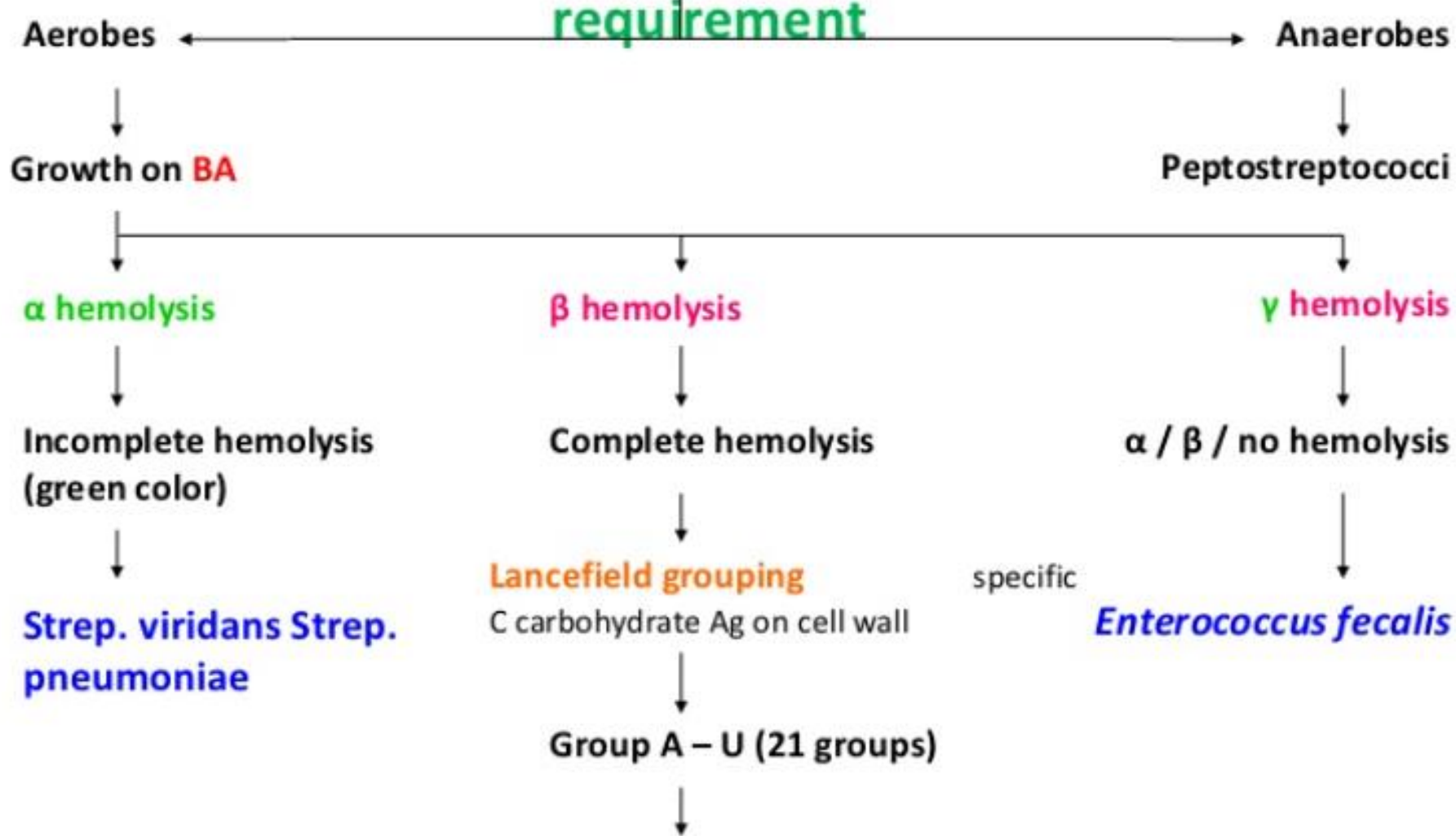
\*A disc contains bacitracin

# Streptococci grown Blood agar



5% Sheep Blood Agar Plate

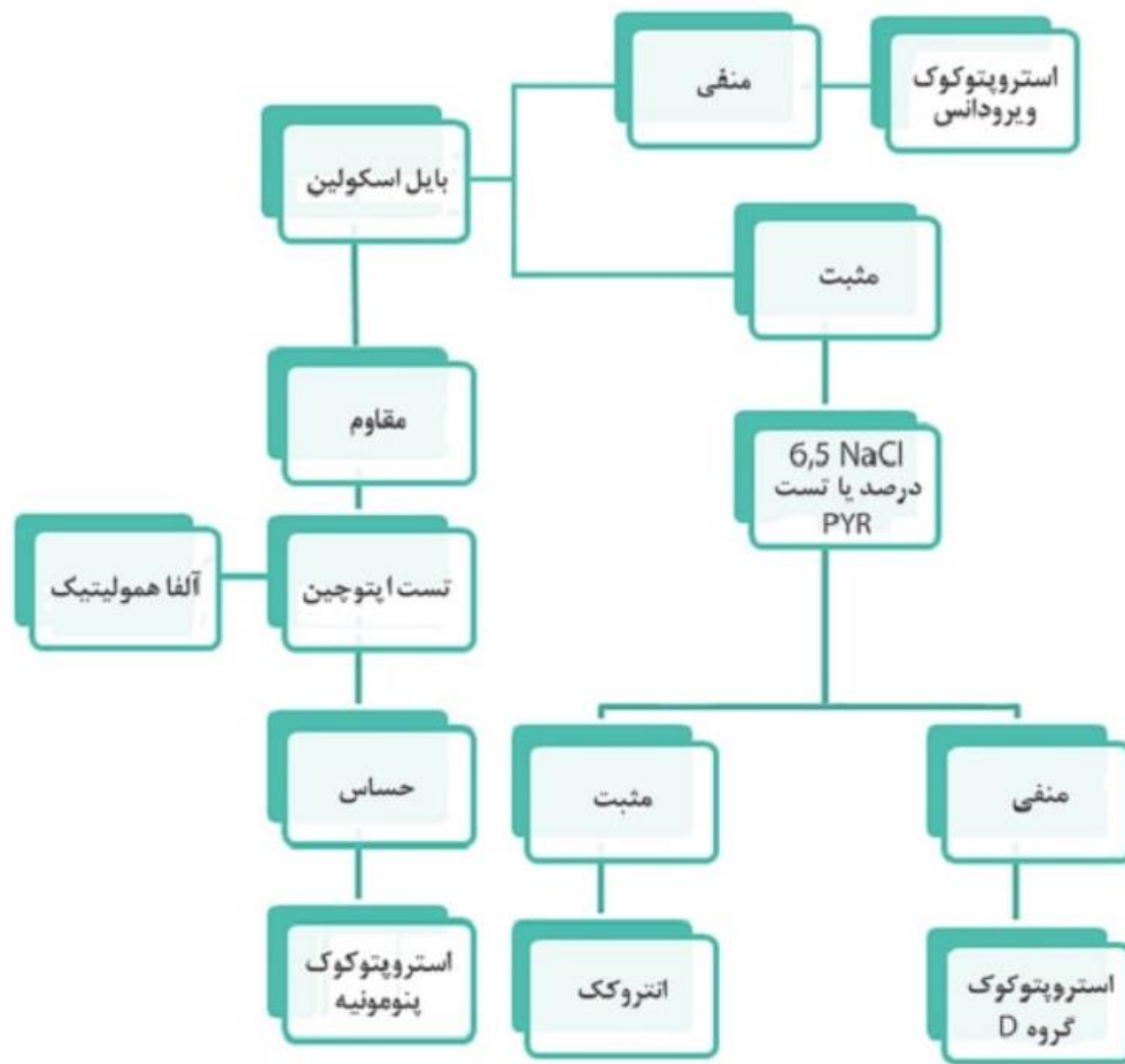
# Classification Based on O<sub>2</sub>



Griffith typing of Group A on MTR proteins into > 100 types

# CLASSIFICATION TABLE

SEROLOGIC	BIOCHEMICAL	HEMOLYTIC PATTERN
A	<i>S. pyogenes</i>	Beta
B	<i>S. agalactiae</i>	Beta, Alpha, Gamma
C	<i>S. equimilis</i>	Beta
D	<i>S. bovis</i> <i>S. faecalis</i>	Alpha, Gamma Alpha, Beta, Gamma
F	<i>S. milleri</i>	Alpha, Beta, Gamma
G	<i>S. milleri</i>	-do-
-	<i>S. pneumoniae</i>	Alpha
VIRIDANS	<i>S. salivarius</i> , <i>S. sanguis</i> , etc	Alpha, Gamma



شکل ۶. چارت برخی از باکتری‌های گرم مثبت



<b>TESTS</b>	<b>RESULTS</b>
<b>Coagulase</b>	-VE (NEGATIVE)
<b>Catalase</b>	-VE (NEGATIVE)
<b>Hyalurodinase</b>	+VE (POSITIVE)
<b>Neuraminidase</b>	+VE (POSITIVE)
<b>Acetoin production</b>	-VE (NEGATIVE)
<b>Indole</b>	-VE (NEGATIVE)
<b>MR (Methyl Red)</b>	+VE (POSITIVE)
<b>VP (Voges Proskauer)</b>	-VE (NEGATIVE)
<b>Alkaline Phosphatase</b>	+VE (POSITIVE)
<b>PYR</b>	+VE (POSITIVE)
<b>Urease</b>	-VE (NEGATIVE)
<b>DNase</b>	+VE (POSITIVE)
<b>Oxidase</b>	-VE (NEGATIVE)



با سپاس از توجه شما

در ستم من! یا خدایم