



دانشگاه اصفهان

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



آزمایشگاه باکتری شناسایی ۱

آزمون CAMP و انجام آزمون های بیوشیمیایی جهت شناسایی استرپتوکوک ها

Diagnostic Laboratory Tests

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

هیدرولیز سریع هیپورات سدیم:

این باکتری می‌تواند هیپورات سدیم را هیدرولیز کرده و اسید بنزوئیک و گلیسین تولید کند. گلیسین توسط عامل اکسیدکننده‌ای به نام نین‌هیدرین، دی‌آمینه شده، احیا می‌شود و تولید رنگ بنفش می‌کند.

If hippurate was not hydrolyzed to glycine, the addition of ninhydrin does not cause a color change.



Hippurase NEG

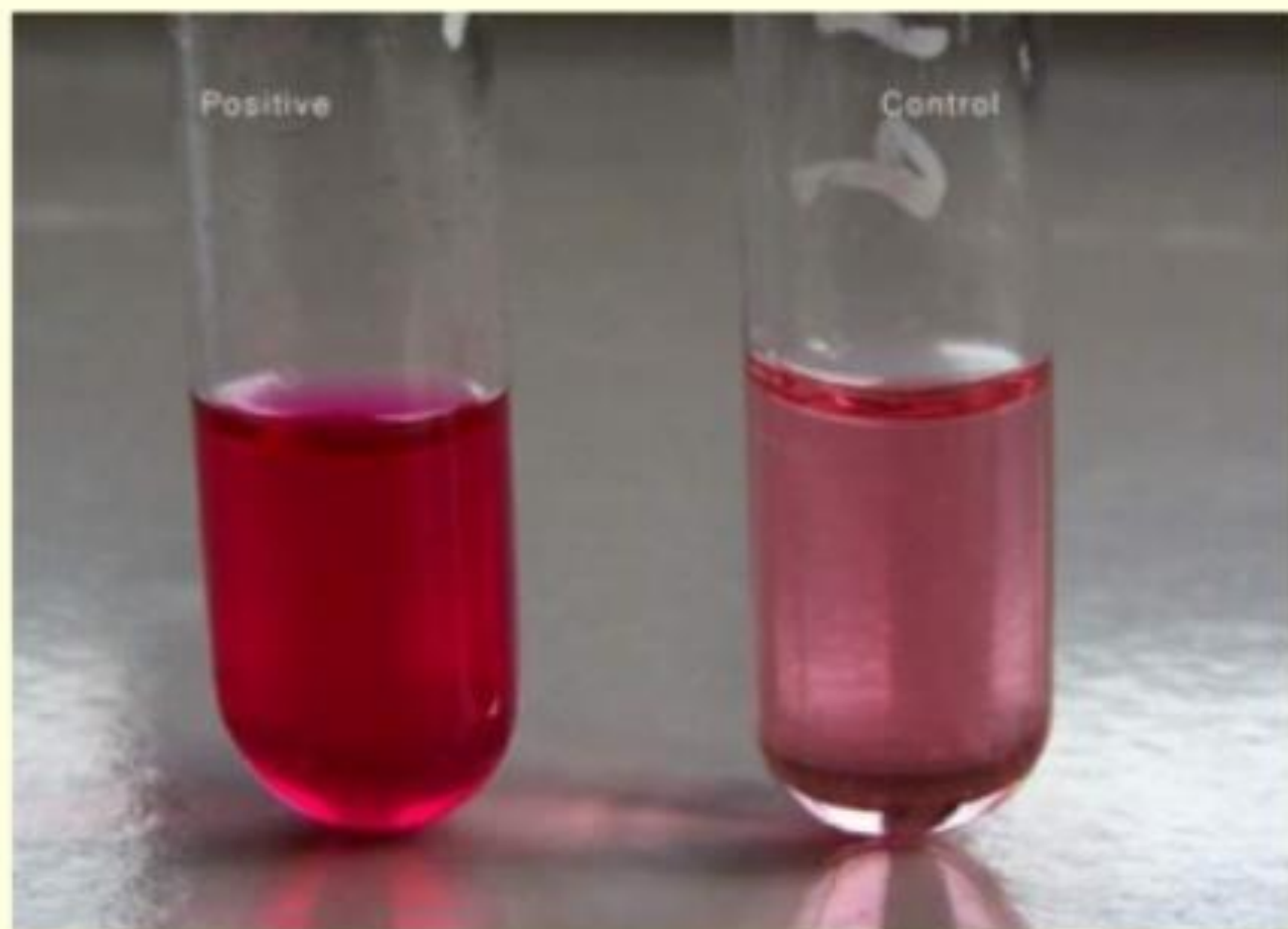
If glycine was produced from hippurate, it reacts with ninhydrin to produce a purple color.

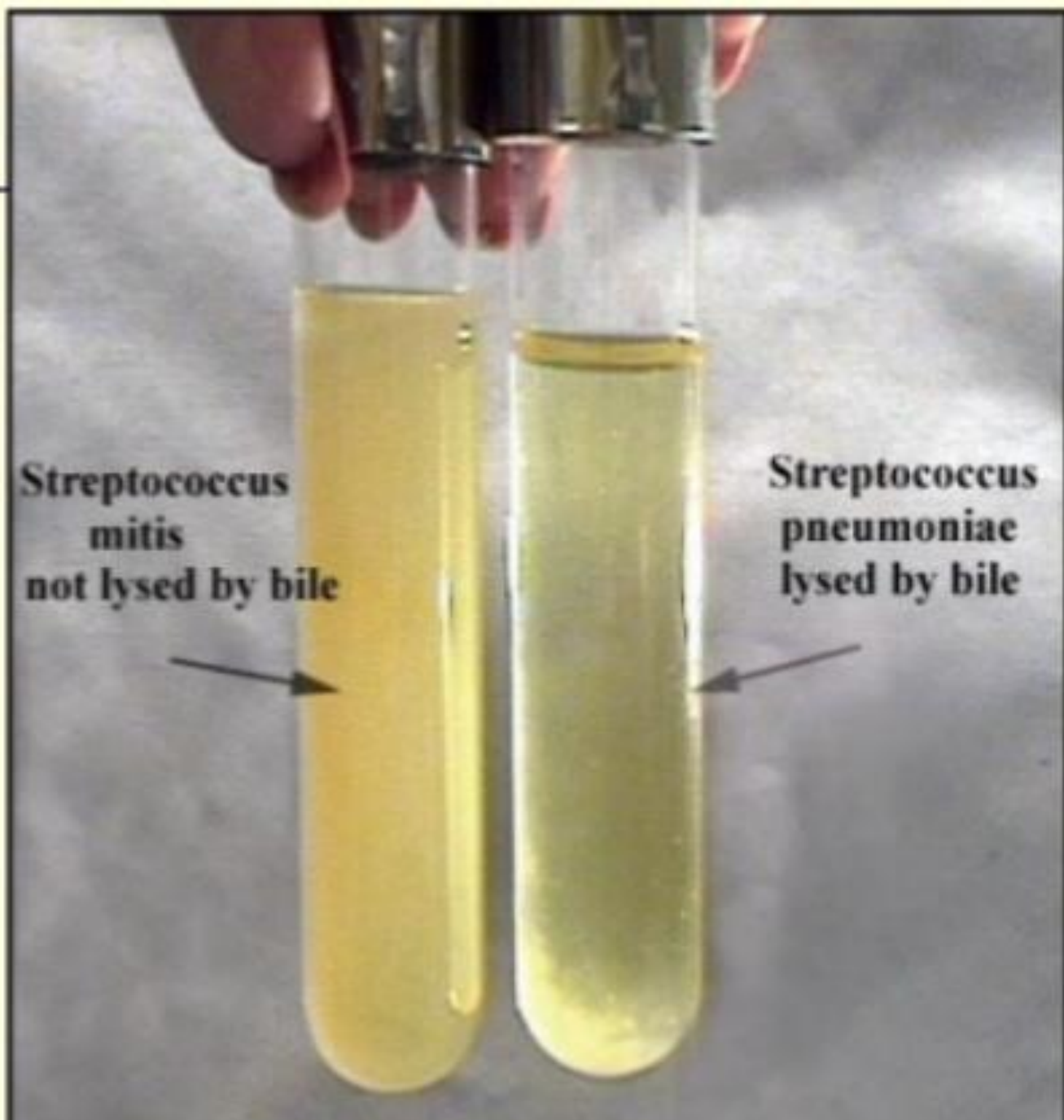
Grp B *Streptococci*



Hippurase POS

Inulin fermentation





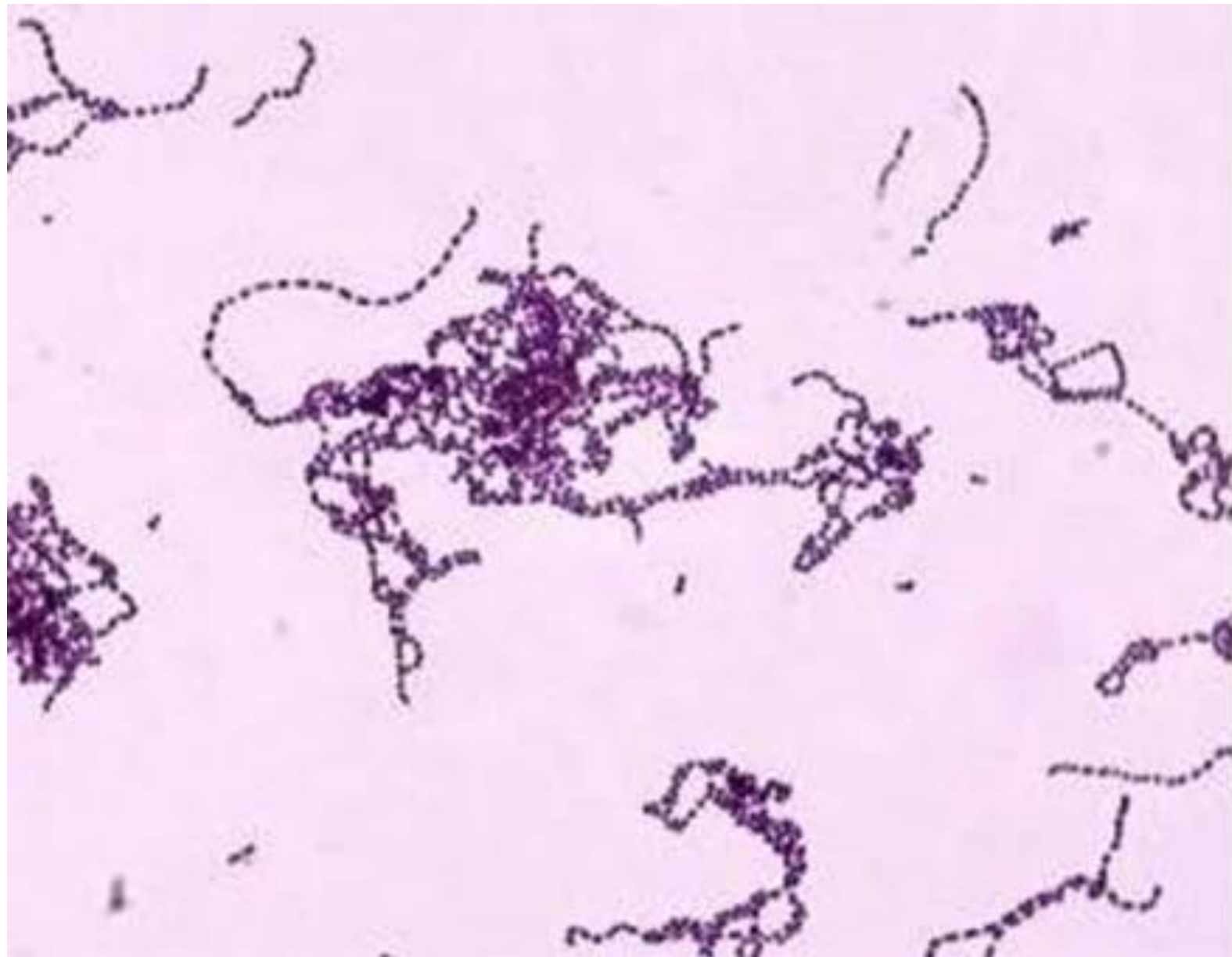
تست هیدرولیز PYR (L پیروگلوتامیک اسید بتانفتیل آمید):

هدف انجام این تست تشخیص احتمالی استرپتوکوک گروه A و انتروکوک از سایر استرپتوکوک هاست. استرپتوکوک پیوژنز و گونه های جنس انتروکوک قادر به هیدرولیز PYR هستند این تست برای تشخیص استرپتوکوک پیوژنز اختصاصی تر از تست حساسیت به باسیتراسین بوده و برای انتروکوک به اندازه تست بای لاسکولین و کلریدسديم اختصاصی می باشد. باکتری توسط پپتیدازهای خود PYR را هیدرولیز می کند و بتانفتیل آمید تولید می شود که با اضافه کردن معرف سیانامید رنگ قرمز تولید می گردد.

روش انجام تست:

به دیسک کاغذی آغشته به PYR که قبلاً مرطوب شده مقداری از باکتری را تلقیح می کنیم و بعد از گذشت دو تا سه دقیقه مقداری از معرف سیانامید به آن اضافه می کنیم. تولید رنگ قرمز نشا ندهنده مثبت بودن تست است.





Positive PYR

واکنش CAMP

اساس واکنش نشان دادن خصوصیات سینرژیستیک بین رها شدن ماده خارج سلولی در حین رشد از استرپتوکوک گروه B و واکنش آن با بتاتوکسین استافیلوکوک اورئوس است که سبب افزایش همولیز در استافیلوکوک اورئوس می‌شود. این واکنش در بعضی از انواع کورینه باکتریوم وجود دارد و به شکل دیگری در لیستریا منوسیتوژنز و ویبریو کلره التور دیده می‌شود. برای انجام آن به استافیلوکوک ATCC ۲۵۹۲۳ نیاز است.

این آزمایش به سه روش انجام می‌شود:

۱. استفاده از سوش‌های aureus تولیدکننده بتا همولیزین

۲. دیسک‌های حاوی بتا همولیزین

۳. بتا همولیزین استخراج‌شده از استافیلوکوک اورئوس

همولیز بتا، در محلی که دو سویه استاف اورئوس و استرپتوکوک گروه B به هم نزدیک می‌شوند، تشدید می‌شود و شبیه به نوک پیکان دیده می‌شود و در این صورت تست CAMP مثبت می‌شود.

روش سوم به این صورت است که اگر یک قطره از بتا همولیزین استخراج‌شده از استافیلوکوک اورئوس، روی محیط کشت استرپتوکوک ریخته شود، بعد از ۲۰ دقیقه انکوبه کردن در دمای ۳۷ درجه سانتی‌گراد، شدت همولیز به خوبی قابل مشاهده می‌گردد.

تست CAMP:

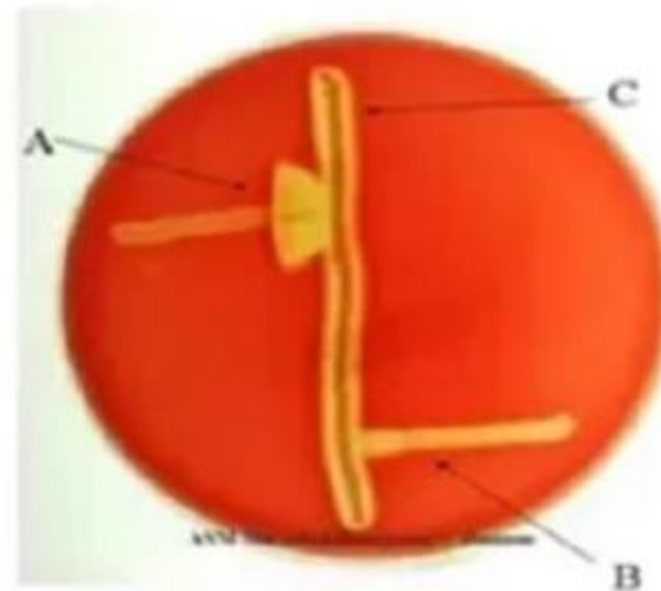
برای شناسایی احتمالی گروه B صورت می‌گیرد. تست CAMP مخفف نام افرادی است که این تست را برای نخستین بار ابداع کردند که شامل Christie-Atkins-Munch-Petersen می‌باشد.

The CAMP test: Identification of *Streptococcus agalactiae*

(A) *Streptococcus* (group B)
positive test
(enhanced hemolysis)

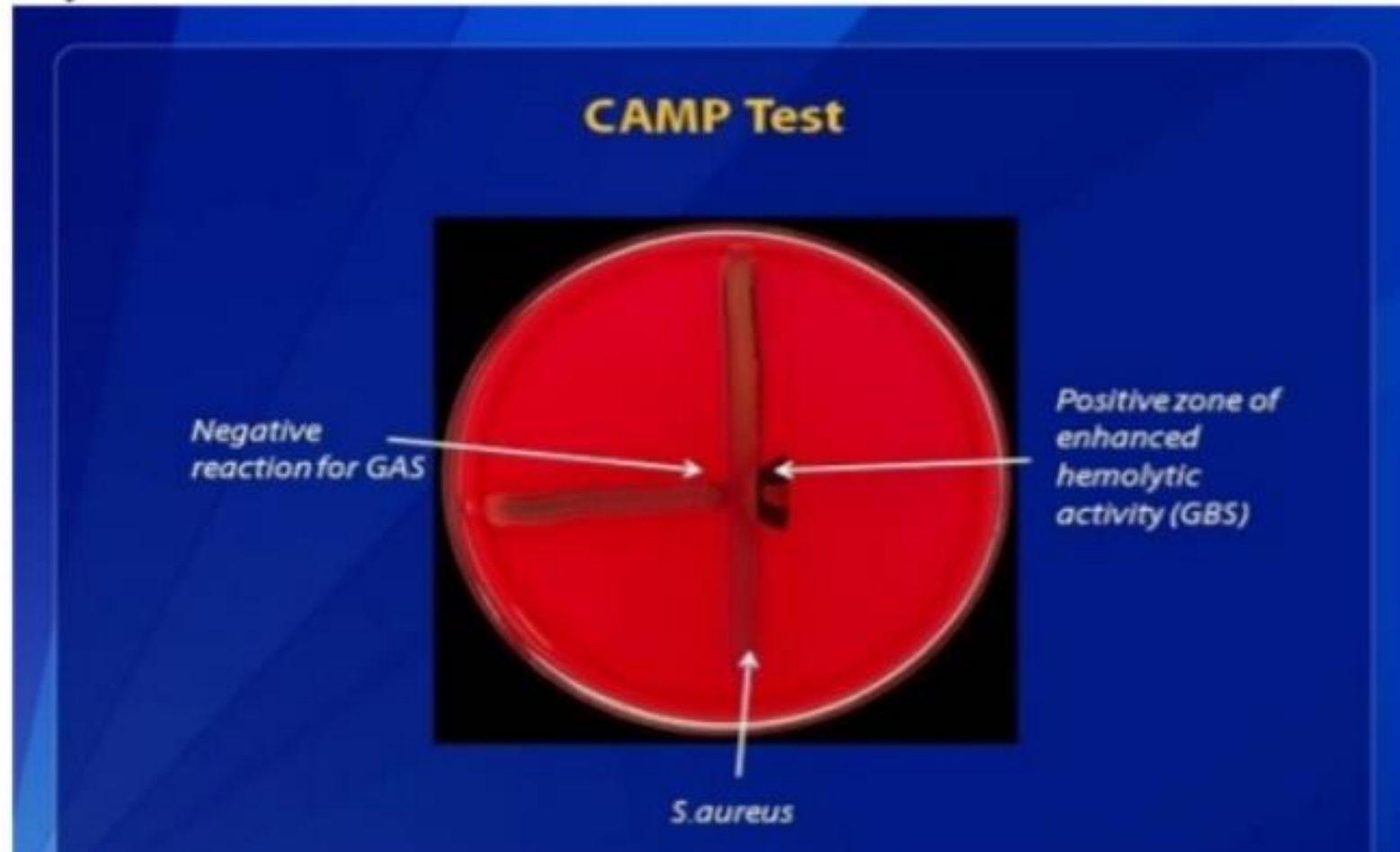
(B) *Streptococcus pyogenes* (group A)
negative test

(C) *Staphylococcus aureus* – replaced by
Clostridium perfringens
in reverse CAMP test



CAMP test

- Christie, Atkins and Munch-Peterson
- When *S. agalactiae* is inoculated perpendicular to a streak of *S. aureus* grown on blood agar → an accentuated zone of hemolysis occurs



Streptococci is Gram-positive spherical/ovoid cocci arranged in long chains; commonly in pairs.

- **Classification** of streptococci includes;

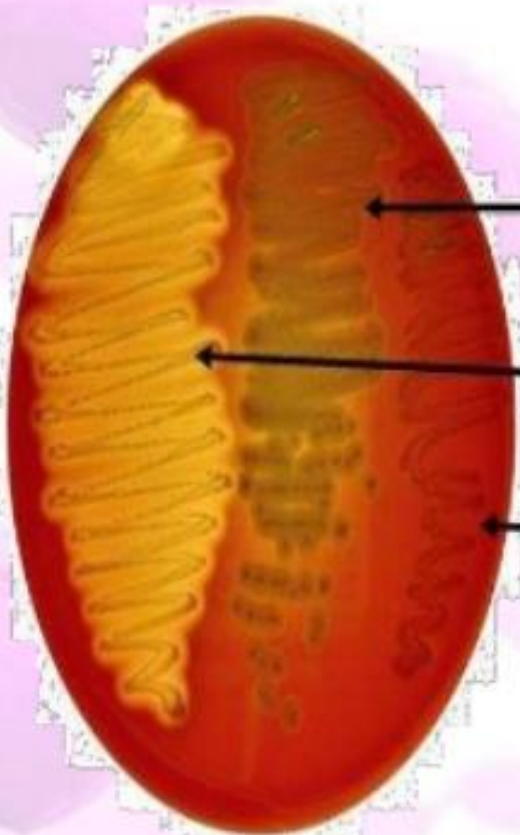
- Classification system is based on hemolysis reactions.

- β - hemolytic - A, B, C, D and G (*S. pyogenes*)

- α - hemolytic - *S. pneumoniae* and *viridans*

- γ - hemolytic - *S. faecalis*

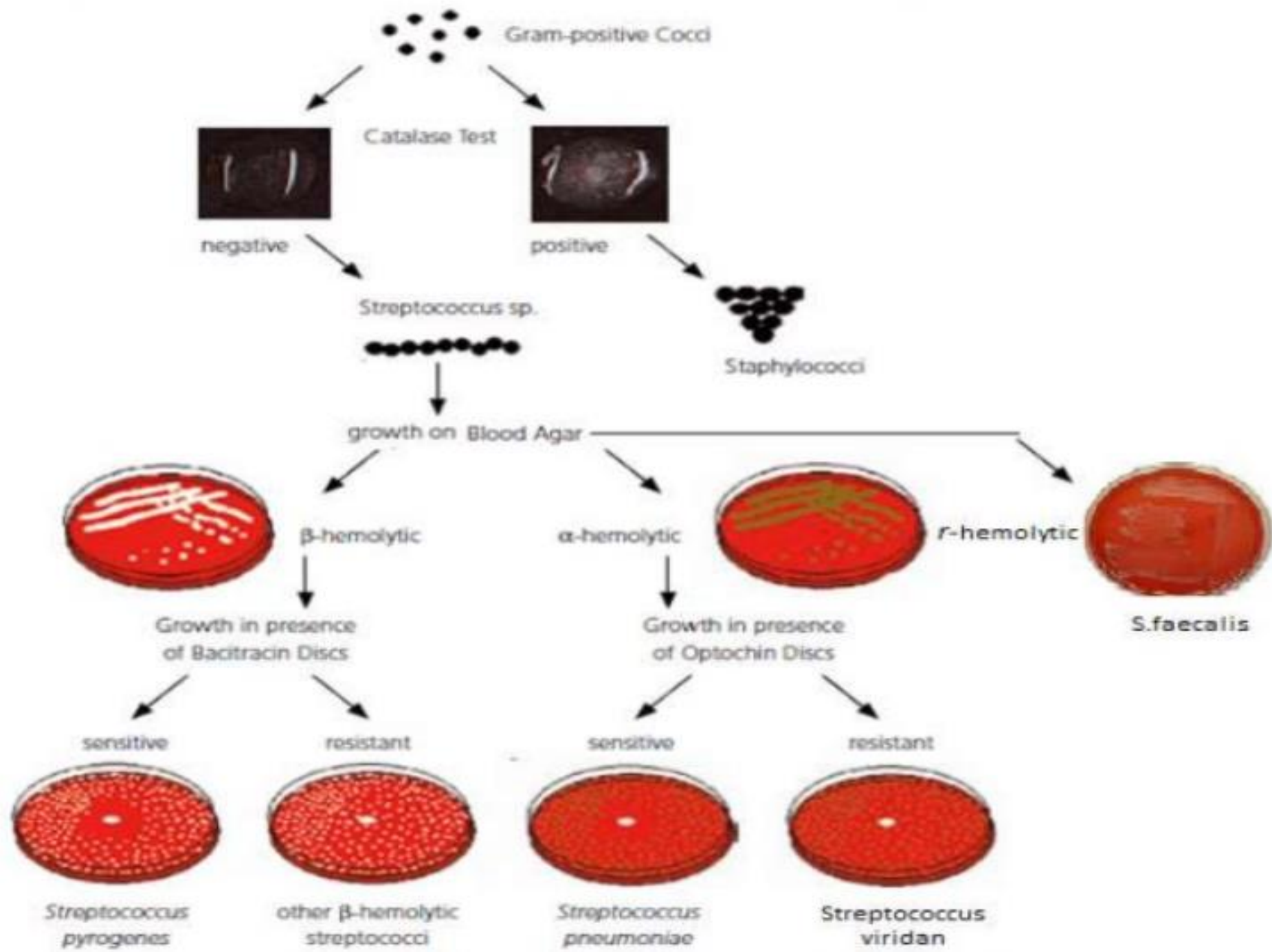
- Lancefield classification of β - hemolytic streptococci based on presence of carbohydrate antigen in cell wall - 17 groups (A, B, C,....)



alpha hemolysis – partial; *S. pneumoniae*

beta hemolysis – complete; *S. pyogenes*

gamma hemolysis – none; *E. faecalis*



General Characteristics of Streptococci

- Gram-positive spherical/ovoid cocci arranged in long chains; commonly in pairs.
- Non-spore-forming, nonmotile.
- Can form capsules
- Facultative anaerobes
- Most parasitic forms are fastidious and require enriched media.
- Small, non pigmented colonies.
- Sensitive to drying, heat, and disinfectants.

Human Streptococcal Pathogens

- **S. Pyogenes**
- **S. Viridans**
- **S. Pneumoniae**
- **S. Faecalis**

S. Pyogenes

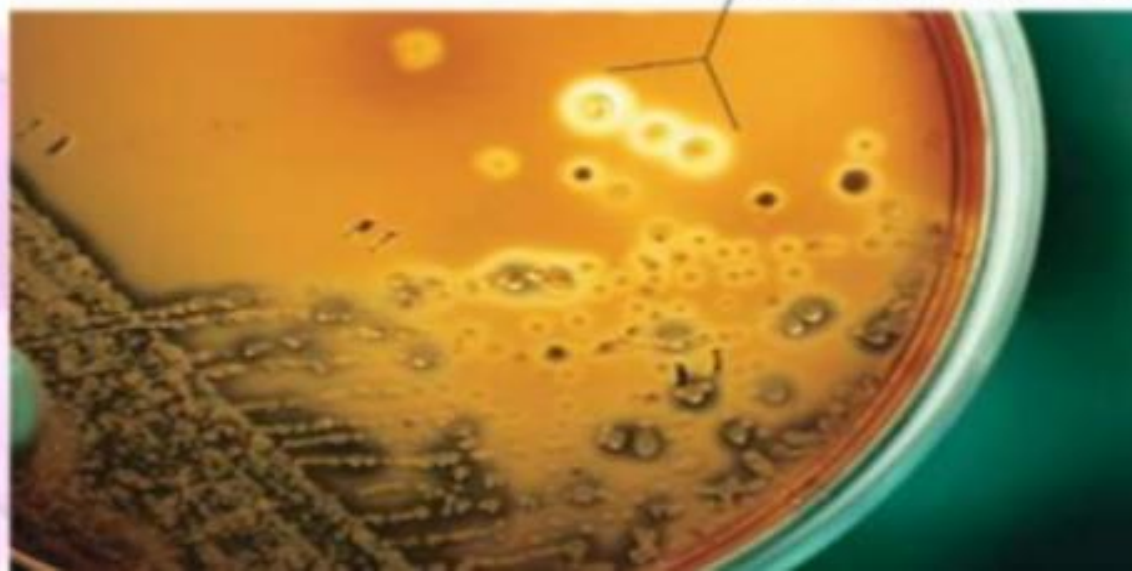
- Most serious streptococcal pathogen. It a parasite and inhabits throat, nasopharynx, occasionally skin.

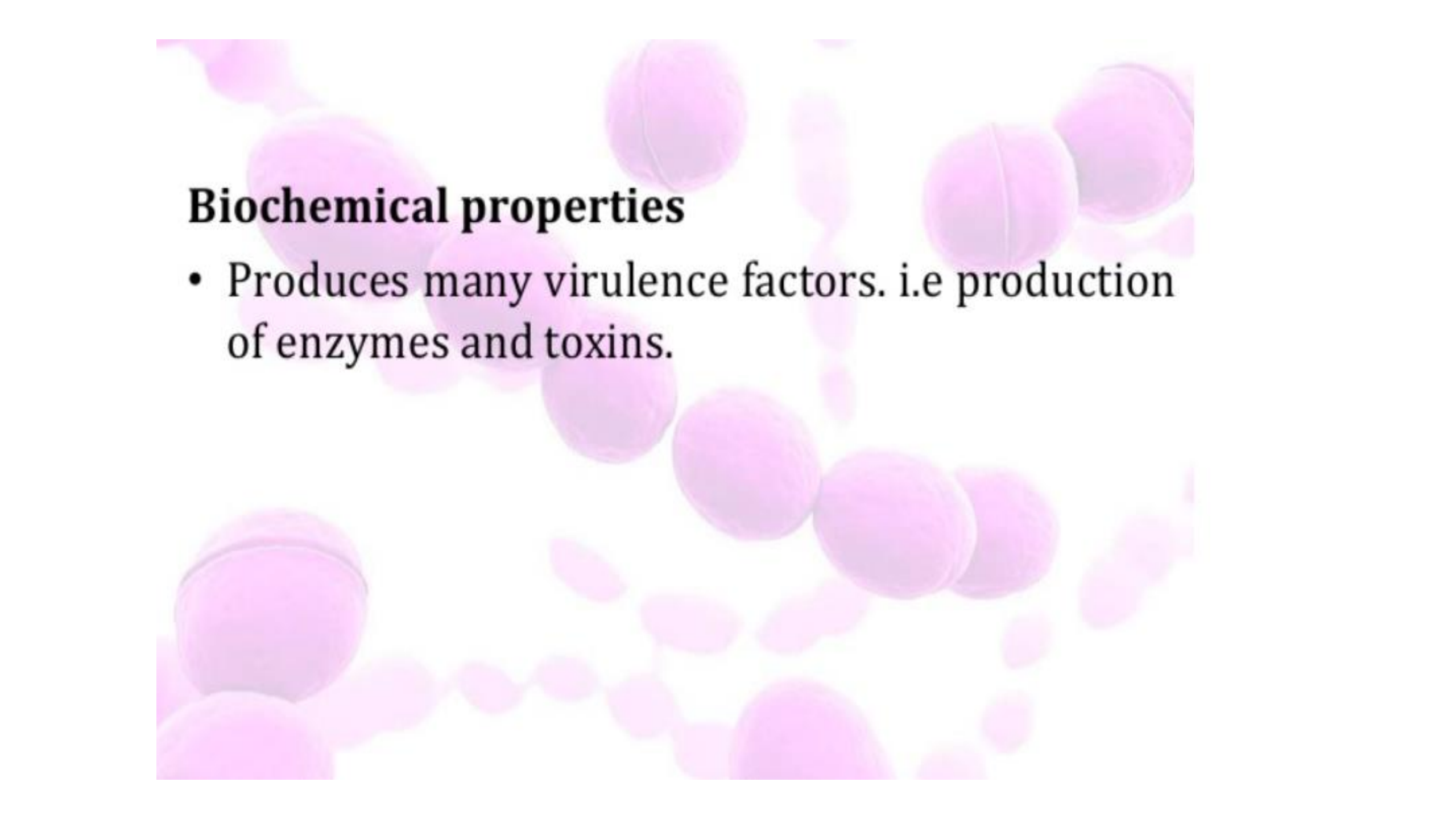
Morphology

- Gram-positive
- Spherical/ovoid cocci arranged in long chains.
- Nonmotile.
- Can form capsules.

Cultural characteristics

- Facultative anaerobes.
- Best growth achieved at pH 7.4-7.6 and temperature 37°C.
- Required enriched media for growth.
- Colonies are around 1mm in diameter, surrounded by a zone of clear hemolysis, semi transparent and vary in appearance.





Biochemical properties

- Produces many virulence factors. i.e production of enzymes and toxins.

Virulence factors of *S. Pyogenes*

➤ **Enzymes:**

- Streptokinase – digests fibrin clots
- Hyaluronidase – breaks down connective tissue
- DNase – hydrolyzes DNA

➤ **Toxins :**

- Streptolysins (hemolysins):- streptolysin O (SLO) and streptolysin S (SLS) – both cause cell and tissue injury.
- Erythrogenic toxin (pyrogenic):- Induces fever and typical red rash.
- Superantigens:- Strong monocyte and lymphocyte stimulants; cause the release of tissue necrotic factor.

Pathogenesis

- Humans are the only reservoir.
- In apparent carriers.(visible carriers of toxins and enzyme)
- Transmission is through contact, droplets, food, fomites
- Portal of entry is generally through skin or pharynx.
- Children are predominant group affected for cutaneous and throat infections
- Systemic infections and progressive diseases may occur if untreated.

S. pyogenes diseases

- **Impetigo (pyoderma)**- It is a skin infection with superficial lesions that break and form highly contagious crust.
- **Throat infections**
- **Rheumatic fever** – It has subclinical pharyngitis in children; carditis with extensive heart valve damage possible, arthritis, chorea, fever.
- **Acute glomerulonephritis** – It is the inflammation of the nephrons.



Impetigo



Pharngitis

Laboratory diagnosis

- *S. pyogenes* is frequently isolated from samples such as skin, throat, sputum, urine, and blood.
- Different methods for laboratory diagnosis of *S. pyogenes* are:
 - ❖ Gram staining
 - ❖ Culture
 - ❖ Catalase test

❖ **Gram staining**

The diagnosis is suggested by the finding of gram positive bacteria cocci in chains in the sample.

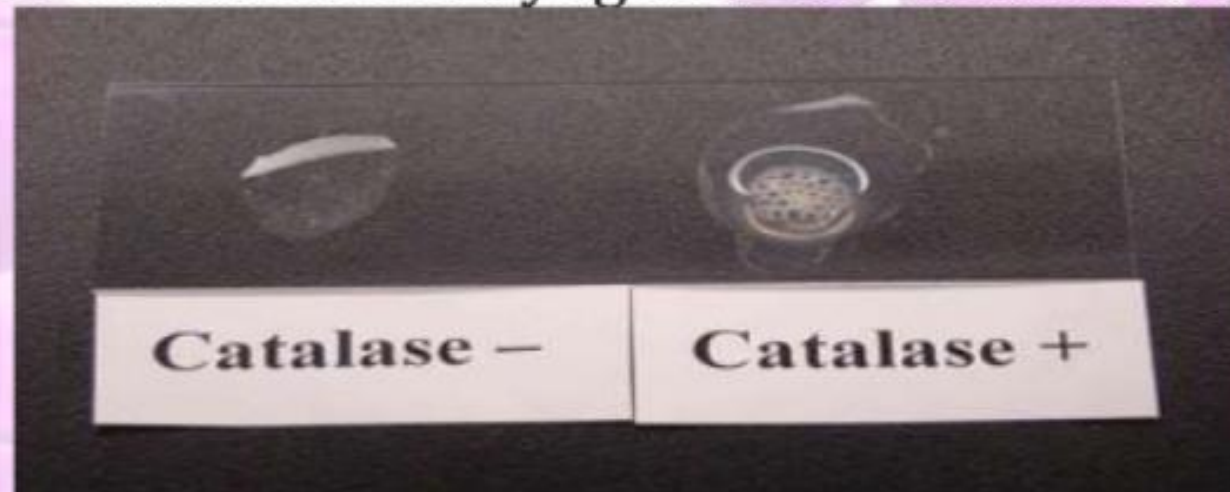
❖ **Culture**

The organism is cultured on blood agar with an added bacitracin antibiotic disk to show beta-hemolytic colonies and sensitivity (zone of inhibition around the disk) for the antibiotic.

❖ Catalase test

Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick. Place a drop of 3% H_2O_2 on to the slide and mix.

A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling. A negative result is no bubbles or only a few scattered bubbles. *S. Pyogenes* are catalase negative.

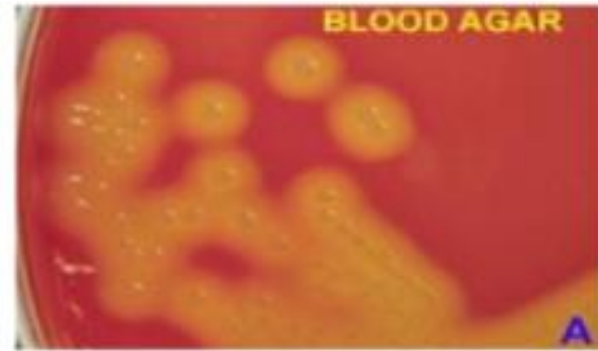


Treatment

- S. Pyogenes infections are treated with penicillin
- Erythromycin is recommended for patients who are allergic to penicillin.

Lab diagnosis – Strep. pyogenes

- Specimens: throat swab, pus, blood
- Microscopy :Gram stain - GPC in chains
- Culture: BA - beta hemolytic colonies
- Identification tests -
 - Catalase Negative
 - Bacitracin sensitive
 - Penicillin sensitive
 - ASO titre / DNAase B test

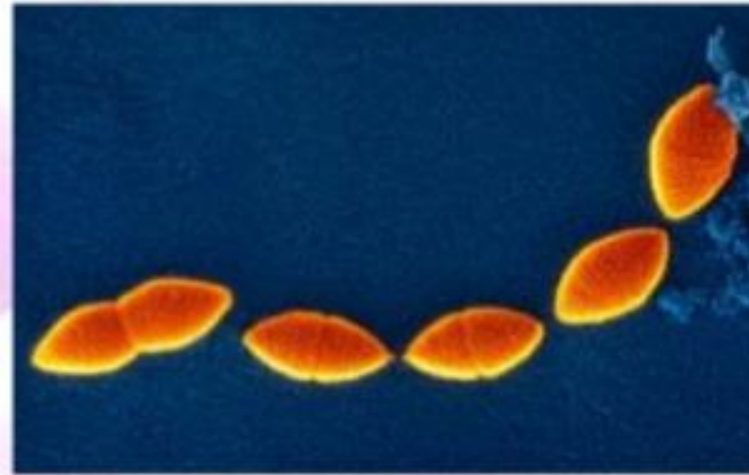


STREPTOCOCCUS PNEUMONIAE

These are commonly seen in nasopharynx of healthy persons. It will not cause any illness itself unless a viral infection or other factors provokes.

Morphology

- They are gram positive cocci.
- Measures 0.5 – 1.25 μm
- Non motile and non sporing
- Capsulated
- Small, oval-shaped cells arranged in pairs and short chains.



Cultural characteristics

- Culture requires blood or chocolate agar.
- Growth improved by 5-10% CO₂.
- Facultative anaerobes.
- Best growth achieved temperature 25 - 40°C.
- Colonies are surrounded by greenish hemolysis under aerobic conditions and clear under anaerobic conditions.



Biochemical properties

- No significant toxin is produced in pneumococci.
- Presence of some of the extra cellular products
 - Haemolysin
 - Immunoglobulin A1 protease
 - Neuraminidase
 - Hyaluronidase
- Lack catalase and peroxidases.
- Ferment glucose, lactose and sucrose with production of acid.

Pathogenesis

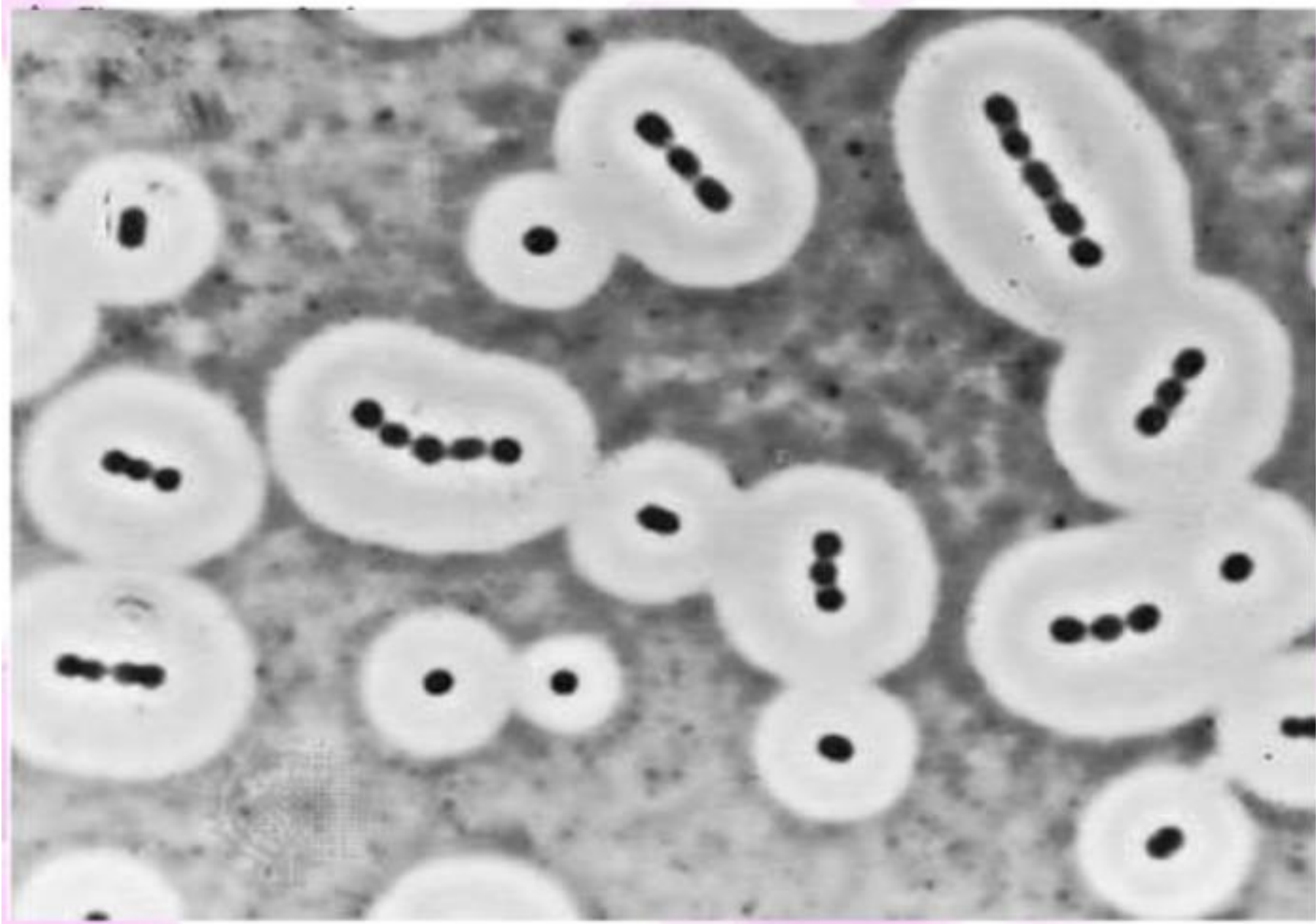
- 5-50% of all people carry it as normal flora in the nasopharynx.
- Young children, elderly, immune compromised, those with other lung diseases or viral infections, are at risk.
- Pneumococci multiply and induce an inflammatory response.
- Gains access to middle ear by way of eustachian tube.

Pneumococci diseases

- Pneumonia : infection of lung parenchyma.
- Otitis media : infection of middle ear

Laboratory diagnosis

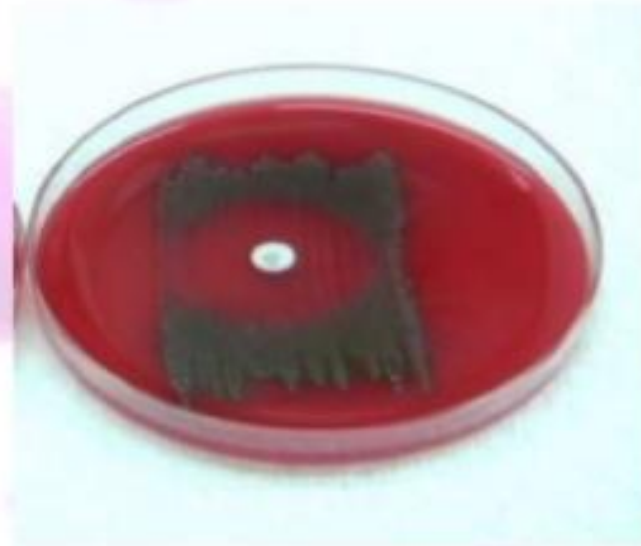
- S. Pneumococci is frequently isolated from samples such as sputum, blood, wound, CSF.
- Different methods for laboratory diagnosis of S. Pneumococci are:
 - ❖ Gram staining
 - ❖ Quellung test
 - ❖ Culture
 - ❖ Catalase test



❖ Culture

The organism is cultured on blood agar or chocolate agar with incubation in an environment of carbon dioxide.

The organism is cultured on blood agar with an added optochin disk to show alpha-hemolytic colonies.



❖ Catalase test

S. pneumoniae is catalase negative.

S. Viridans

- It is alpha haemolytic streptococci.
- It is oval in shape and found in short chains.
- It has six species groups (viridans group);
S. mutans, S. oralis, S. salivarius,
S. sanguis, S. milleri, S. mitis
- Found in gums and teeth, oral cavity, and also in nasopharynx, genital tract, skin.
- Not very invasive; dental or surgical procedures facilitate entrance.
- It causes dental caries, sepsis and endocarditis.
- Persons with preexisting heart conditions should receive prophylactic antibiotics before surgery or dental procedures.

S. Faecalis

- It is also known as enterococci.
- It is a Gram-positive
- Oval cocci in pairs or short chains.
- Identified in MaConkey agar. Colonies are magenta in color and pin point.
- It can grow in the range of 10 to 45°C and survive at temperatures of 60°C for 30 min.
- It is non-motile, facultative anaerobic microbe.
- It ferments glucose and does not produce a catalase.
- It is associated with urinary tract infections, biliary tract infections, septicemia, endocarditis and intra abdominal abscess.
- Aminogycosides are drug of choice.

Table 6.1. *First-stage table for Gram-positive bacteria*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Shape	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Acid fast	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	w	+
Spores	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Motility	-	-	-	-	D	-	-	d	-	-	+	-	-	-	-	-	D	D	-	-	-
Growth in air	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	d	+	+	+	+
Growth anaerobically†	-	+	w	w	+	+	+	-	+	+	+	+	-	+	+	+	+	D	-	-	?
Catalase	+	+	w	-	-	-	-	+	+	+	+	-	+	+	-	-	-	+	+	+	+
Oxidase	+	-	-	-	-	-	-	-	-	-	-	-	?	?	?	?	?	d	-	-	-
Glucose (acid)	D	+	+	+	+	+	+/-	-	-	+	+	+	+	+	+	-	D	D	+	+	+
Carbohydrates [F/O/-]	O/-	F	F	F	F	F	F/-	-	-	F	F/-	F	F	F	F	-	F/-	F/O/-	O	O	O/NT

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Haemolysis	β	β/-	β	α	β	β	β	β/-	β	α/-	β/-	β	α	α/β	α/β	-	-
Requires CO ₂ for growth	-	-	-	-	-	-	-	d	-	-	-	-	d	-	-	-	+
Growth at 45 °C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth in 6.5% NaCl broth	-	d ^a	-	-	-	-	- ^a	- ^a	-	-	- ^a	-	-	-	-	-	-
Growth on 40% Bile Agar	-	+	-	-	-	-	-	d	-	-	d	d	-	d	-	-	-
Leucine aminopeptidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile-aesculin test	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-
Voges-Proskauer test ^b	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
Pyrryldonylarylamidase	+	-	-	-	-	-	-	-	-	d	d	-	d	-	-	+	+
Phosphatase	+	+	+	+	+	+	+	+	+	d	-	+	-	D	d	-	-
Pyridoxal or cysteine dependence	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Hydrolysis of																	
hippurate	-	+	-	-	-	-	-	-	-	+	-	d	-	-	-	-	-
aesculin	d	-	-	-	d	d	d	d	+	+	+	-	d	+	d	-	-
arginine	+	+	+	+	+	+	+	+	+	+	+	+	d	+	-	-	-
starch ^c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sensitive to																	
bacitracin (0.1 unit)	+	d	d	d	-	-	-	-	-	d	d	d	+	d	d	-	-
optochin	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
H ₂ O ₂ production	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-
Fermentation of																	
pyruvate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ribose	-	+	-	+	d	+	+	-	+	+	-	+	-	-	-	-	-
arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
mannitol	D ^d	-	-	-	-	-	-	d	+	+	-	-	-	-	-	-	-
sorbitol	-	-	-	d	+	-	-	-	+	+	-	-	-	-	-	-	-
adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d	(w)
lactose	+	d	-	+	+	d	d	d	d	+	+	+	+	+	+	d	-
trehalose	+	+	-	+	-	+	+	+	+	+	+	+	+	+	d	d	(w)
raffinose	-	-	-	-	-	-	-	d	-	d	D ^e	-	d	d	d	d	-
inulin	-	-	-	-	-	-	-	-	-	+	d	-	d	d	-	-	-
starch	+	d	+	+	+	+	+	d	d	d	+	+	d	d	d	d	(w)
Polysaccharide from sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-	Dx/-	-/Dx	-	-
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yellow pigment	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lancefield antigen	A	B	C	C	C	C	G	-/F/ G/C/A	E/P/ U/V/-	-/E	R/S/ T/-	L	-	-/H	-/O/K-	-	-

1 *Streptococcus pyogenes*; *S. haemolyticus*
2 *Streptococcus agalactiae*
3 *Streptococcus equi*
4 *Streptococcus dysgalactiae*
5 *Streptococcus zooepidemicus*
6 *Streptococcus equisimilis*
Streptococcus spp. group C

7 *Streptococcus* spp. group G (large colony variety); *Streptococcus canis*
8 *Streptococcus anginosus*; '*S. milleri*'; minute colony haemolytic streptococci; *S. intermedius*-MG; *S. constellatus*
9 *Streptococcus porcinus*; *S. lentus*
10 *Streptococcus uberis*
11 *Streptococcus suis*

12 *Streptococcus* spp. group L
13 *Streptococcus pneumoniae*; pneumococci
14 *Streptococcus sanguis*
15 *Streptococcus oralis*; '*S. mitior*'
16 *Streptococcus* spp. pyridoxal or cysteine dependent
17 *Streptococcus morbillorum*

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Haemolysis	-/β	α/-	-	α/-	β	α	-/α/β	-/α	α/-	α	-/α/β	-	-/α	-/α	-/α	α	β/α/-
Requires CO ₂ for growth	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Growth at 45 °C	+	+	+	+	+	+	+	d	d	d	-	-	-	-	+	-	-
Growth in 6.5% NaCl broth	+	+	+	+	+	+	+	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	- ^a	-	+	-
Growth on 40% Bile agar	+	+	+	+	+	+	+	+	+	+	d	d	+	d	+	+	-
Leucine aminopeptidase	+	+	+	+	+	+	+	+	+	+	+	+	+	d	+	-	+
Bile-aesculin test	+	+	+	+	+	+	+	+	+	+	d	d	d	d	+	d	-
Voges-Proskauer test ^b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d	w
Pyrrrolydonylarylamidase	+	+	+	+	+	+	+	-	-	-	-	-	d	d	-	d	+
Phosphatase	-	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	+
Pyridoxal or cysteine dependence	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrolysis of																	
hippurate	d	d	-	-	+	d	d	-	-	-	-	-	d	-	d	d	-
aesculin	+	+	+	+	+	+	+	+	+	+	+ ^f	+	+	+	+	d	-
arginine	+	+	+	+	+	-	+	-	-	-	- ^g	-	+	-	+	-	-
starch ^c	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Sensitive to																	
bacitracin (0.1 unit)	-	-	-	-	-	-	-	-	-	-	-	d	d	-	d	d	d
optochin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H ₂ O ₂ production	-	d	-	+	-	d	-	-	-	-	- ^f	-	-	d	+	+	-
Fermentation of																	
pyruvate	+	-	-	-	-	(w)	-	-	-	-	-	-	-	-	-	-	-
ribose	+	+	+	+	+	+	+	-	-	-	-	-	+	d	+	d	-
arabinose	-	+	+	+	+	d	-	d	-	-	-	-	d	d	d	-	-
mannitol	+	+	+	+	+	+	-	d ^h	-	-	+	-	d	d	-	d	-
sorbitol	+	d	d	-	-	+	-	-	-	-	+ ^f	-	-	-	-	d	-
adonitol	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
sucrose	+	+	+	+	+	+	-	+	+	+	+	+	d	+	-	+	d
lactose	+	+	+	+	+	+	+	+	+	-	+	+	d	d	d	d	-
trehalose	+	+	+	+	+	+	d	+	d	-	+	d	d	+	+	+	d
raffinose	-	d	d	+	+	-	+	+	d	+	+	+	-	d	-	d	-
inulin	-	d	-	+	+	d	-	d	d	+	d	+	-	-	-	d	-
starch	+	d	-	-/w	+	-	-	+	-	-	-	d	+	-	-	-	d
Polysaccharide from sucrose	-	-	-	-	-	-	-	Dx/-	-	-	Dx	Lv/Dx	-	Dx/-	-	-	-
Motility	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Yellow pigment	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Lancefield antigen	D	D	D	D	D	Q/D ^j	D	D	D	D	D	-/E	-/K	N	-	D/-	-

18 *Enterococcus faecalis*; *S. faecalis*

19 *Enterococcus faecium*

20 *Enterococcus mundtii*

21 *Enterococcus casseliflavus*

22 *Enterococcus gallinarum*

23 *Enterococcus avium*

24 *Enterococcus durans*; *S. faecium*

var *durans*; *S. durans*

25 *Streptococcus bovis* biotype I

26 *Streptococcus bovis* biotype II

27 *Streptococcus equinus*

28 *Streptococcus mutans*, serotypes

c, e and f

29 *Streptococcus salivarius*

30 *Lactococcus lactis*

31 *Leuconostoc* spp.

32 *Pediococcus* spp.

33 *Aerococcus* spp.; *Pediococcus*; *Gaffkya* spp.

34 *Gemella haemolysans*; *Neisseria haemolysans*

The background of the slide is a light, ethereal space filled with numerous spheres of varying sizes and colors. The colors range from soft pinks to vibrant purples. Some spheres are in sharp focus, while others are blurred, creating a sense of depth and movement. The overall aesthetic is clean, modern, and celebratory.

THANK YOU