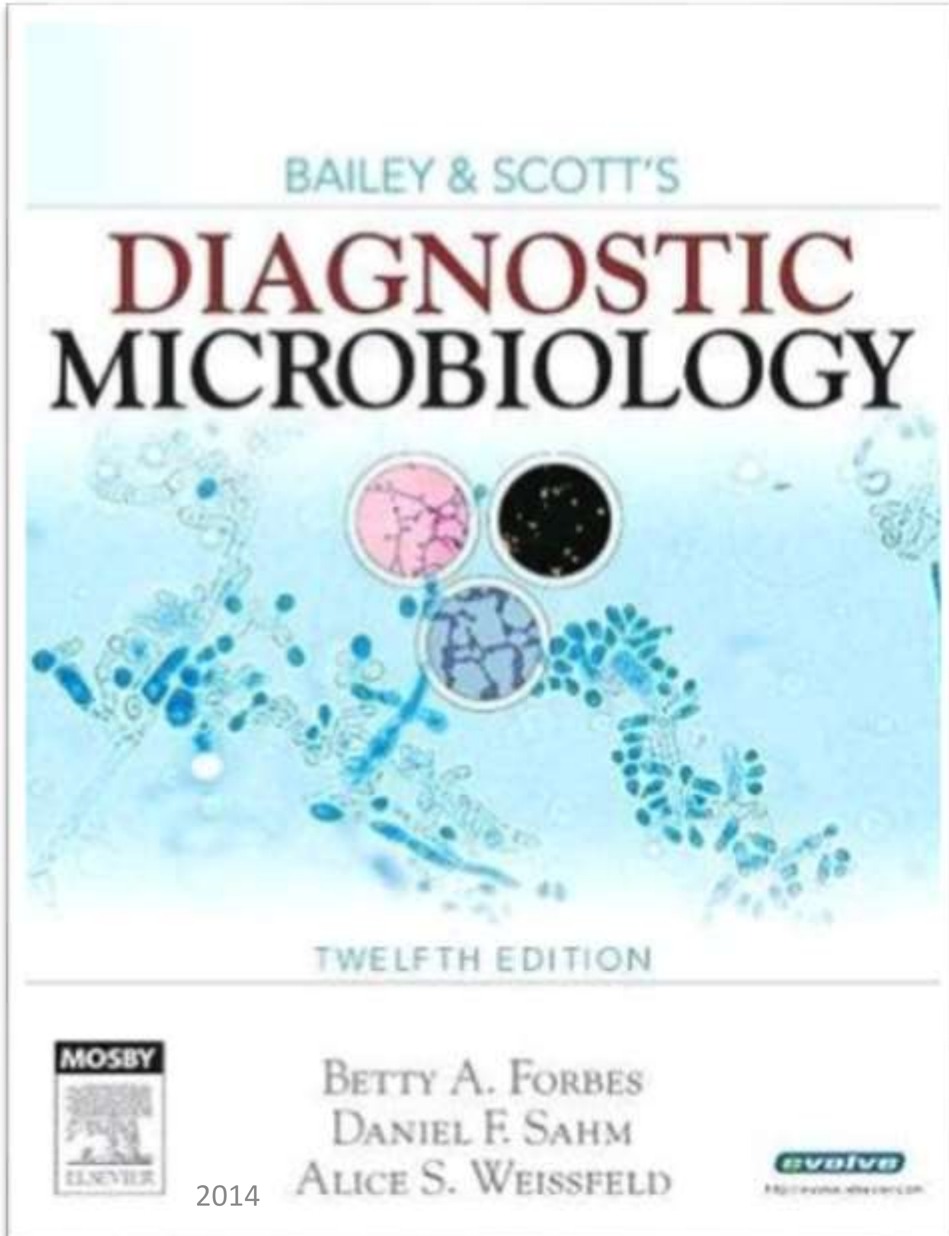
King Saud University
Dept. of Bot. & Microbiology



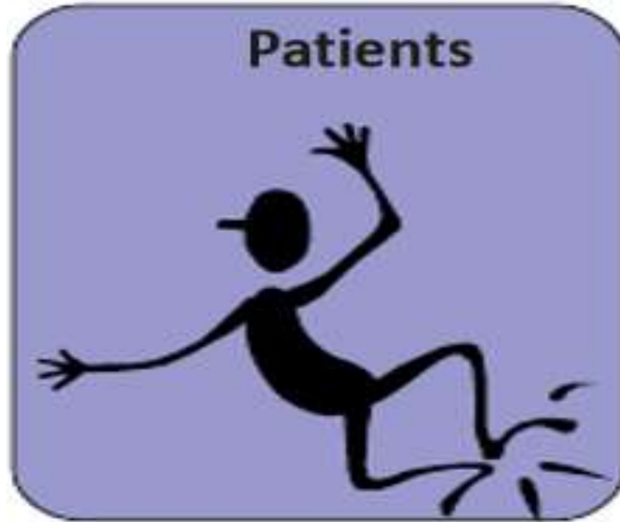

# Diagnostic Microbiology

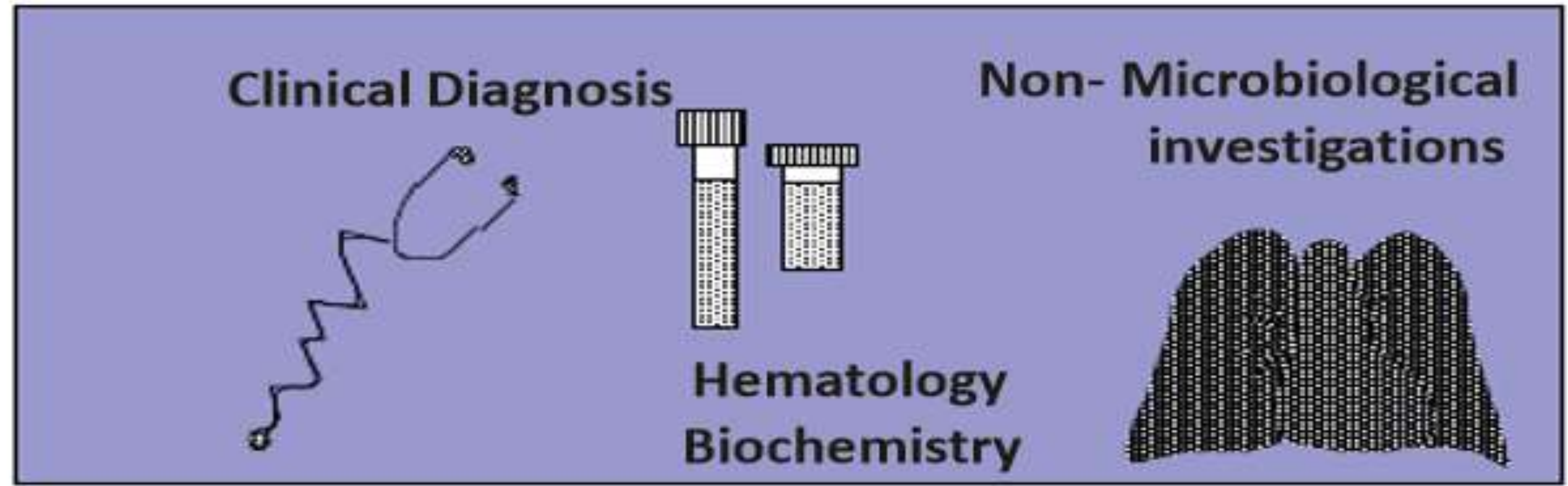
## 320 MIC

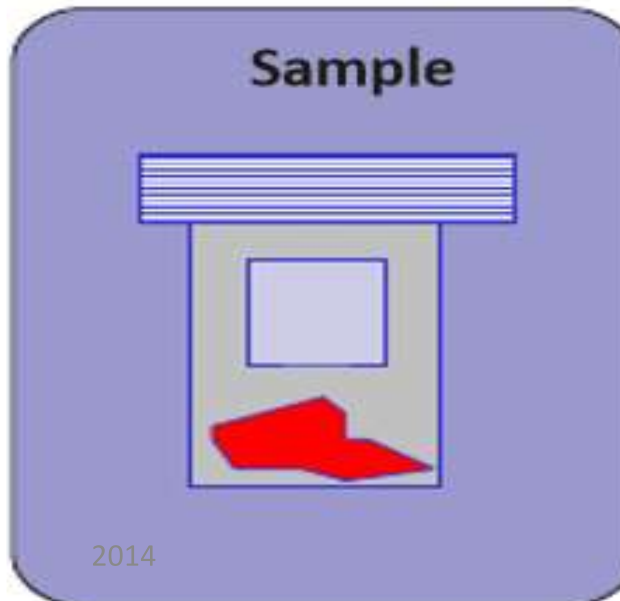
## Lecture: 9

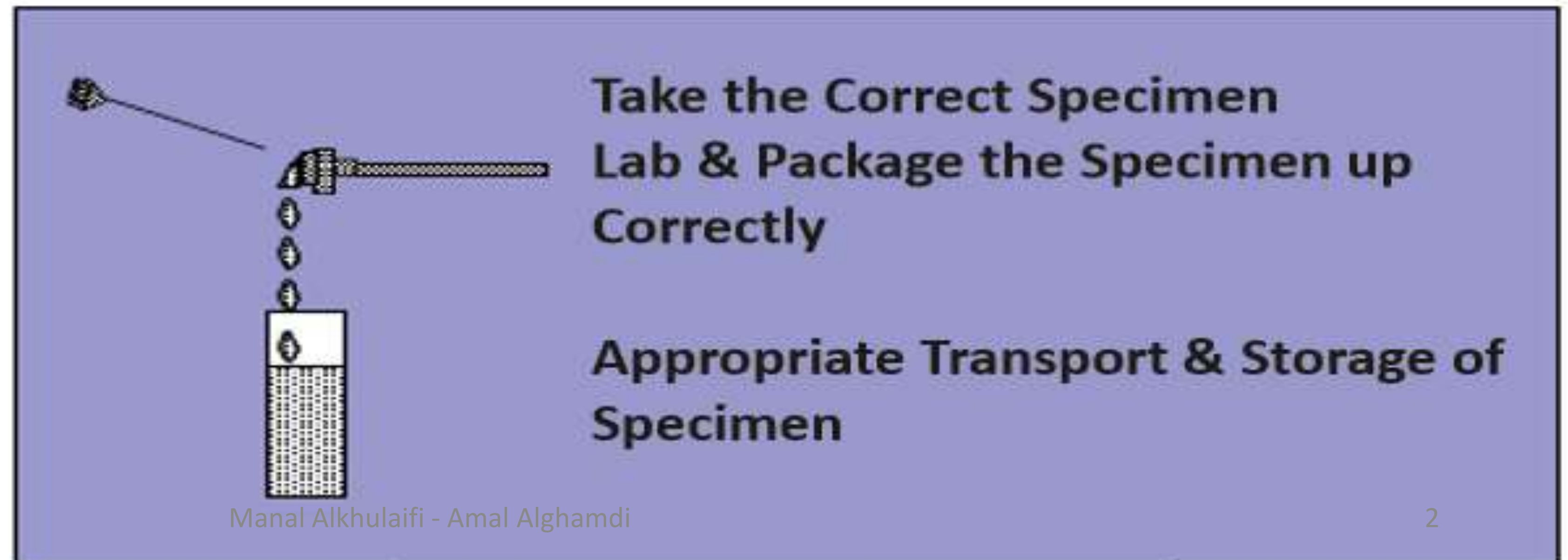
# Diagnosis of Bacterial Infections I

# Diagnosis of Bacterial infections



# COLLECTION OF SPECIMENS (Sample):

1. Determine agents of the disease.
2. Choose the appropriate specimen.
3. Obtain specimen properly, avoiding contamination.
4. Transport quickly to laboratory.
5. Store properly.
6. Provide all information needed by lab. Staff.

# BACTERIOLOGICAL METHODS:

- Naked eye examination.
- Microscopic examination.
- Specimen culture.
- Isolate identification by:

a)Biochemical reactions.

b)Growth on selective media.

c)Anti-sera testing.

d)Microscopic examination.

- Serological tests to detect antibodies.
- Sensitivity testing of isolate.
- Isolate typing for epidemiological studies,

e.g. : phage typing.

**The method should be:**

- Sensitive and specific.
- Rapid.
- Easy to perform, not labor intensive.
- Data easy to interpret.
- Widely available.
- Cost effective.
- Automation high-throughput analysis.
- Upload of the results

# BACTERIOLOGICAL METHODS:

## Traditional bacterial identification:

- Phenotypic identification (gram staining- growth characteristics- antibiogram- biochemical methods- fully or partly automated identification methods (Vitek, Phoenix,...)).
- Cultivation ( Pure cultures- Unique characteristics- Highly related species cannot be phenotypically differentiated- Corresponding databases are often limited, hampering- accurate identification).

**In the last 15 years molecular and chemotaxonomic methods have proven beneficial in overcoming some of these limitations**

## Identification of bacteria:

- Comparison of microbial identification methods
- traditional methods
- molecular methods
- chemotaxonomic methods
- Database identifications.
- Summary and general considerations.

# BACTERIOLOGICAL METHODS:

- Molecular methods:

DNA and RNA present in all bacteria , multilocus, whole genome, Sensitive and specific, Directly on specimen samples (blood, sputum,..), Extensive validation is needed, PCR, fingerprint methods, micro-arrays, oligonucleotide probes,...

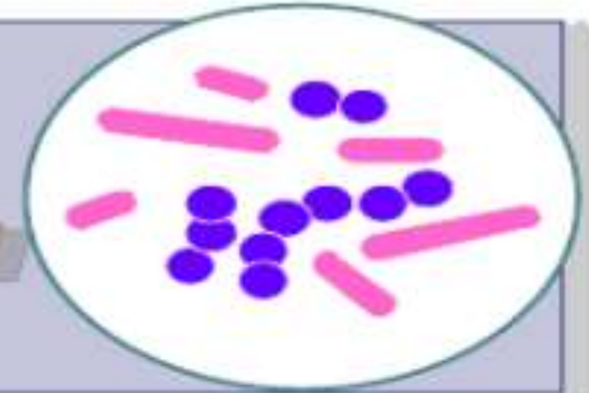
- Chemotaxonomic methods:

Classify organisms based on differences and similarities in chemical markers (cell wall constituents, lipids, whole cell proteins)- Chemotaxonomic fingerprints- SDS-PAGE of whole cell proteins - spectroscopy.

# BACTERIOLOGICAL METHODS:

microscopy

unstained or stained with e.g.
Gram stain



culture

Stain

Decolorize Counter stain

identification by biochemical or serological tests
on pure growth from single colony

sensitivities

on plates or in broth

by disc diffusion
methods,
breakpoints or MICs

Sero diagnosis

DNA technologies

# BLOOD CULTURE:

*1. Diseases suspected:*

septiceamia, endocarditis, osteomyelitis, meningitis, pneumonia, enteric fever, brucellosis, etc.

*2. Organisms suspected:*

*Staphylococcus aurous, Streptococcus pneumoniae , Escherichia coli, Klebsiella pneumoniae , Pseudomonas aeruginosa , Salmonella typhi , Brucella , etc.*

*3. Collect at least:*

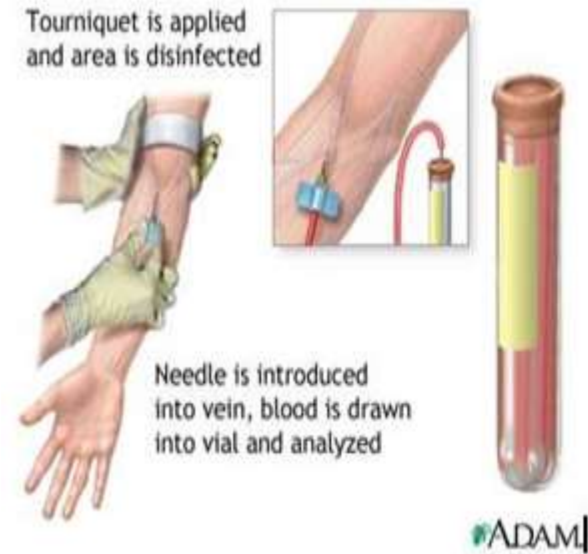
10 ml. blood every 24 hours, because bacteria in blood are scanty and intermittent.

*4. Collect blood:*

aseptically to avoid contaminants.

*5. The blood culture bottle obtained from the lab.* must contain 100 ml. of a suitable growth medium, e.g.: **brain- heart infusion broth**.

*6. In the lab.,* blood culture bottles are checked daily for turbidity up to 21 days.



# BLOOD CULTURE:

*7. Bottle is subcultured* after 24 hrs., 72 hrs, one week, 2 weeks, and 3 weeks.

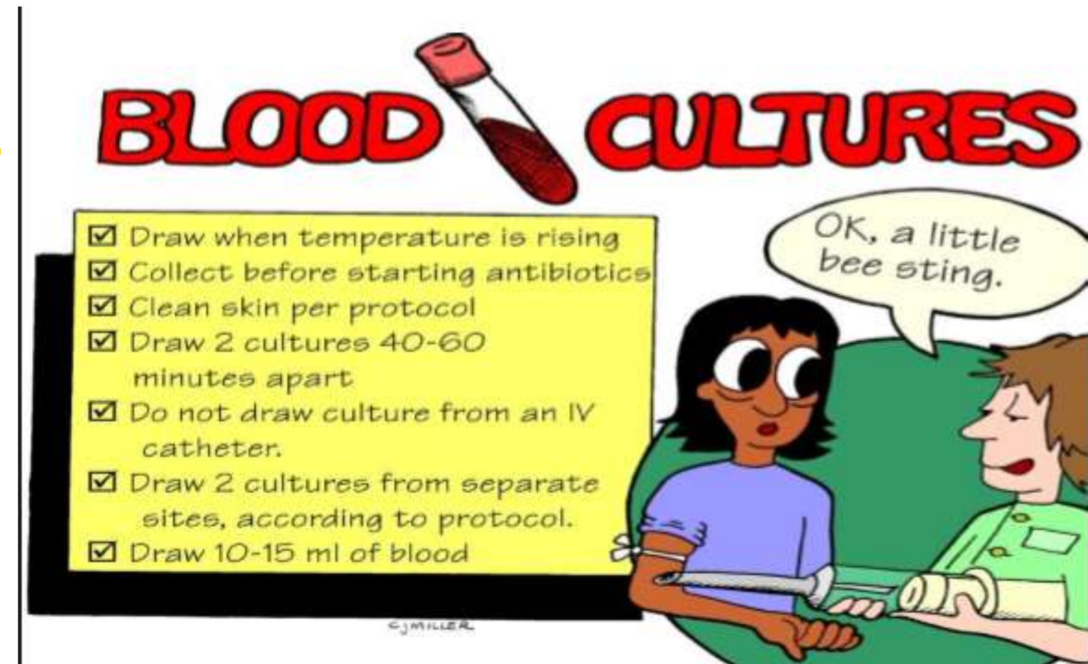
Standard media used for subculture are:

**Blood agar (B.A.), MacConkey, chocolate agar, Sabouraud agar, etc.**

*8-Identification of isolate* is by standard methods, and sensitivity tests are performed.

*9. If no growth after three weeks, discard bottle.* Bottles are automatically tested every 10 minutes. Positive results are tagged for quick processing.

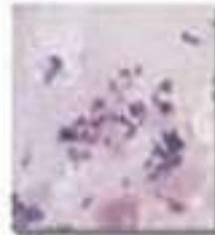
Negative bottles can be batch-scanned out of the system and unloaded at the end of protocol.



Positive Blood Culture

15 Min.

Gram Stain



GPCC

Yeast

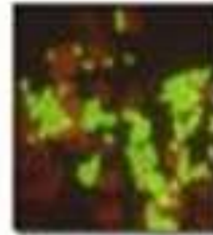
GPCPC

S. aureus → PNA FISH™

C. albicans → PNA FISH™

E. faecalis → PNA FISH™

Results (2.5 Hrs.)



S. aureus

non-S. aureus GPCC

C. albicans

non-C. albicans Yeast

E. faecalis - Green
other enterococci - Red

non-enterococci GPCPC

# THROAT CULTURE:

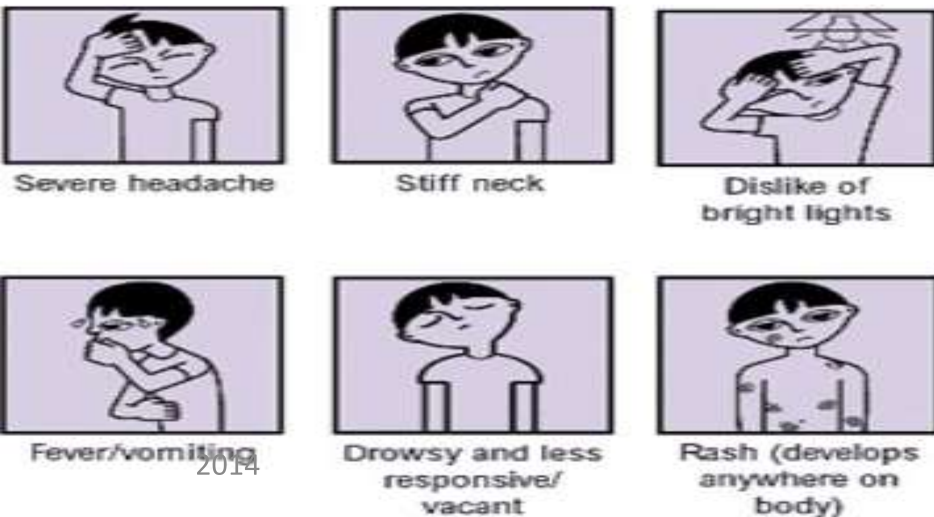
1. Mainly used to isolate ß-haemolytic *Streptococcus pyogenes* that cause pharyngitis.

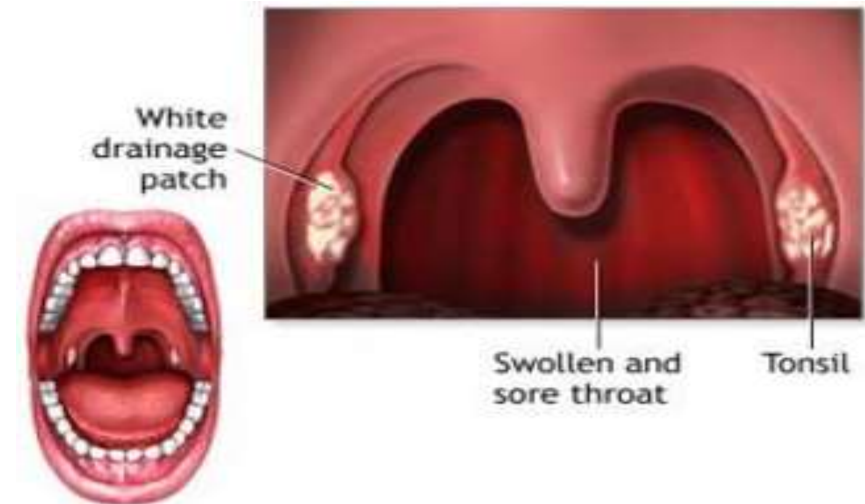
Requested to diagnose diphtheria, gonorrhoea, and candidosis.

2. Swab posterior pharynx, tonsils, & tonsillar fossae.
3. Swab is inoculated on B.A. and bacitracin disc is added.

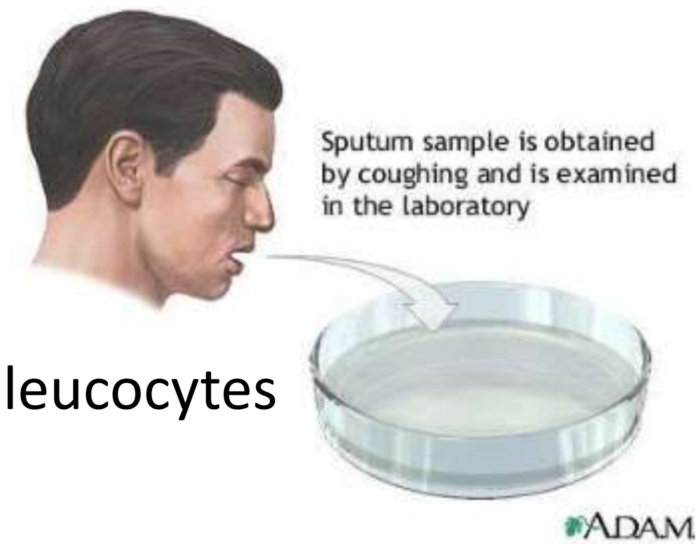
Then incubate for 18-24 hrs at 37˚C.

4. Colonies of gp. (A) *S. pyogenes* are ß-haemolytic and bacitracin sensitive.



# SPUTUM CULTURE:



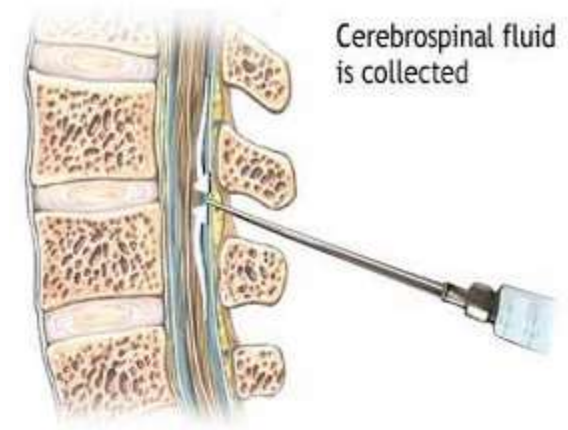

1. Performed to diagnose pneumonia, TB, lung abscess.
2. Sputum must be real not saliva.
3. Gram stain will show if it is saliva or not. Good sputum shows (25) leucocytes less than (10) epithelial cells per 100x field.
1. If the patient cannot cough you may choose:

a) Induction of sputum. b) Transtracheal aspirate

c) Bronchial lavage. d) Lung biopsy.

5. Do gram stain to assess cause of pneumonia (large numbers of organisms).
6. Culture is made on **B.A. and other selective media**. Identify by serological and biochemical tests.
7. ***Mycoplasma*** is diagnosed by antibody rise on serology. **TB** is diagnosed by ZN (Ziehl–Neelsen) stain (acid-fast stain) and culture on **Löwenstein–Jensen medium (L.J. medium).**

# SPINAL FLUID (CSF) CULTURE:




1. CSF is collected to diagnose meningitis, encephalitis, brain abscess, subdural empyema.

1. Causes of meningitis are: (3 encapsulated organisms)

*Neisserria meningitidis , Streptococcus pnuemoniae , Haemophilus influenzae*.

3. Send specimen immediately to laboratory. Gram stain may give a presumptive diagnosis.

4. Identification is made by antisera and capsule swelling reaction (Quelling), and immunofluorescence.

5. Culture is on Blood Agar (B.A.) & **chocolate agar.** Incubate plates at 35˚C in 5% $Co_2$.

6. *Mycobacterium tuberculosis* & Cryptococcus neoformans cause sub-acute meningitis.

ZN is made to identify Myco. TB.

*Cryp. neoformans* capsule may be detected by India Ink staining.

7. Heamatin (X-factor) and NAD (V-factor) may help in identification of *H. influenzae*.

8. Serological tests (latex agglutination) are used to identify organism causing meningitis.

# STOOL CULTURE:

1. Pathogenic organisms *are Shigella, Salmonella, and Campylobacter*.

2. Stool general may reveal:

a) Leukocytes and pus cells by methylene blue stain.

b) Gram stain is not performed.

3. Culture on **MacConkey** & **Eosin- methylene blue** & other selective media.

Identify by biochemical reactions and antisera.

- Widal test is made for enteric fever.

4. *C. jejuni* is cultured on **selective Skirrow agar** at 42˚C in 10% $Co_2$ .

# URINE CULTURE:

1. Performed to diagnose pyelonephritis & cystitis.
2. Organisms isolated are: *E. coli, Proteus, Enterobacter, Enterococcus faecalis, Pseudomonas,and Klebsiella*.
3. Midstream, morning urine sample is collected after washing external orifices. Suprapubic aspiration and catheterization may also be used for urine collection.
4. If there is delay culture urine within one hour after collection, or store at 4˚C for no more than 18 hrs.
5. Bacterial urine counts are made by inoculating the sample on MacConkey agar using a 0.001(1µl) loop .

   Then multiply number of colonies by 1000 ($10^3$).

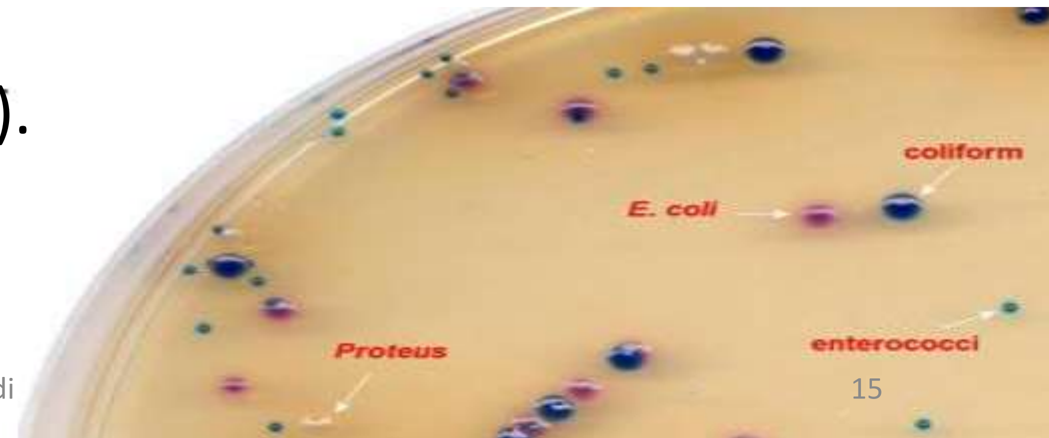
**@ Count interpretation :**

***a) For symptomatics:***

significant count is 100 × $10^3$ colonies/ml.

***a) For asymptomatics***:

significant count is only 100 colonies /ml.



# GENITAL TRACT CULTURE:

1. Performed to diagnose gonorrhea caused by *Neisseria gonorrhoeae*, using culture and microscopic examination.
2. Discharge is swabbed from urethra, cervix, & anal canal.
3. It is inoculated quickly on **Thayer-Martin, chocolate agar, or** transported in **trans-grow or Stuart media**.
4. *N. gonorrhoeae* is identified microscopically as gram negative intracellular, diplococci within the pus (neutrophil) cell
5. *Chlamydia trachomatis* is may cause Non-Specific Urethritis.

It is cultured on **yolk sac of chick embryo or human tissue culture**.

6. Syphilis *Treponema pallidum* is seen by D-F microscopy of a chancre fluid.

Syphilis is diagnosed by non-specific serological tests.

*N. Gonorrhoeae* colonies

# WOUND AND ABSCESS CULTURES:

1. Abscess is caused by *Bacteroides , S. aurous, S. pyogenes* .

Wound infections are due to *Clostridium perfringens , S. aurous, Pasteurella multocida*.

2. Swab is transported immediately to lab. in **thioglycolate enrichment broth** or **Robertson's Cooked Meat Medium (RCMM)**.

Several aerobic and anaerobic media are inoculated.

# Appendices

# Blood Agar

*Inoculation method:* surface streak and stab with loop

*Contains:* BHIA, sheep blood

Discriminates organisms that have the ability to hemolyse red blood cells:

1- completely through production of hemolysins (streptolysins or alpha-toxin)

**or** 2- partially through ability to degrade hemoglobin pigment into green products (biliverdin)

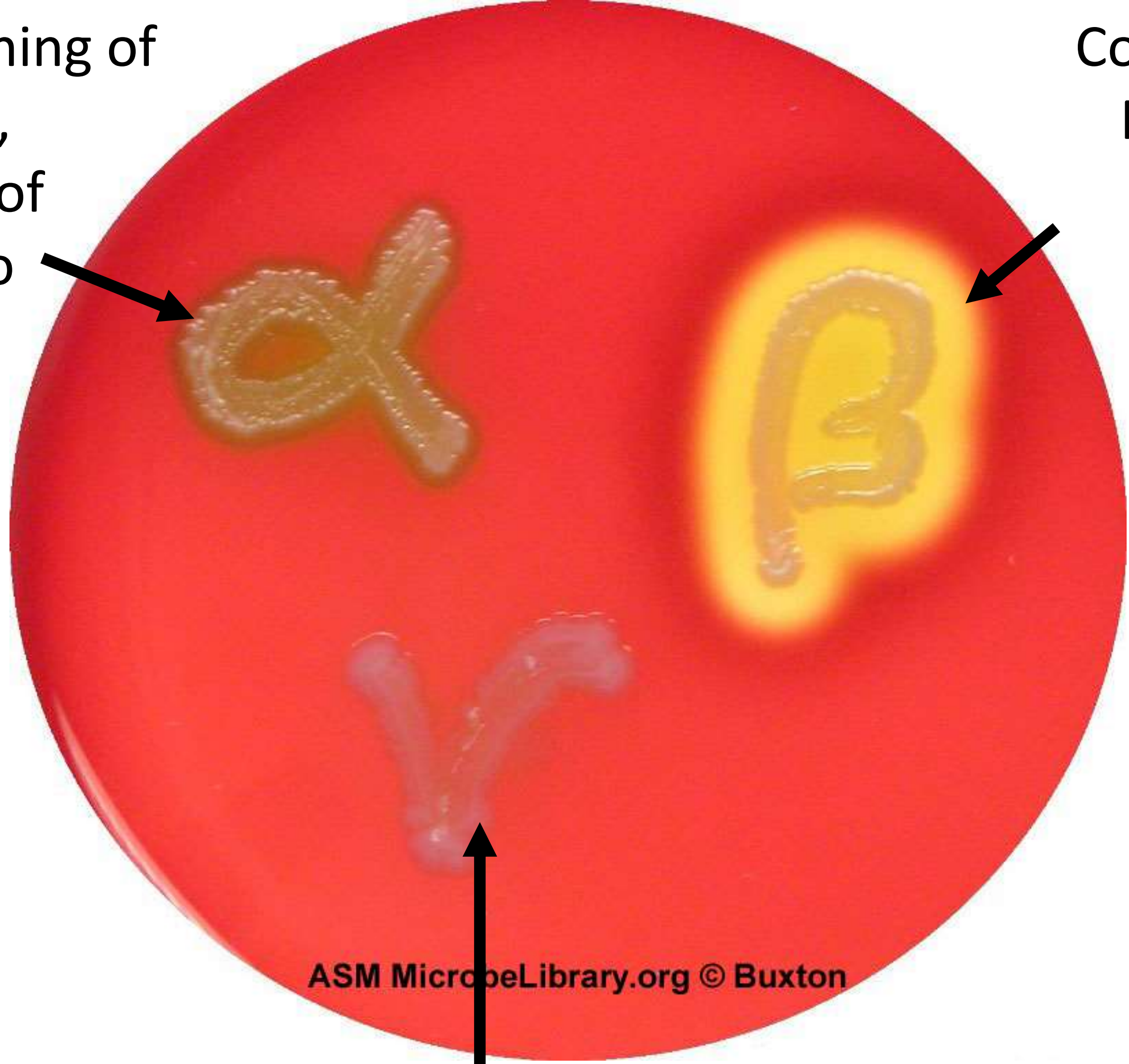
**Results:**

1- Complete clearing of RBCs = Beta-hemolysis, positive for production of hemolysins

**or** 2- Partial clearing and greening of blood = Alpha-hemolysis, positive for degradation of hemoglobin pigment into biliverdin

3- No clearing with or without rusting = Gamma-hemolysis, negative for hemolysis

Partial clearing and greening of blood = Alpha-hemolysis, positive for degradation of hemoglobin pigment into biliverdin




Complete clearing of RBCs = Beta-hemolysis, positive for production of hemolysins

No clearing with or without rusting = Gamma-hemolysis, negative for hemolysis

## Blood Agar

Inoculation method: surface streak and stab with loop

Contains: BHIA, sheep blood

Discriminates organisms that have the ability to hemolyse red blood cells completely through production of hemolysins (streptolysins or alpha-toxin) or partially through ability to degrade hemoglobin pigment into green products (biliverdin)

Results:

- Complete clearing of RBCs = Beta-hemolysis, positive for production of hemolysins
- Partial clearing and greening of blood = Alpha-hemolysis, positive for degradation of hemoglobin pigment into biliverdin
- No clearing with or without rusting = Gamma-hemolysis, negative for hemolysis

Gamma

Alpha

Beta

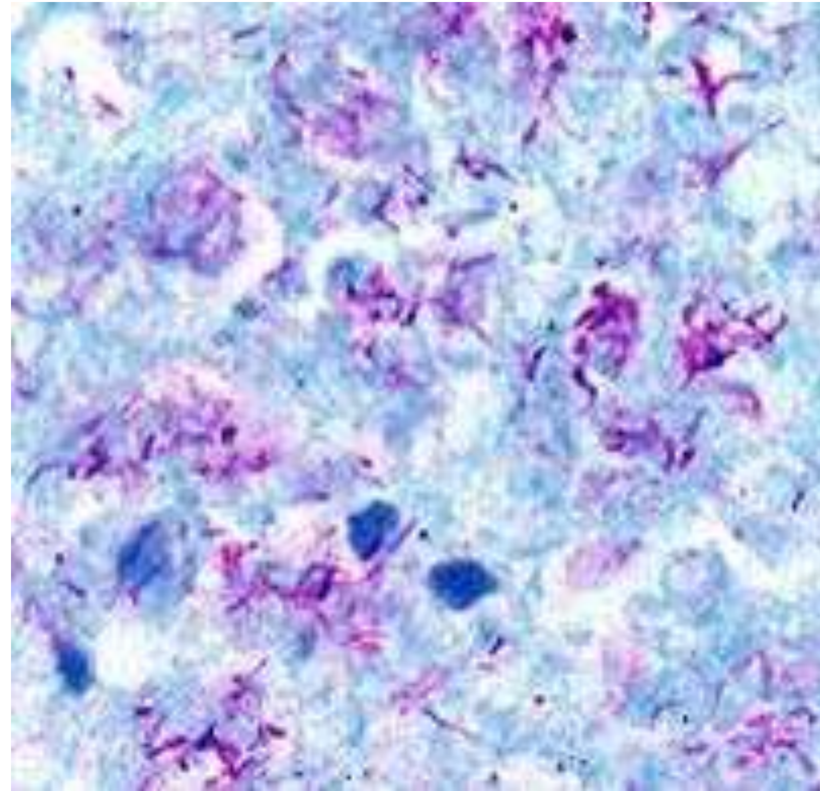
# Bacitracin Susceptibility or Resistance

*Susceptible* = organism killed bacitracin: zone of no growth around disk

*Resistant =* organism growth not affected by bacitracin: organism grows around and under disk

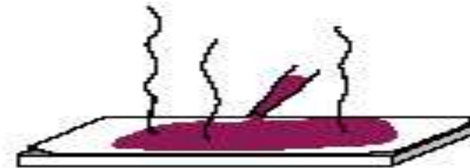
# Acid-fast Stain

- Takes advantage of mycolic acid in the cell wall of slow growing mycobacterium
- Use *M. smegmatis* and *S. aureus* on the same slide using Ziehl-Neelsen method.

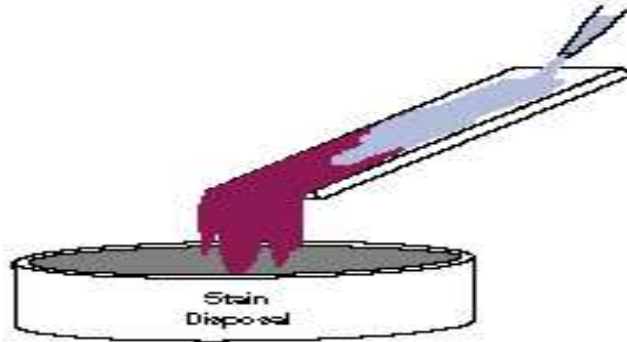


# Procedural Diagram
# Acid-Fast Stain (Ziehl-Neelsen Method)

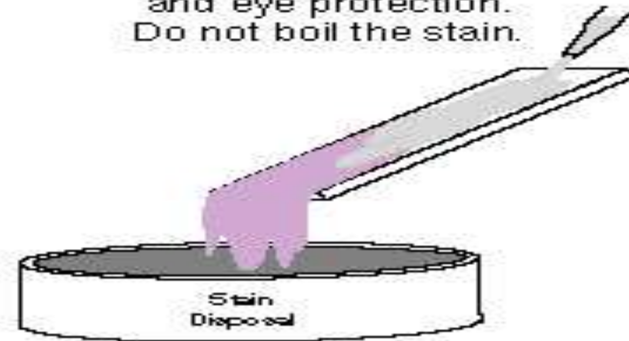
1. Begin with a heat-fixed emulsion.
(The emulsion can be prepared
in a drop of sheep serum.)



2. Cover the smear with a strip of bibulous paper.
Apply ZN carbolfuchsin stain.
Steam (as shown in Figure 3-54) for 5 minutes.
Keep the paper moist with stain.
Perform this step with adequate ventilation
and eye protection.
Do not boil the stain.



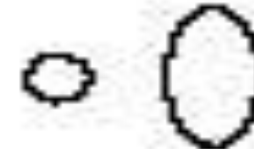

3. Grasp the slide with a slide holder.
Remove the paper and dispose of it properly.
Gently rinse the slide with distilled water.




4. Continue holding the slide with a slide holder.
Decolorize with acid-alcohol (CAUTION!)
until the run-off is clear.
Gently rinse the slide with distilled water.

5. Counterstain with Methylene Blue stain for 1 minute.
Rinse with distilled water.

6. Gently blot dry in a tablet of bibulous paper.
Do not rub.
Observe under oil immersion.

| | Acid-Fast | Nonacid-Fast |
|---|---|---|
| Cells prior to staining are transparent. | | |
| After staining with carbolfuchsin, cells are reddish-purple. Steam heat enhances the entry of carbolfuchsin into cells. | | |
| Decolorization with acid alcohol removes stain from acid-fast negative cells. | |  |
| Methylene blue is used to counterstain acid-fast negative cells. | | |

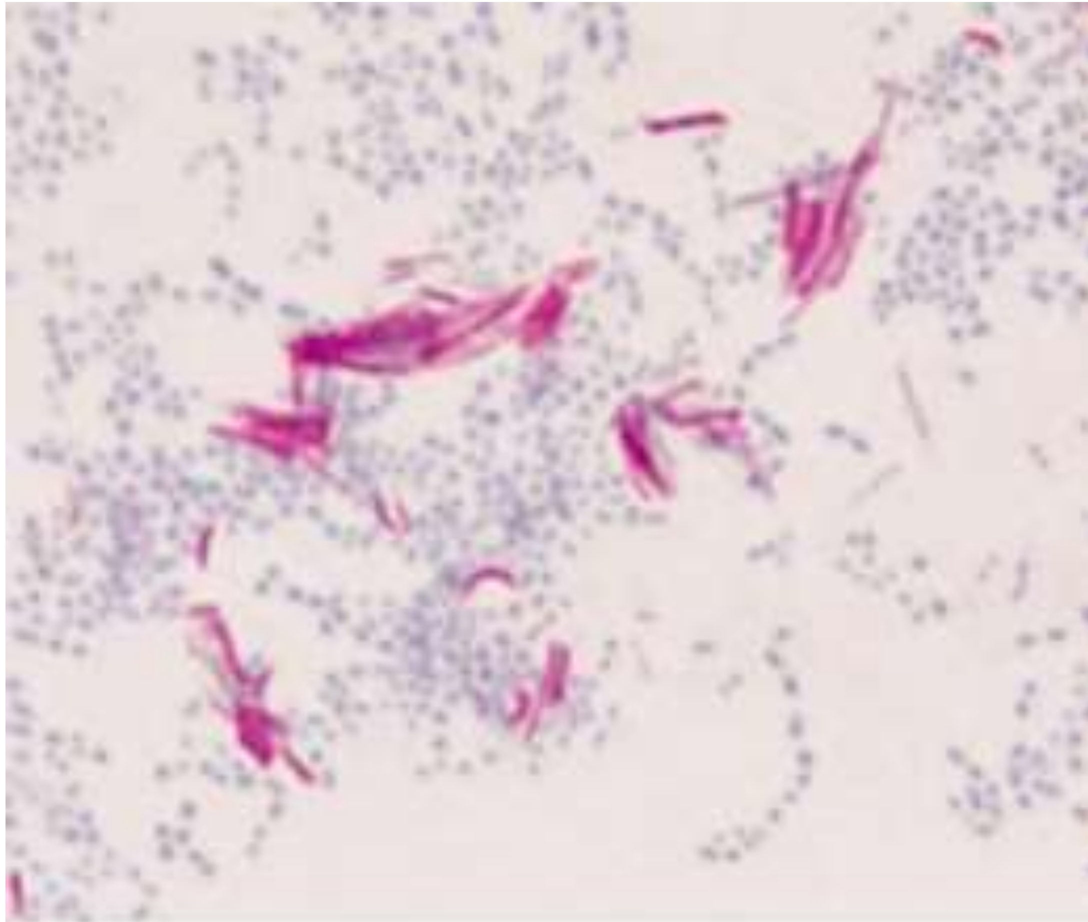
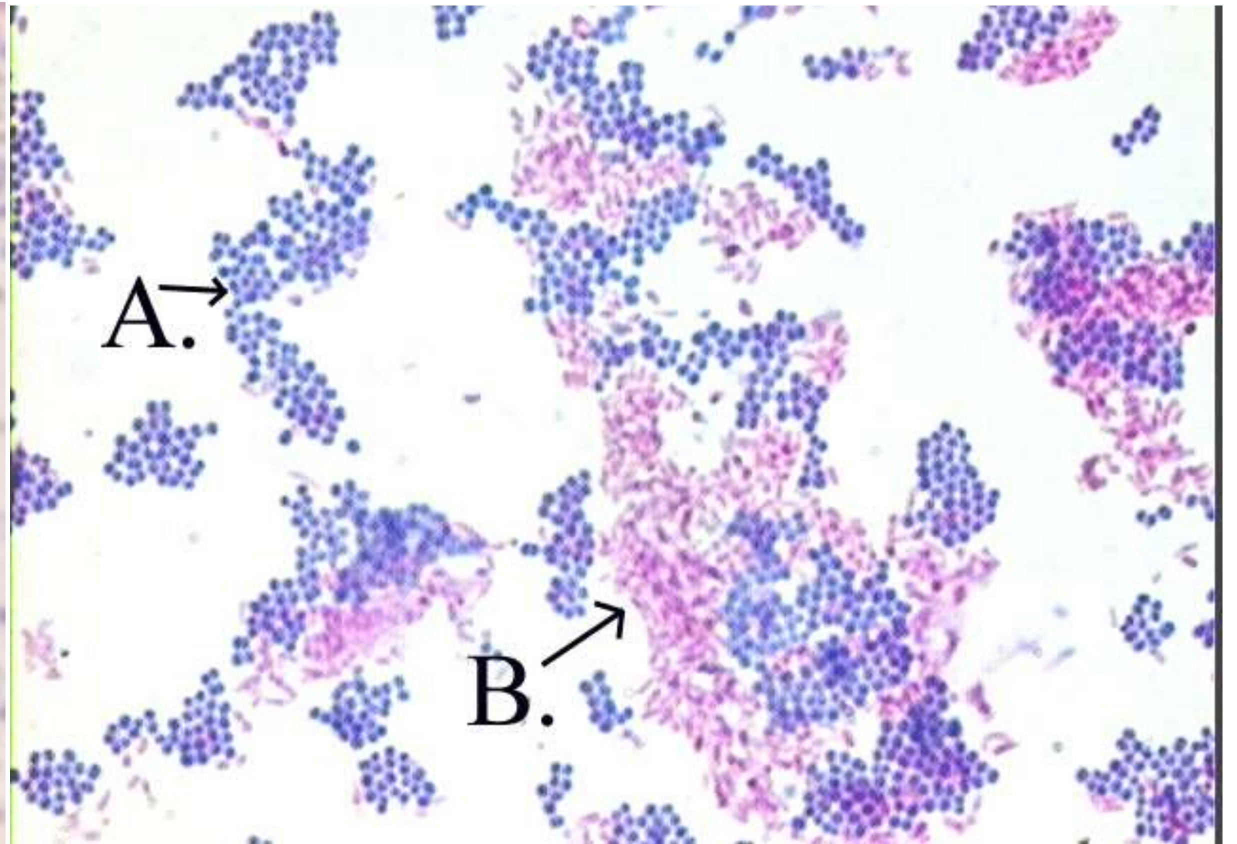


Figure 3-50




A = Non acid fast

B = Acid Fast Bacteria

# *Mycobacterium* growth on L.J. Medium