Identification of Bacterial Pathogens

basic skills in diagnostic bacteriology

Dr.T.V.Rao MD

Identification of Microorganisms

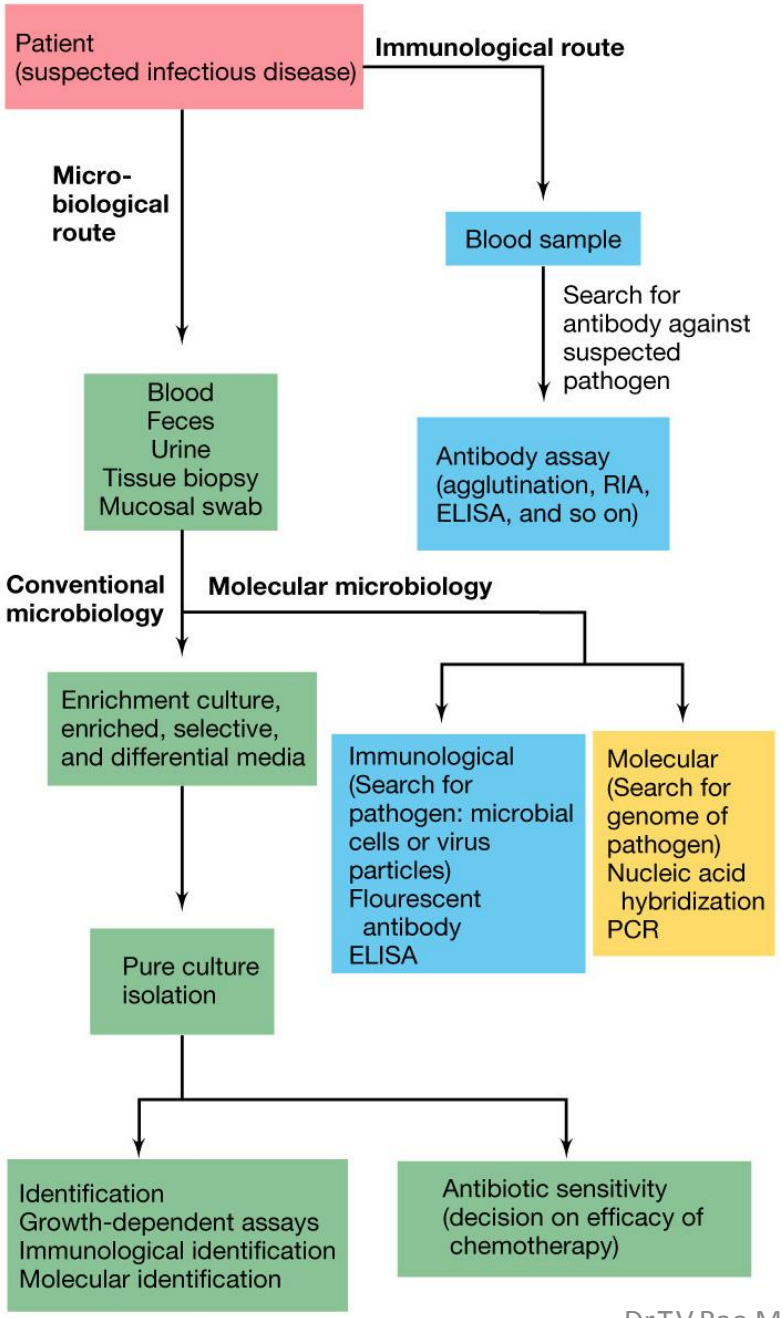
- For many students and professionals the most pressing topic in microbiology is **how to identify unknown specimens**.
- **Why is this important?**
- Labs can grow, isolate and identify most routinely encountered bacteria within **48 hrs** of sampling.
- The methods microbiologist use fall into **three categories**:
  - ♣**Phenotypic**- morphology (micro and macroscopic)
  - ♣**Immunological**- serological analysis
  - ♣**Genotypic**- genetic techniques

Microbe Identification

- The **successful identification** of microbe depends on:
  - Using the proper **aseptic techniques**.
  - **Correctly** obtaining the specimen.
  - Correctly **handling the specimen**
  - **Quickly transporting** the specimen to the lab.
  - Once the specimen reaches the lab it is **cultured and identified**.

Microbe Identification

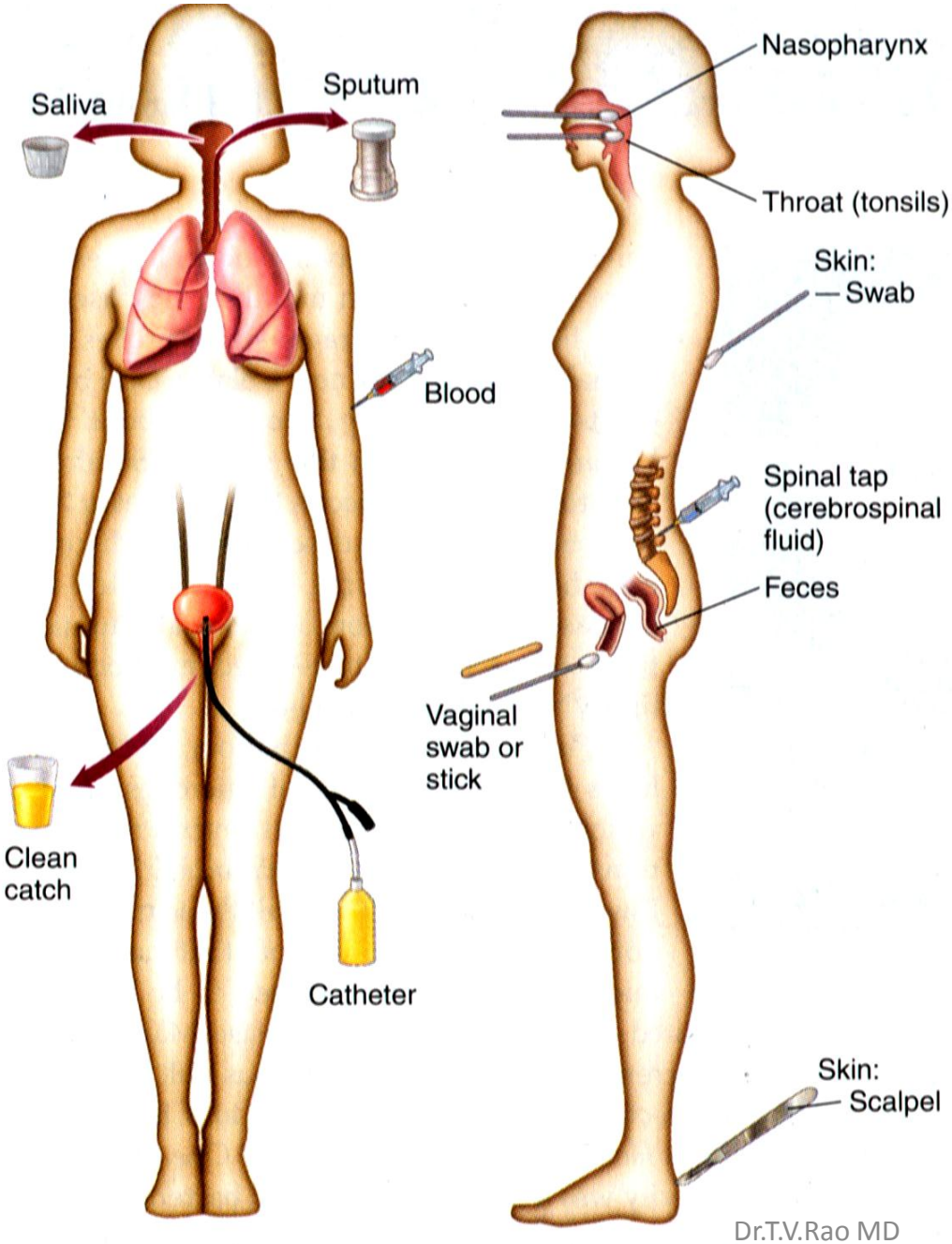
- Identification measures include:
  - **Microscopy (staining)**
  - **growth on enrichment, selective, differential or characteristic media**
  - **specimen biochemical test (rapid test methods)**
  - **immunological techniques**
  - **molecular (genotypic) methods**.
- After the microbe is identified for clinical samples it is used in **susceptibility tests** to find which method of control is most effective.



Microbe Identification

Specimen Collection

- Successful identification depends on how the specimen is **collected**, **handled** and **stored**.
- It is important that general **aseptic procedures** be used including **sterile sample containers** and **sampling methods** to prevent contamination of the specimen.
- E.g. Throat and nasopharyngeal swabs should not touch the cheek, tongue or salvia.
- **What other precautions must be taken when collecting specimens?**
- After collection the specimen must be **taken promptly to the lab** and **stored appropriately** (e.g. refrigeration).



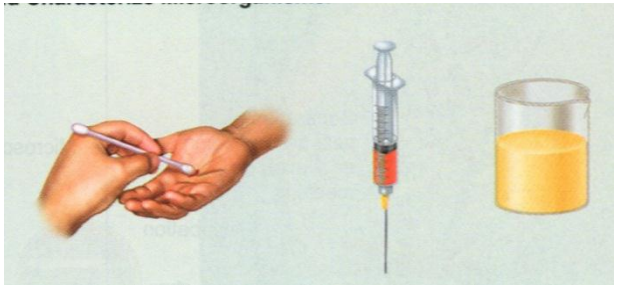
Specimen Collection

Phenotypic Methods of Identification

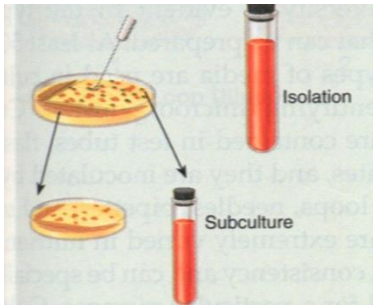
- Microbiologists use 5 basic techniques to **grow, examine and characterize microorganisms** in the lab.
- They are called the **5 'I's**: **inoculation, incubation, isolation, inspection and identification**.
- Inoculation: to **culture microorganisms** a tiny sample (inoculum) is introduced into medium (inoculation).
- Isolation involves the **separating one species from another.**

Phenotypic methods of Identification

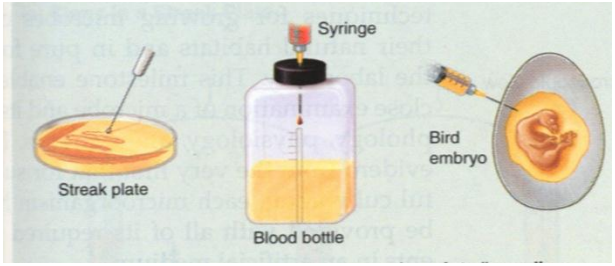
- **Incubation**: once the media is inoculated it is incubated which means putting the culture in a controlled environment (incubation) to **allow for multiplication**.
- After incubation the organisms are **inspected** and i**dentified** phenotypically, immunologically or genetically.



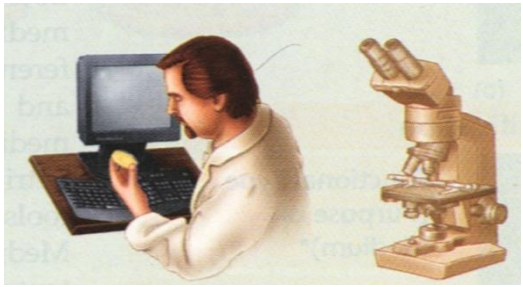
Specimen collection



isolation

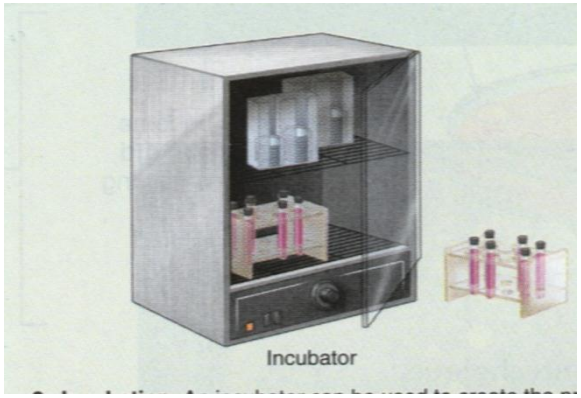


inoculation

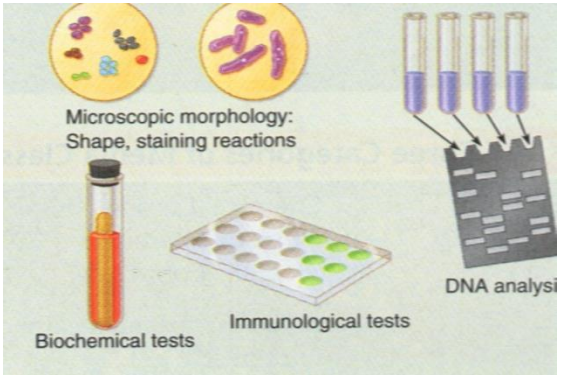


inspection

5 “I” s



incubation



identification

Phenotypic Methods

- ‘Old fashioned’ methods via biochemical, serological and morphological are still used to identify many microorganisms.
- **Phenotypic Methods**
- Microscopic Morphology include a combination of cell shape, size, Gram stain, acid fast rxn, special structures e.g. endospores, granule and capsule can be used to give an **initial putative identification**.

Phenotypic Methods

- **Macroscopic morphology** are traits that can be accessed with the naked eye e.g. appearance of colony including texture, shape, pigment, speed of growth and growth pattern in broth.
- **Physiology/Biochemical** characteristic are traditional mainstay of bacterial identification.
- These include enzymes (catalase, oxidase, decarboxylase), fermentation of sugars, capacity to digest or metabolize complex polymers and sensitivity to drugs can be used in identification.

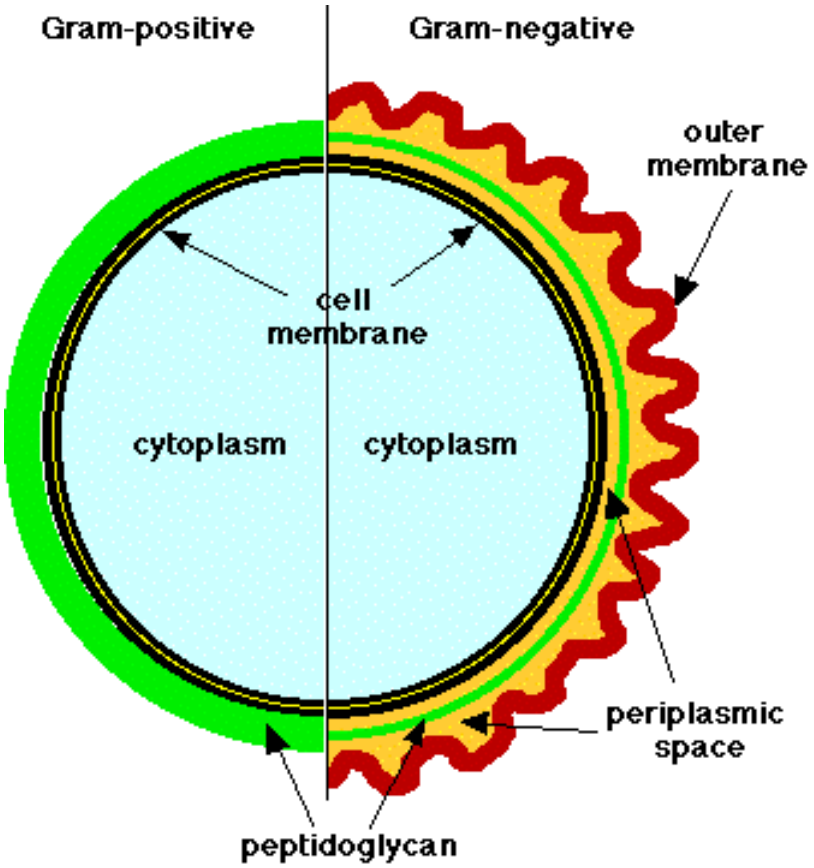
Microscopy

- **Magnification**
  - enhancement of size using ocular and objective lenses.
    - Ocular: eyepiece (10X)
    - Objective: 4X – 100X
  - allows for visualization of bacteria, fungi, and parasites, not viruses
- **Resolution**
  - ability to distinguish two objects as distinct
  - resolving power is closest distance between two objects
  - immersion oil is added when using 100X objective to prevent light scatter
- **Contrast**
  - use stains to enhance visualization; allow organism to stand out from background

Staining techniques by Grams Method

- make slide by smear, drop, or cytocentrifuge
- dry, then fix by heat (flame, 10 min at 60ºC) or fix by methanol (95% 1min)
- Gram stain
  - Crystal violet: primary stain
  - Gram's iodine: mordant/fixative
  - Acetone-ethanol: decolorizer
  - Safranin: counterstain

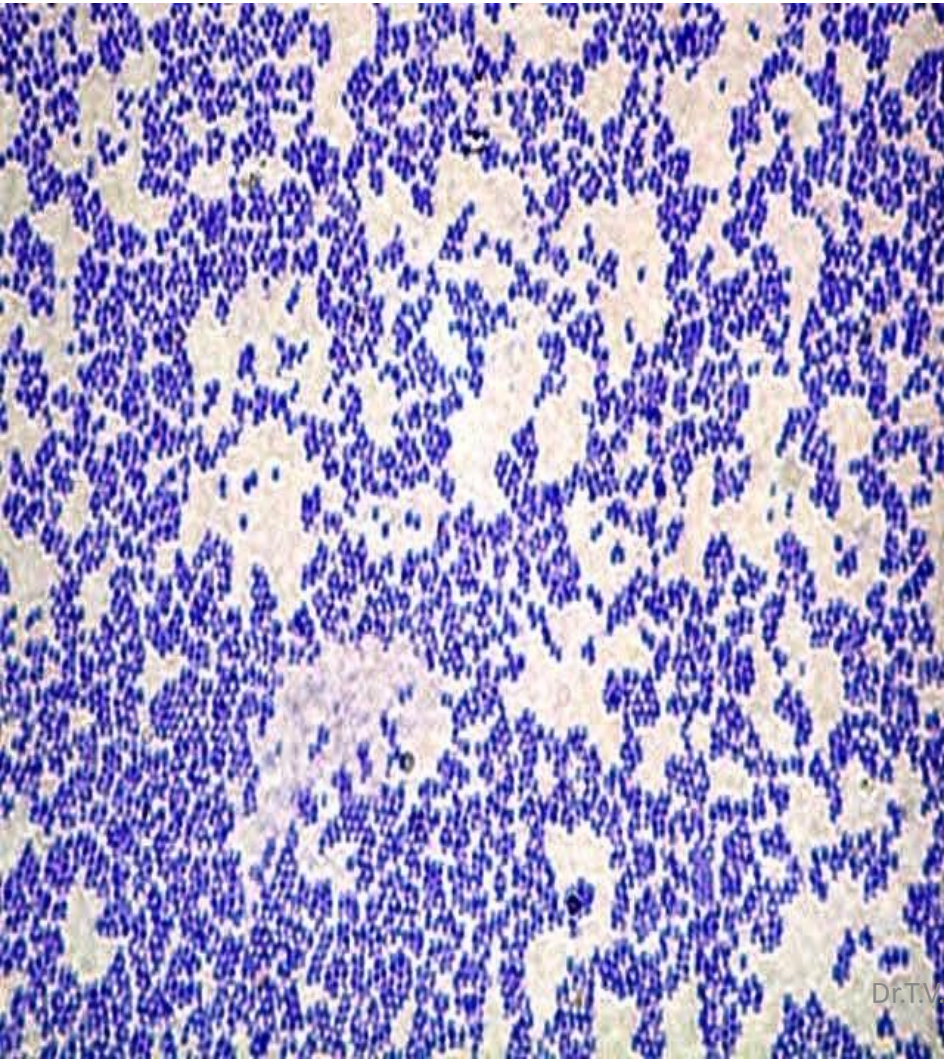
Bacteria differ as per structure

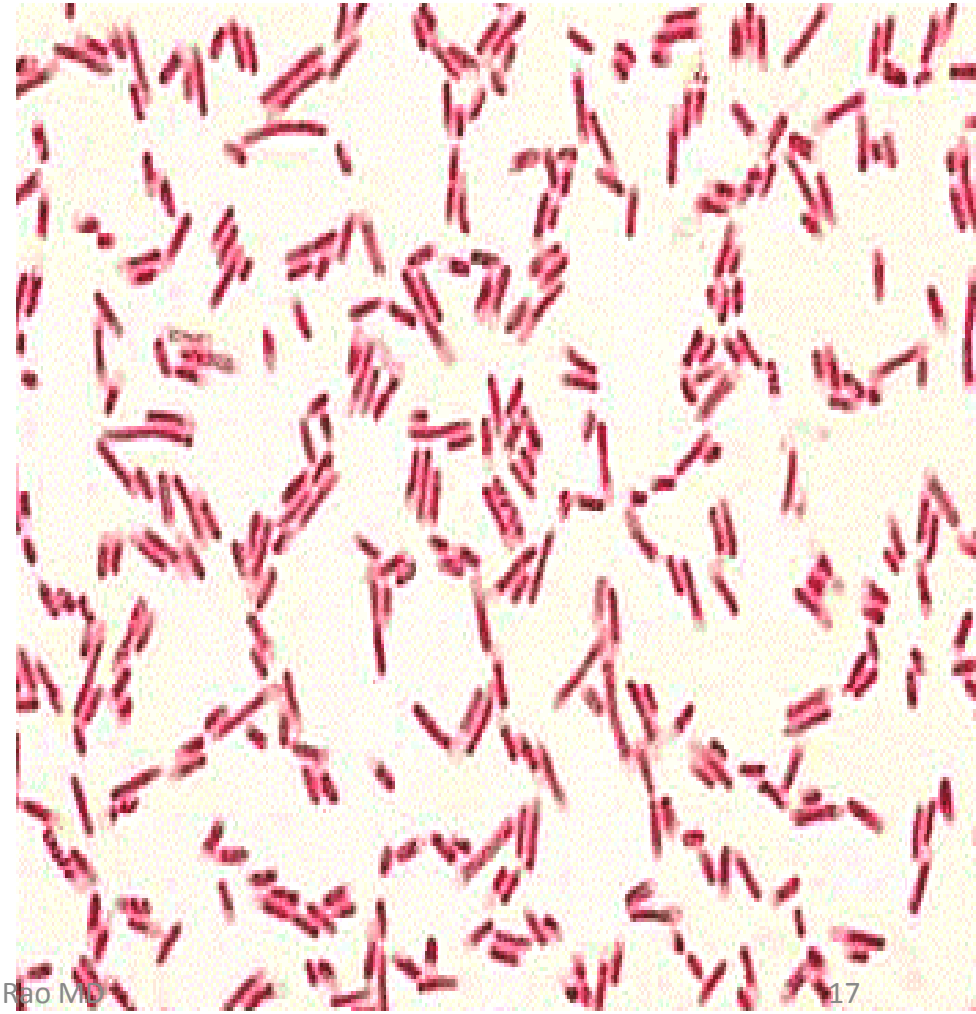


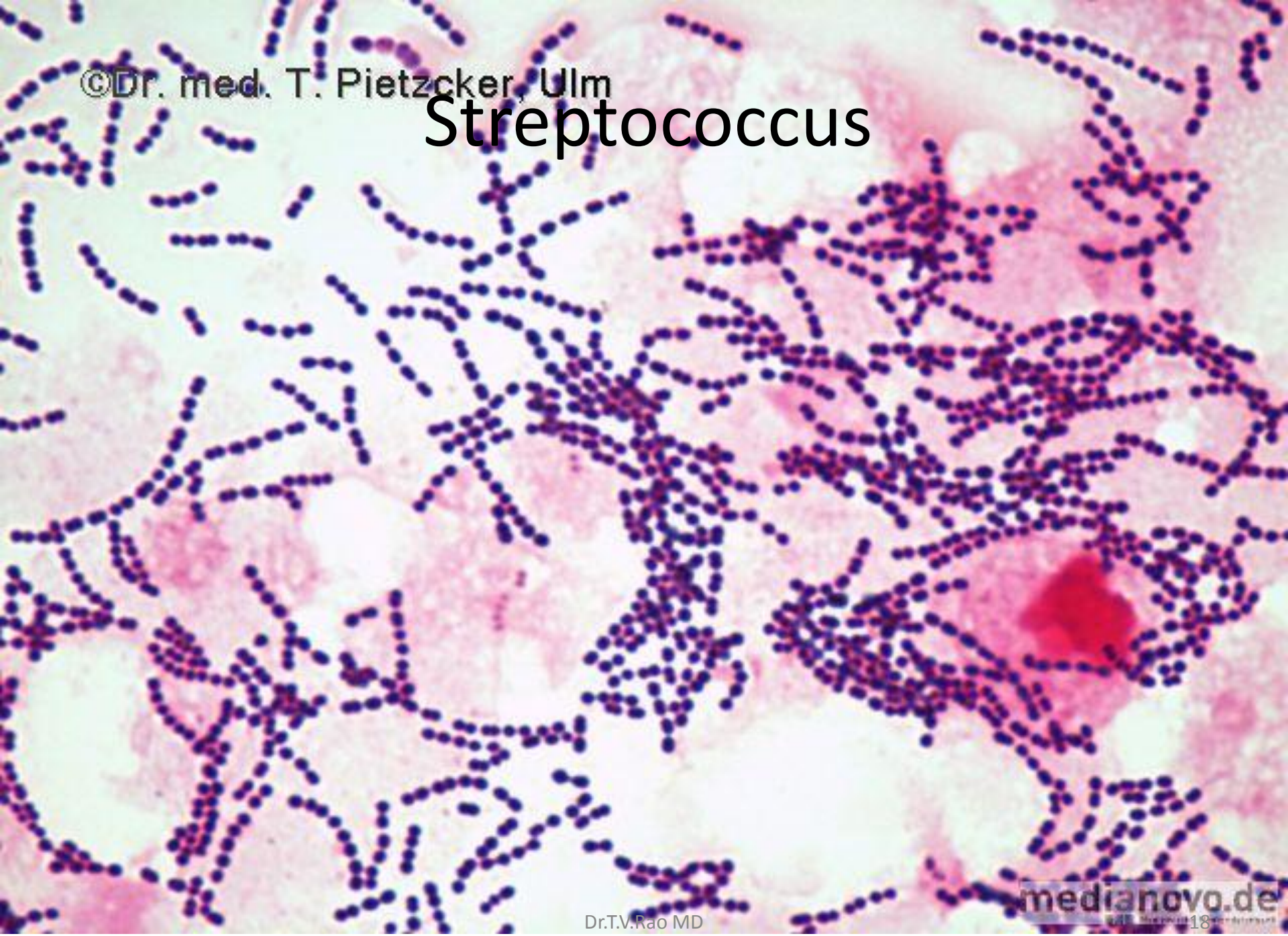
http://www.sp.uconn.edu/~terry/229sp02/lectures/Lect2.html

Gram Staining Procedure

Gm+ve cocci & Gm-ve bacilli

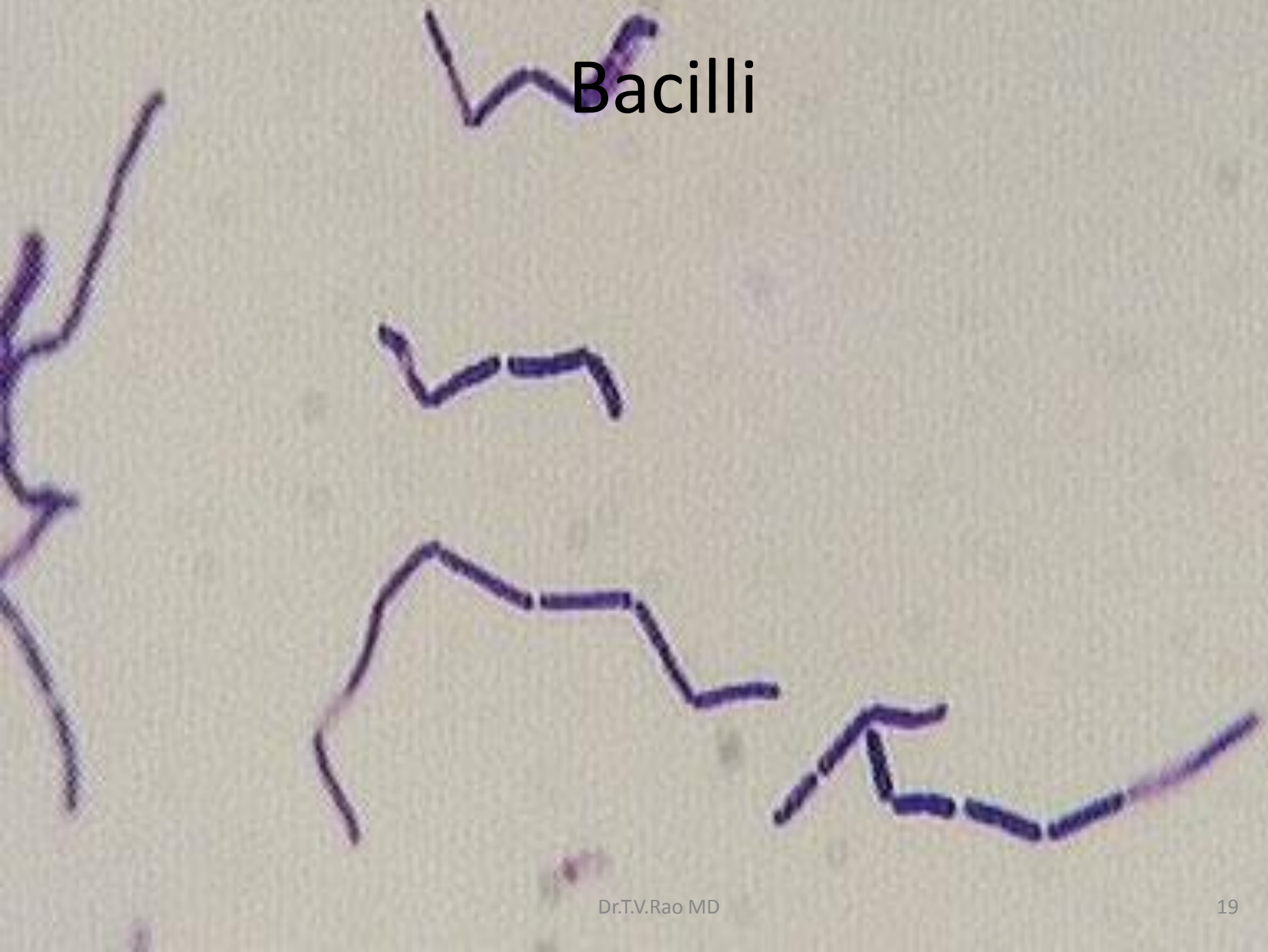


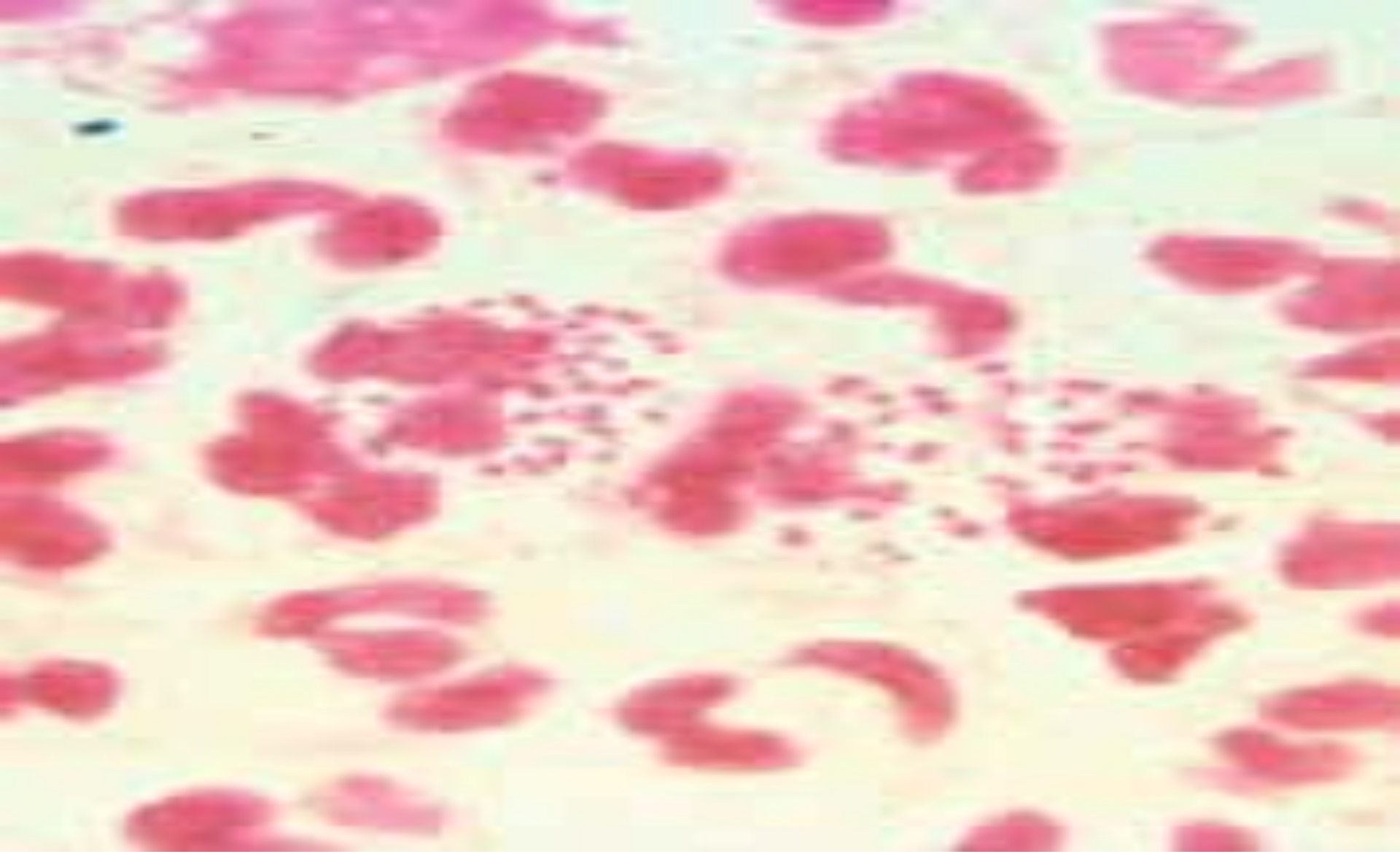




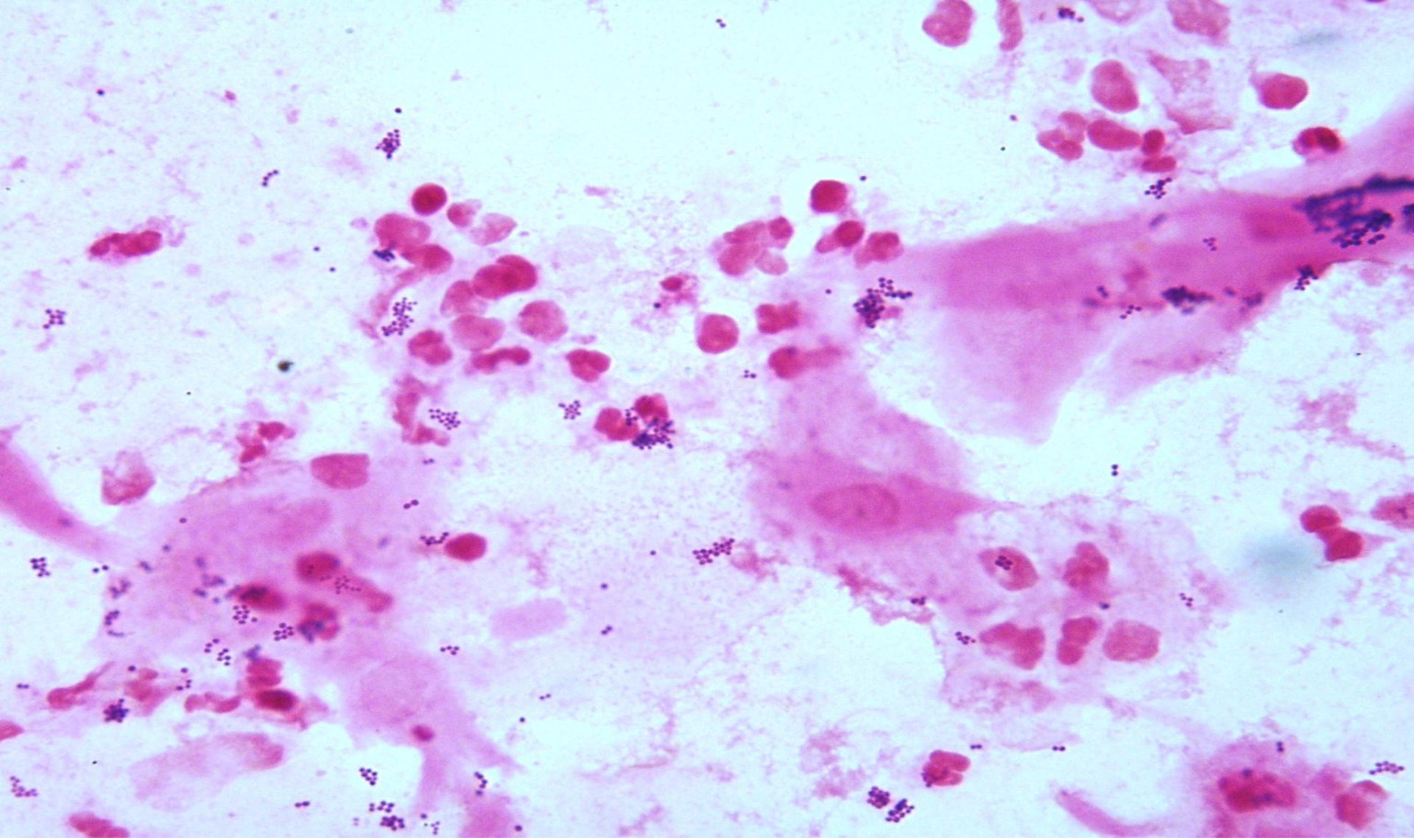
Streptococcus

Bacilli

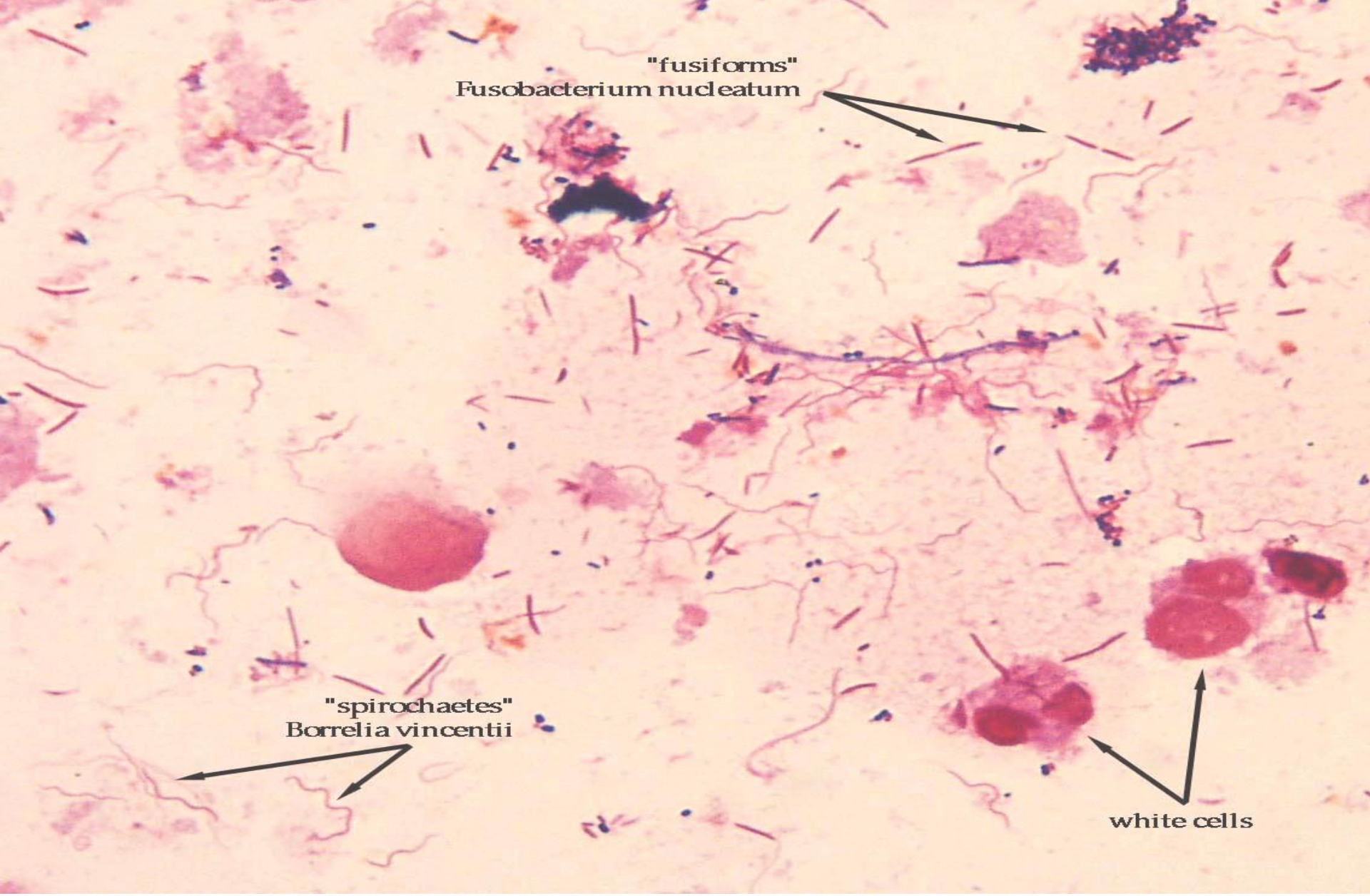




Neisseria gonorrhea - Gram stain
http://www.cdc.gov/STD/LabGuidelines/default.htm

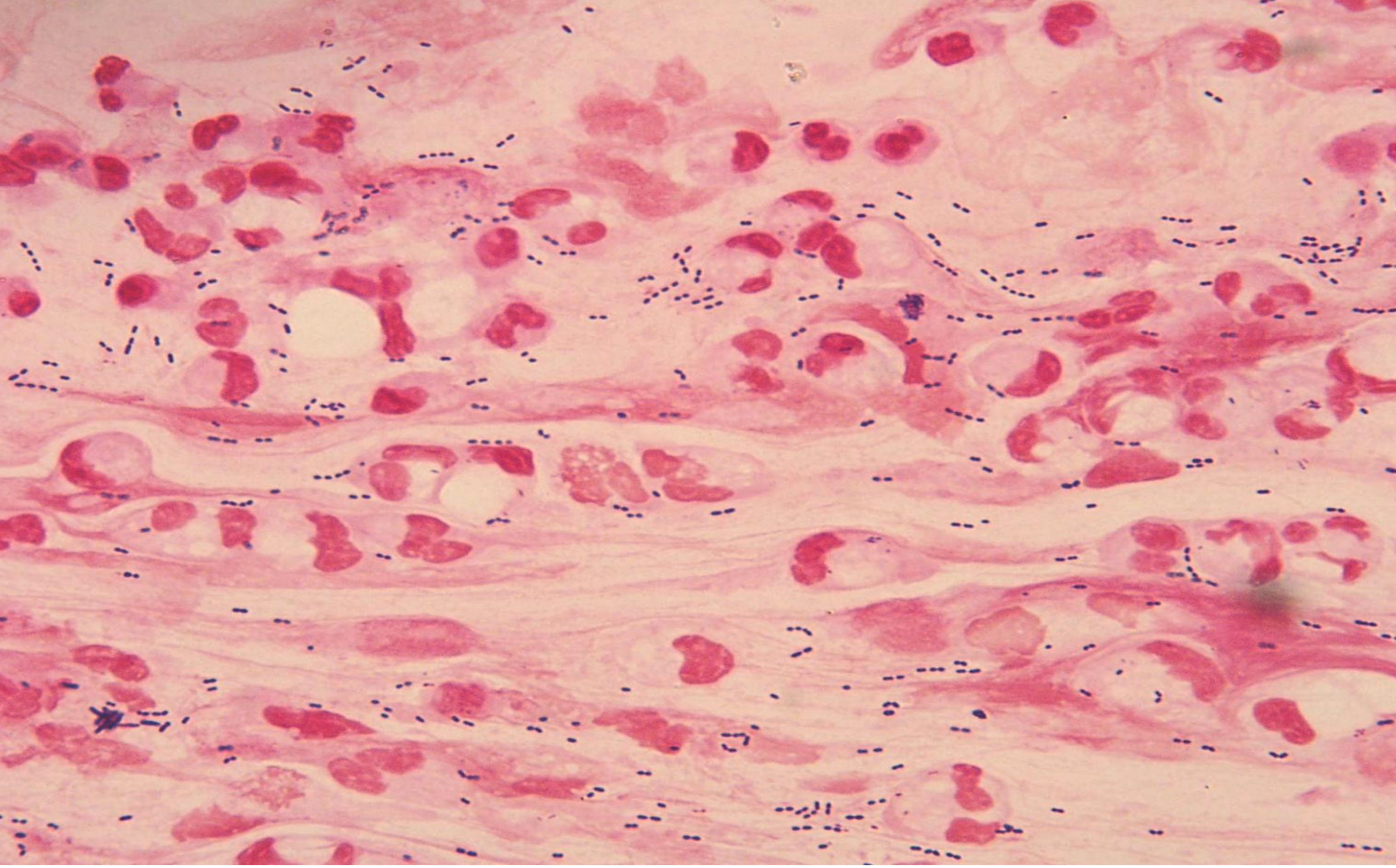


Wound specimen - Gram stain
http://www.healthsci.utas.edu.au/hls/teaching/micro/mma.html

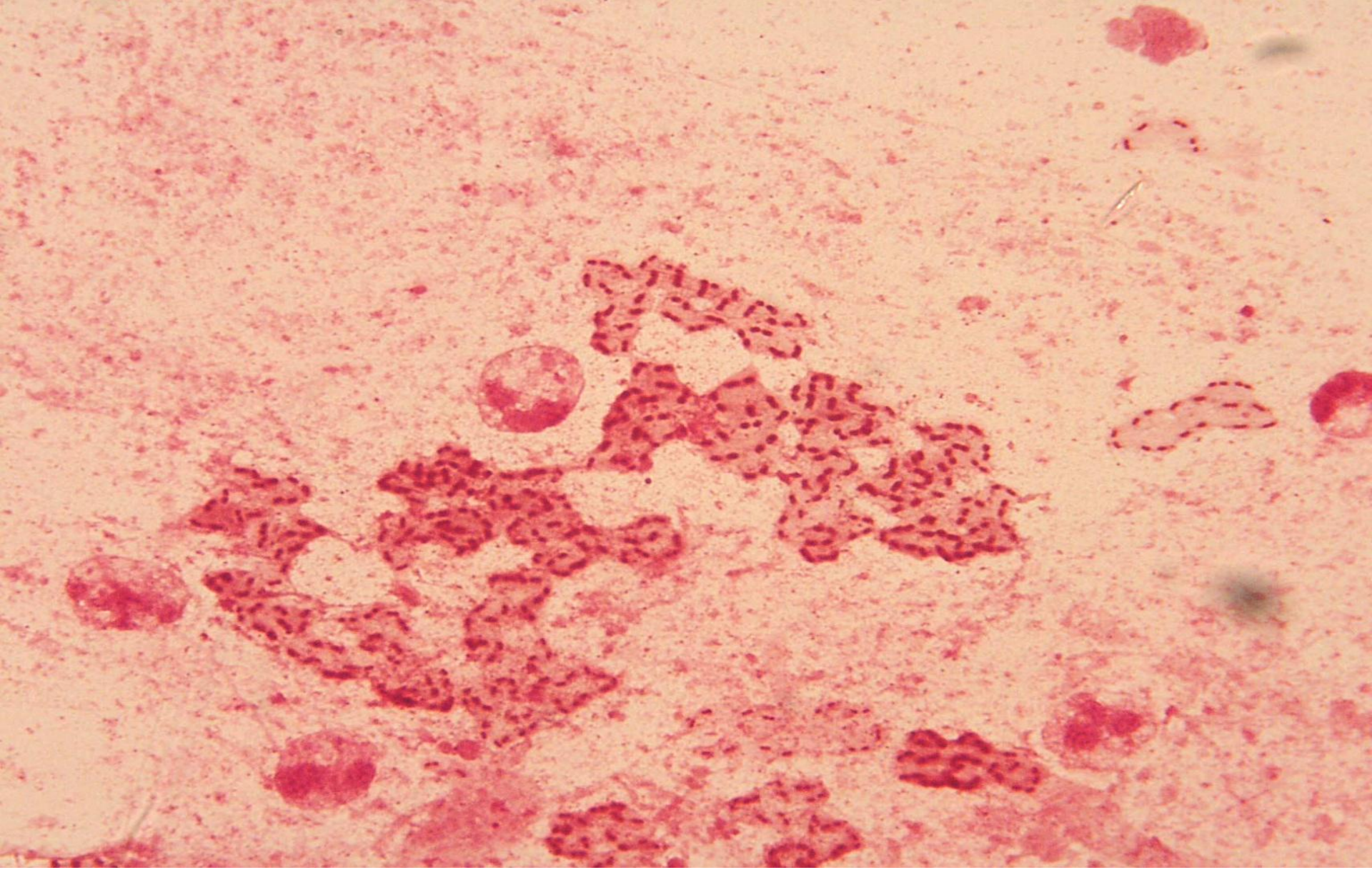


Oral specimen - Gram stain

http://www.healthsci.utas.edu.au/his/teaching/micro/mma.html



Sputum specimen Gram stain—*Streptococcus pneumoniae*
http://www.healthsci.utas.edu.au/hls/teaching/micro/mma.html



Sputum—cystic fibrosis patient, encapsulated gram negative rods
http://www.healthsci.utas.edu.au/hls/teaching/micro/mma.html

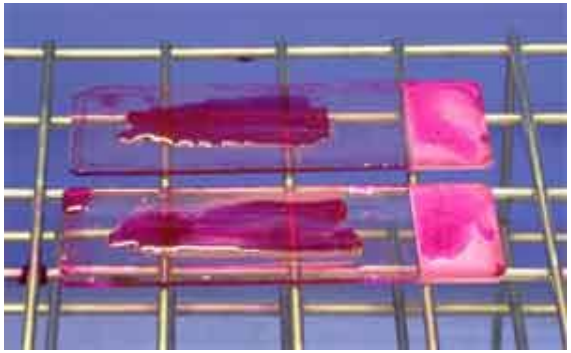
Staining techniques for Mycobacterial spp

- Acid-fast stains
  - for staining of organisms with high degree of fatty (mycolic) acids—waxy
  - render the cells resistant to decolorization: “acid-fast”
  - *Mycobacterium* sp., *Nocardia* sp., *Cryptosporidium* sp. are acid-fast
  - Procedure
    - Ziehl-Neelsen: heat drives in primary stain (carbolfuchsin)
    - Kinyoun: higher conc. of phenol does not require heat
    - Decolorize with acid-alcohol
    - Counterstain with methylene blue or malachite green

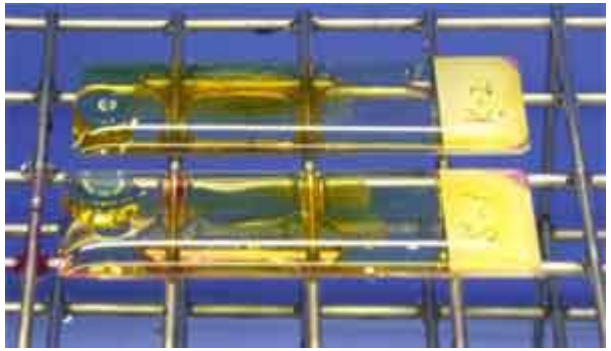
1

2

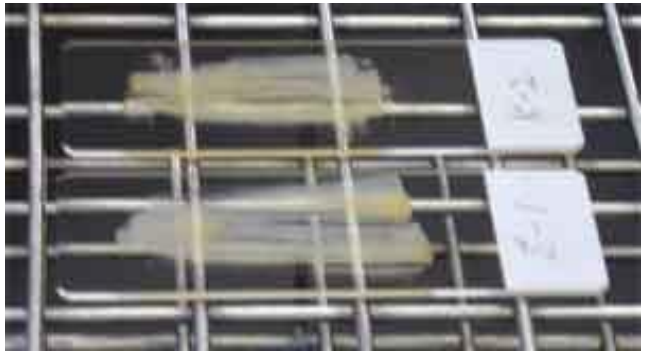
3



4

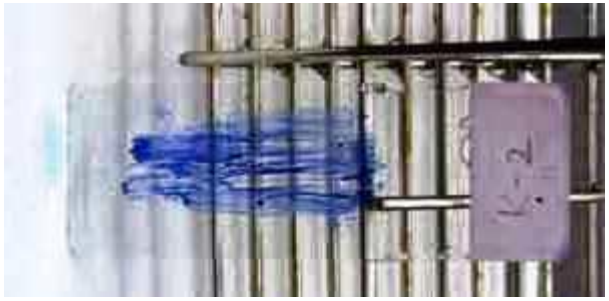


5



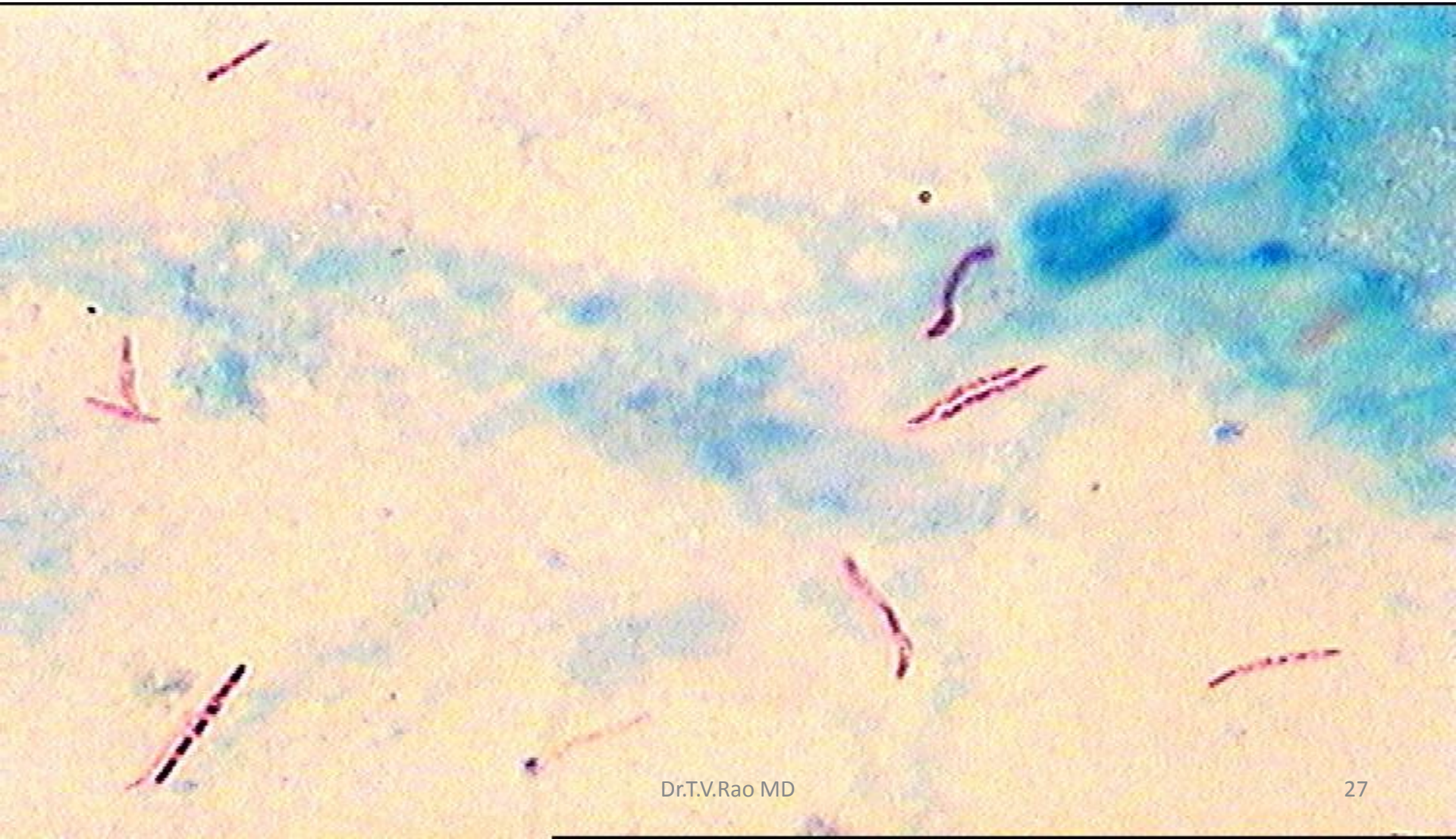
6

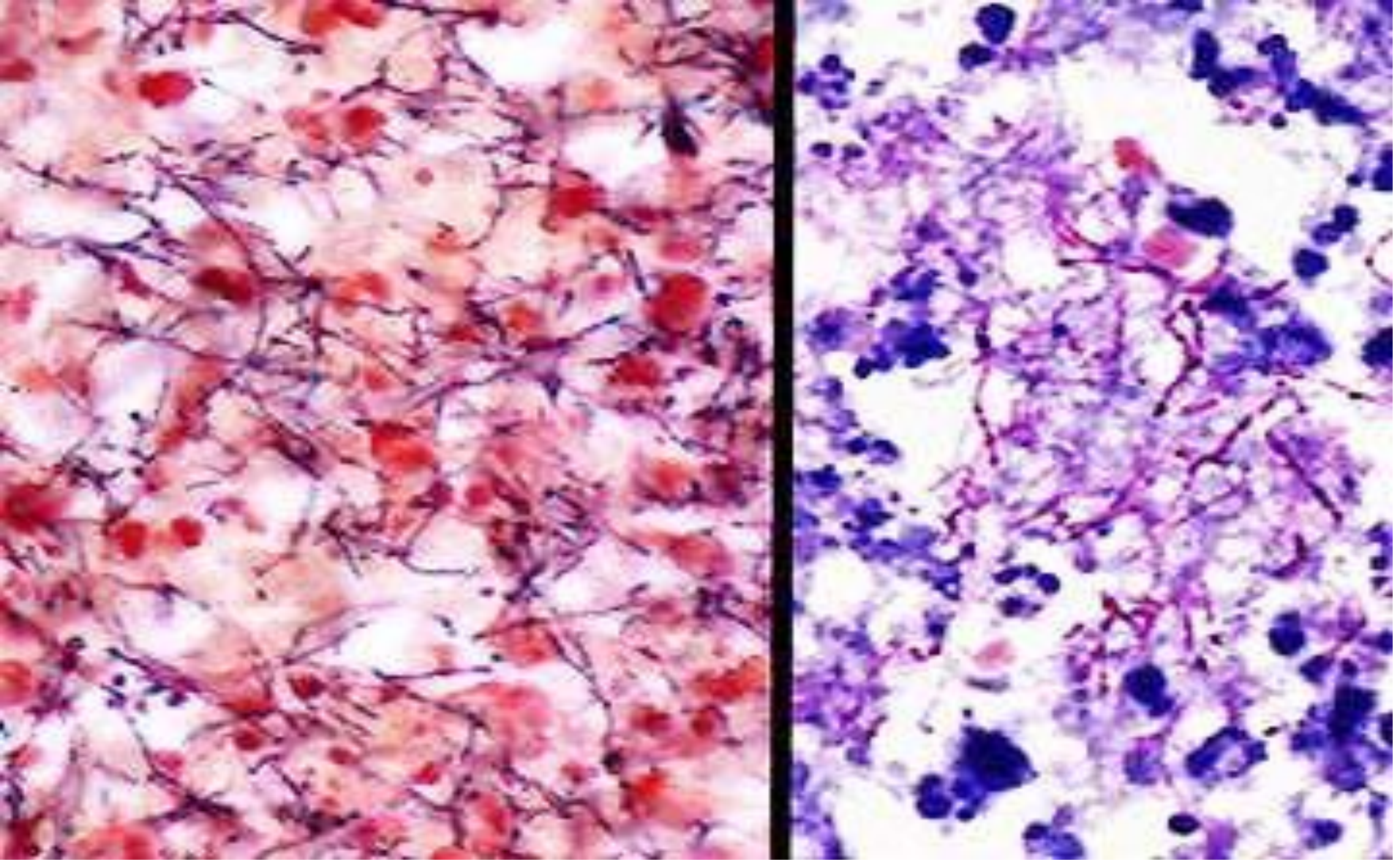
7



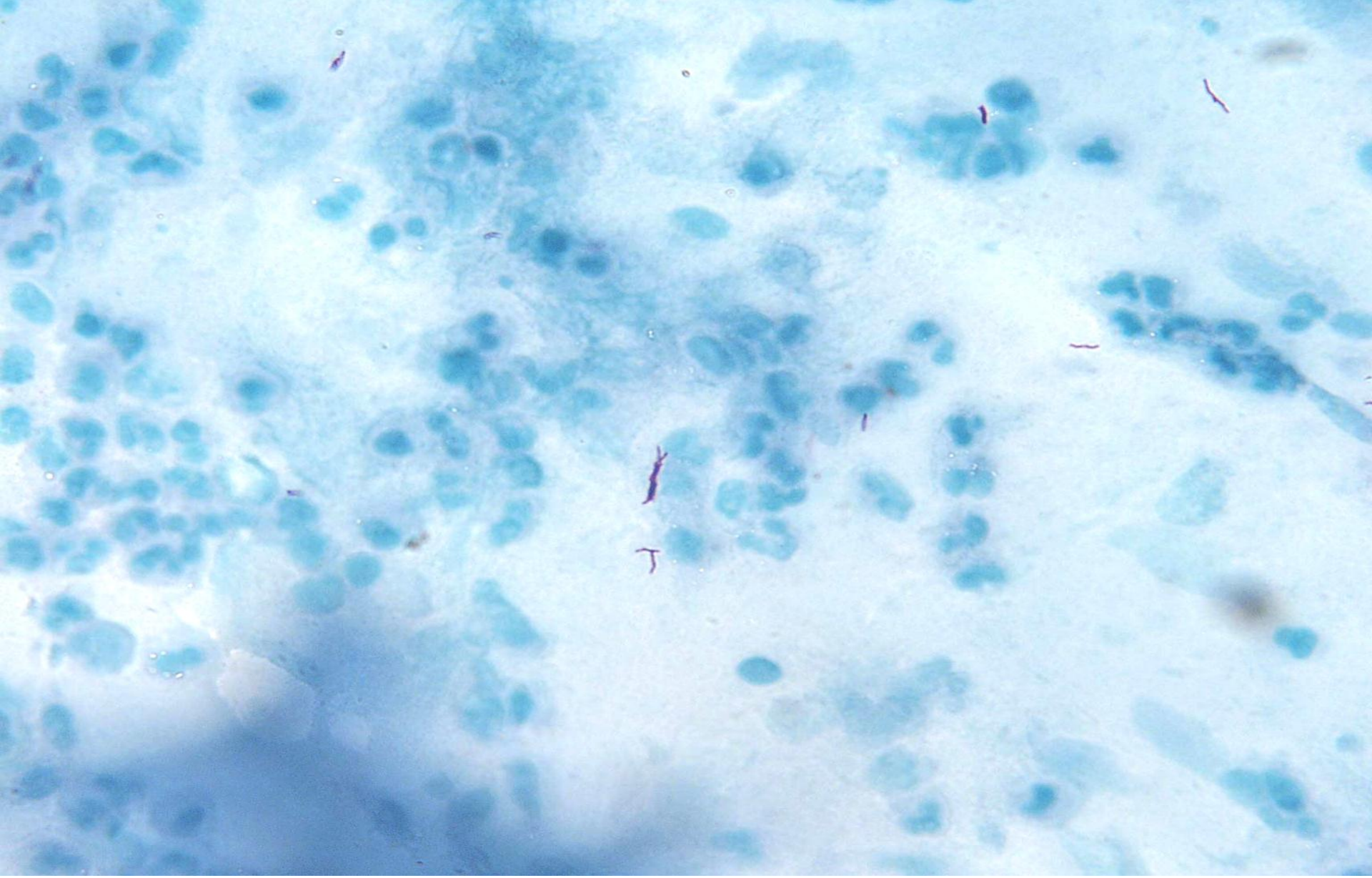
Ziehl-Neelsen stain

How the Acid fast bacteria appear

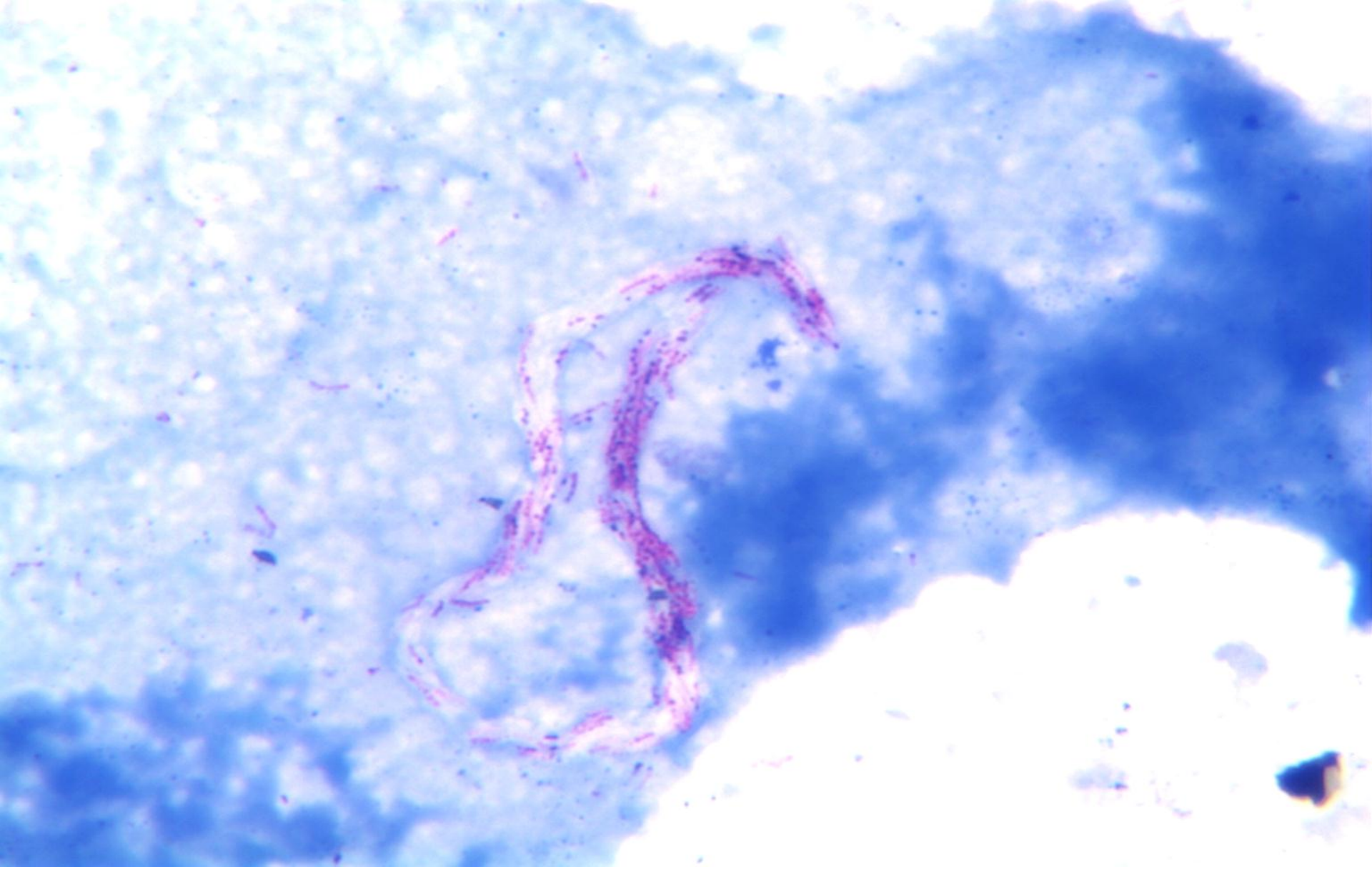




A Gram stain (left) of the abscess shows thin, gram positive rods in chains.
An acid fast stain (right) was also positive.
http://pathhsw5m54.ucsf.edu/overview/bacteria3.html



Sputum specimen—Acid fast stain
http://www.healthsci.utas.edu.au/hls/teaching/micro/mma.html

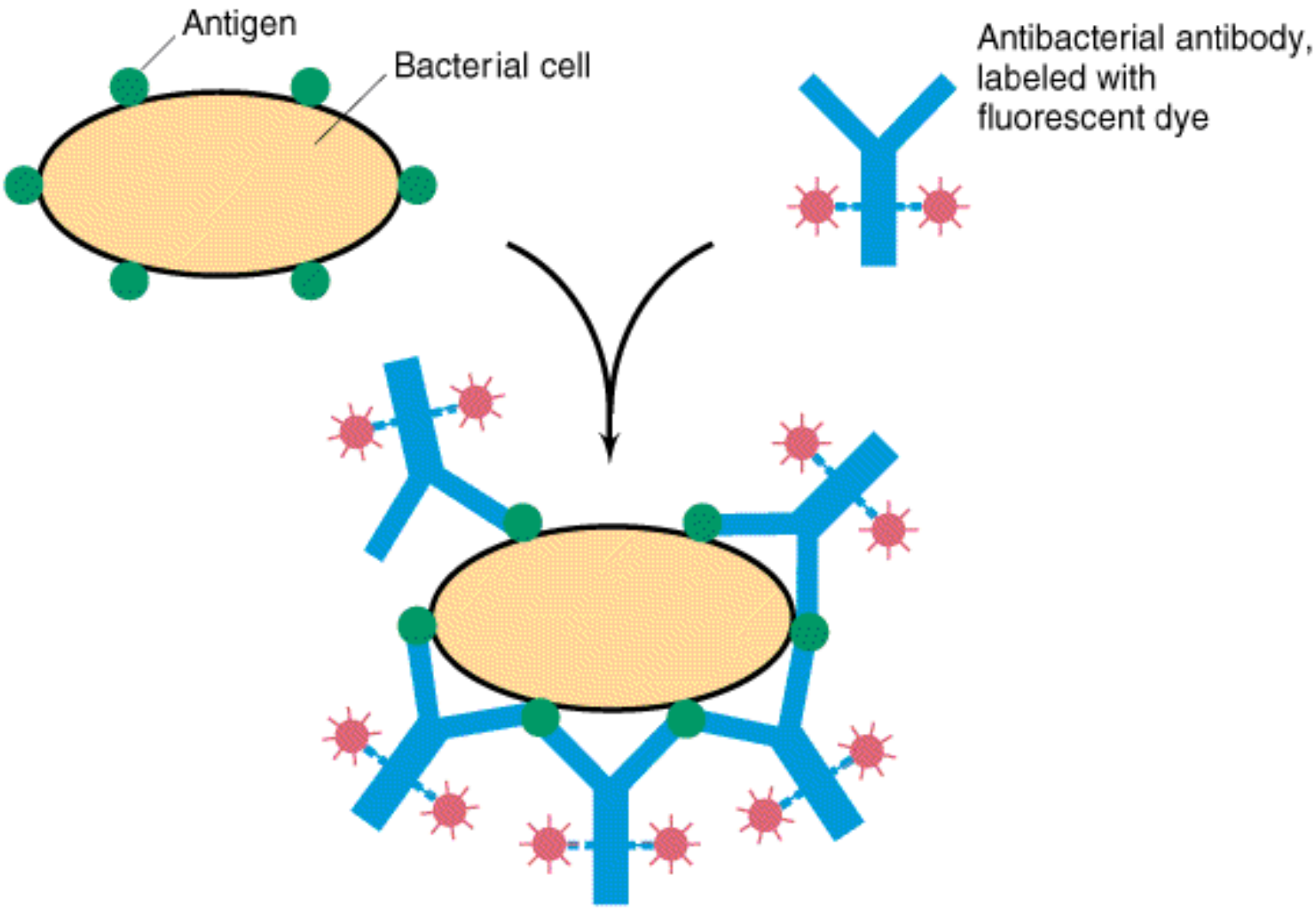


Acid fast stain demonstrating chording

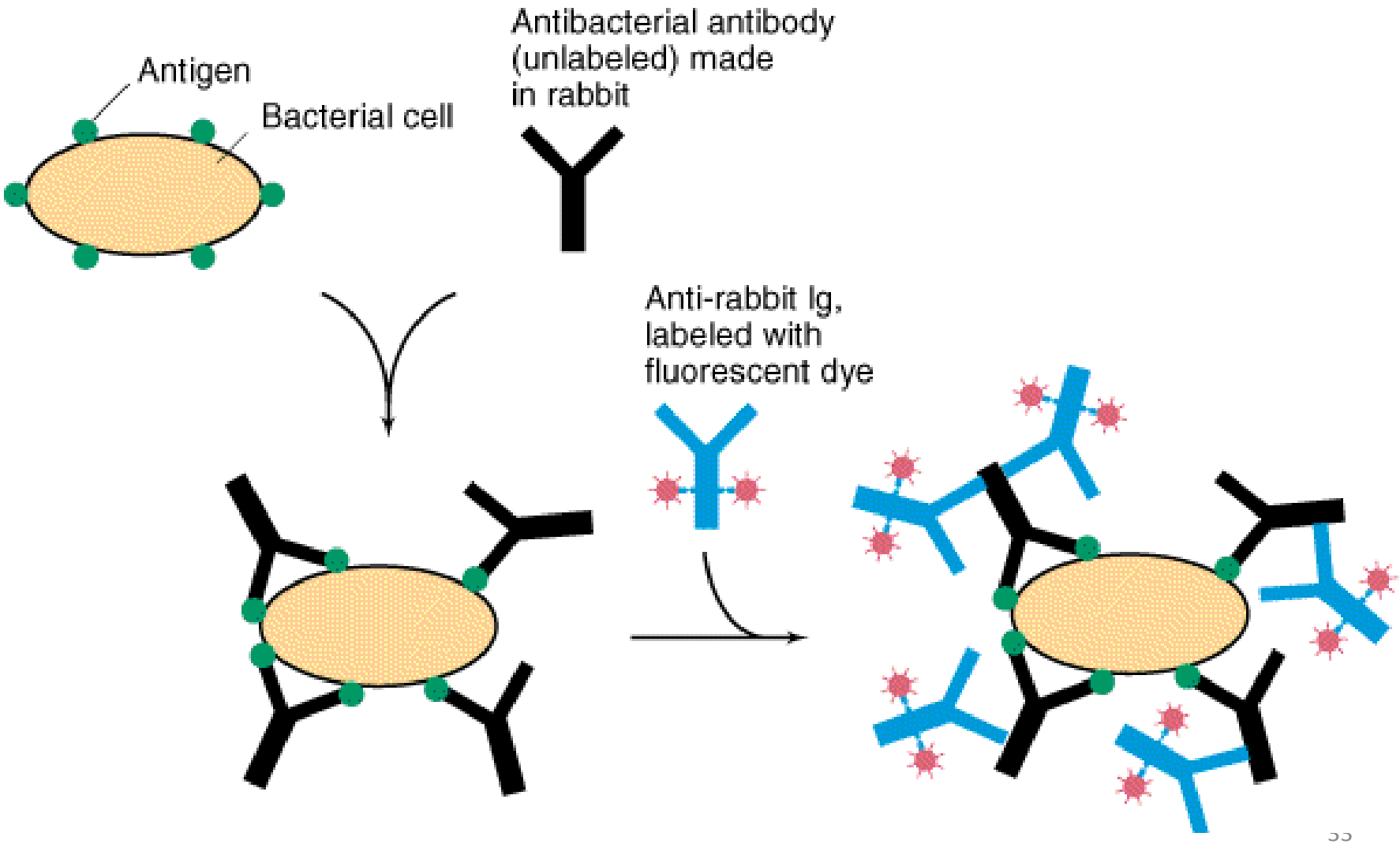
Fluorochrome AFB Microscopy

- More rapid and sensitive
- Specificity : same with sufficient expérience
- Equipment cost , bulbs, technical demands
- for busy labs
- External quality assessment should be done if this method is performed

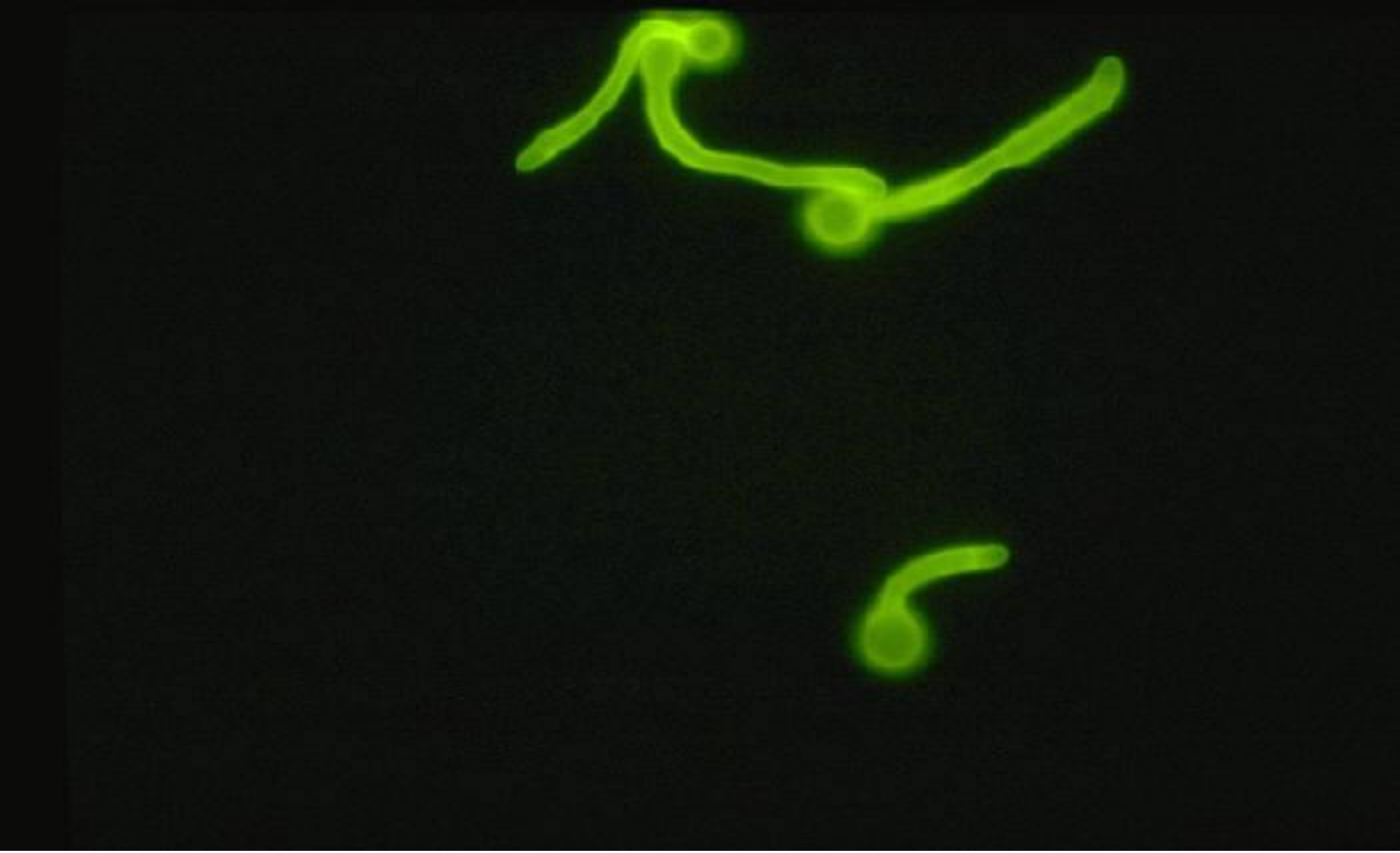
Direct Fluorescent Antibodies



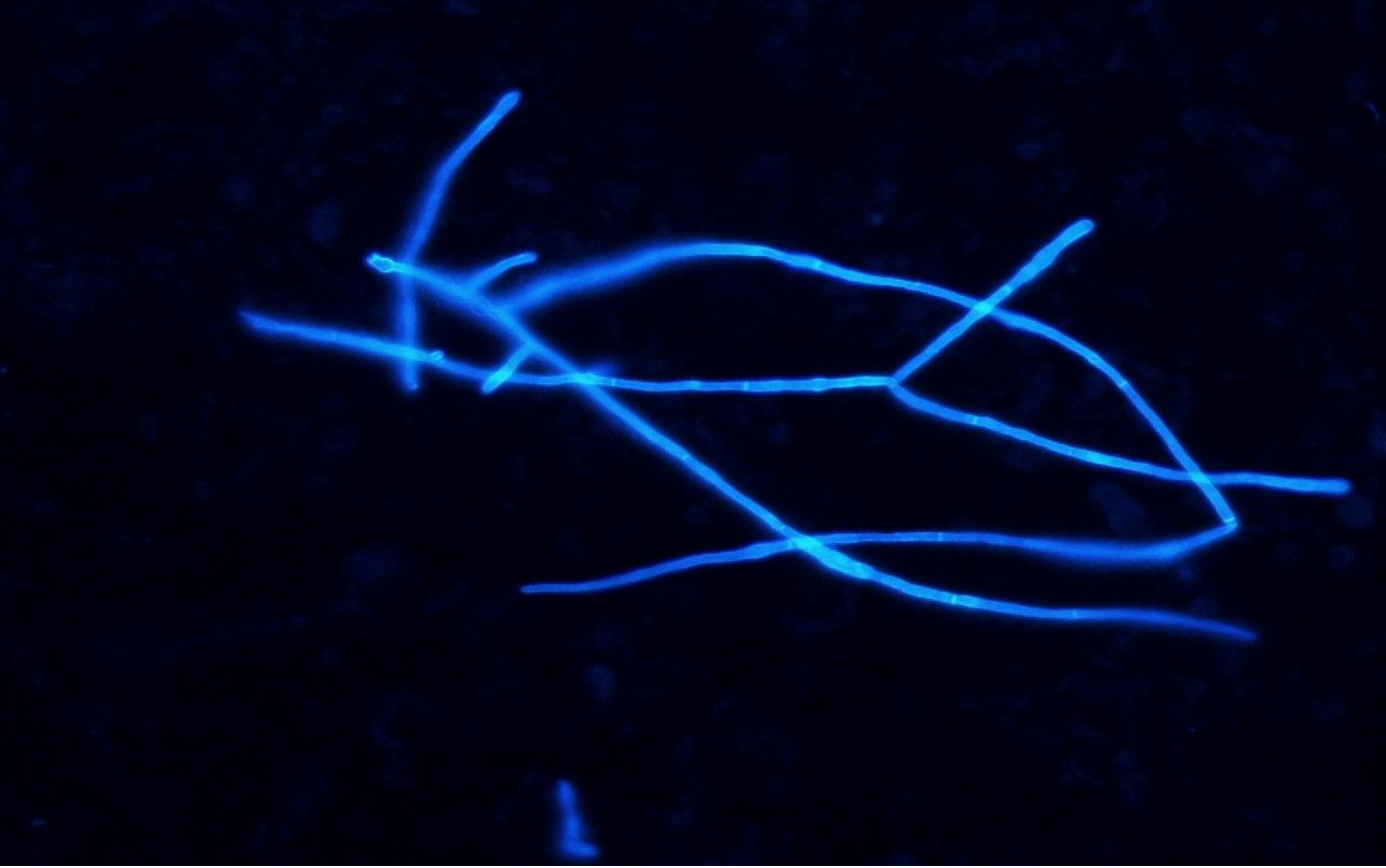
Indirect Fluorescent Antibodies



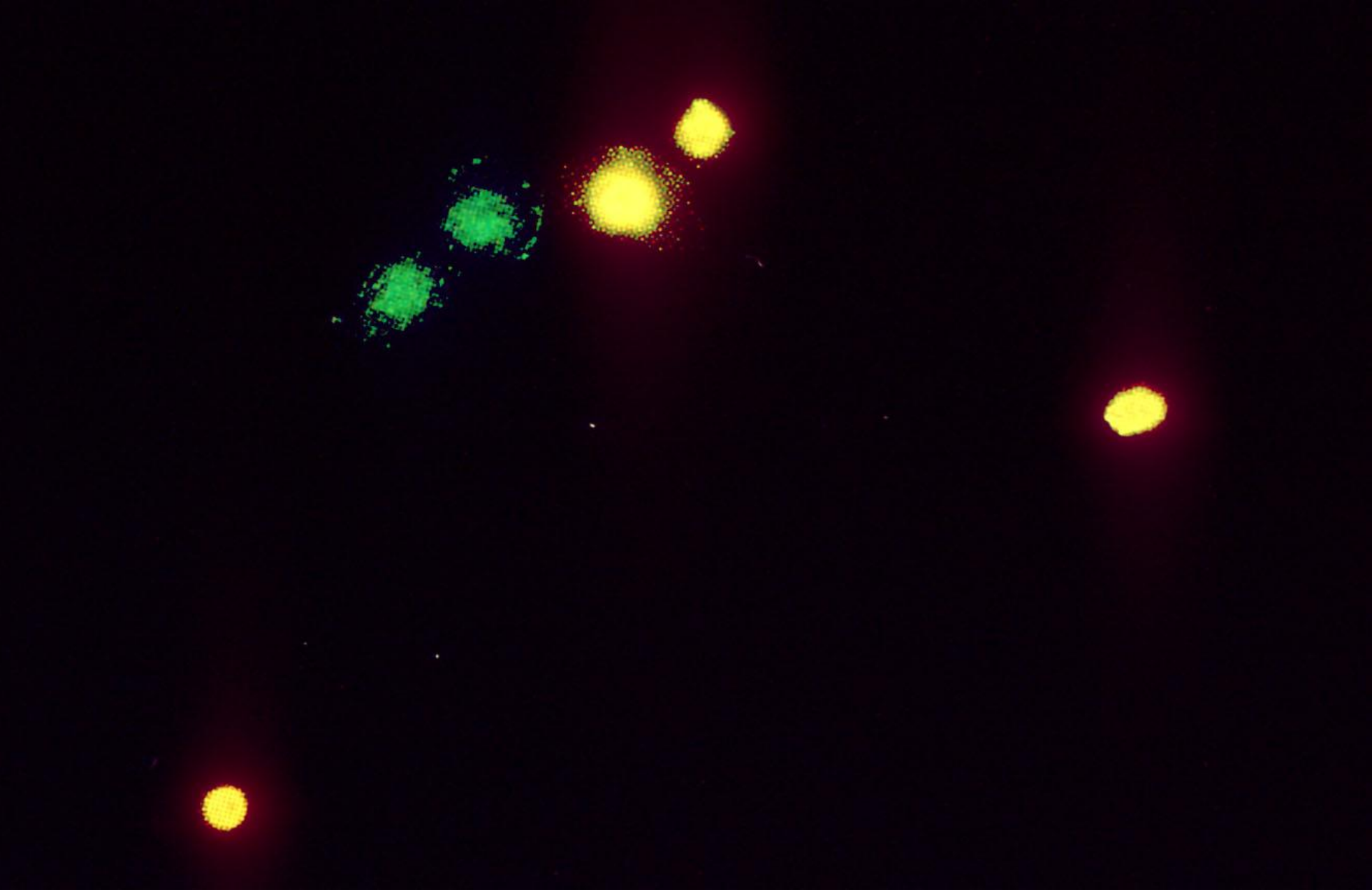
Mycobacterium – auramine stain
http://www.lung.ca/tb/abouttb/what/causes_tb.html



Yeast—calcofluor white
http://www.med.sc.edu:85/mycology/mycology-3.htm



Mould—calcofluor white



Influenza virus infected cells, fluorescent antibody stain

Better Use Microscopy

- Phase Contrast Microscopy
  - shift in light allows visualization of organism; can visualize viable organisms
- Fluorescent Microscopy
  - certain dyes (fluorochromes) give off light when excited (fluorescence)
  - color of light depends on the dye and the filters used
  - Staining techniques
    - Fluorochroming: direct chemical interaction with organism
      - Acridine orange: stains nucleic acid; useful for cell-wall deficient organisms
      - Auramine-rhodamine: bind to mycolic acids in nearly all *Mycobacteria*
      - Calcoflour white: binds to chitin in cell walls of fungi
    - Immunofluorescence: fluorochrome is bound to an antibody; can detect/identify specific organisms

Culture and isolation of bacteria

- Principles of Cultivation
  - Nutritional requirements
    - General concepts
      - non-fastidious: simple requirements for growth
      - fastidious: complex, unusual, or unique requirements for growth
    - Phases of growth media
      - solid → agar; boil to dissolve, solidifies at 50ºC
      - liquid, broth

- **Media classifications and functions**
  - **Enrichment**
    - used to enhance growth of specific organisms
  - Supportive
    - support growth of most non-fastidious organisms
  - **Selective**
    - contains agents that inhibit the growth of all agents except that being sought (dyes, bile salts, alcohols, acids, antibiotics)
  - Differential
    - contains factor(s) that allow certain organisms to exhibit different metabolic characteristics

According to Use

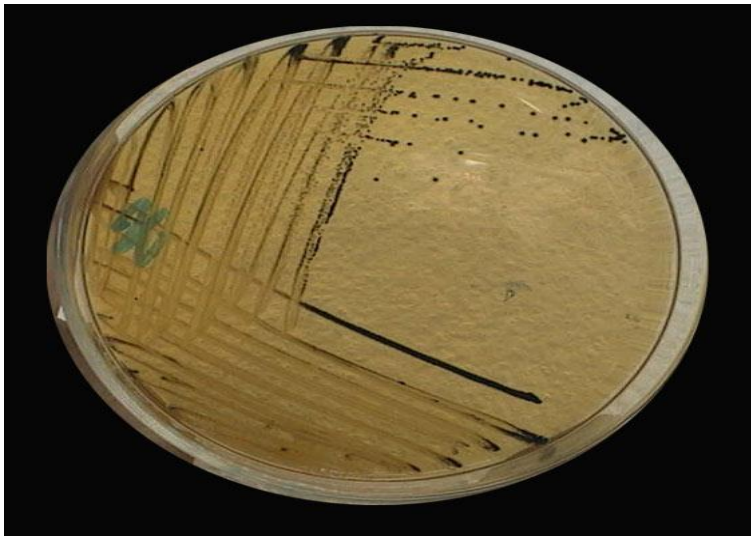
- Enriched Medium – broth or solid, contains rich supply of special nutrients that promotes growth of a particular organism while not promoting growth of other microbes that may be present (e.g BAP & chocolate agar)

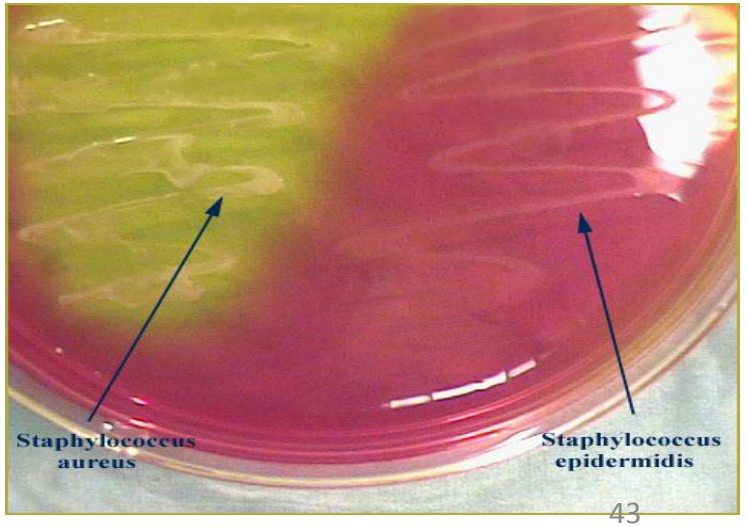
• Types of artificial media

- Brain-heart infusion
  - nutritionally rich supportive media used in broths, blood culture systems and susceptibility testing
- Sheep blood agar
  - supportive media containing 5% sheep blood for visualization of hemolysis
- Chocolate agar
  - same as sheep blood agar except blood has been "chocolatized" RBCs lysed by heating; releases X (hemin) and V (NAD) factors for *Neisseria* and *Haemophilus*
- MacConkey agar
  - selective for Gram-negative rods (GNRs) because of crystal violet and bile salts; differential due to lactose, fermenters lower pH changing neutral red indicator pink/red

According to Use

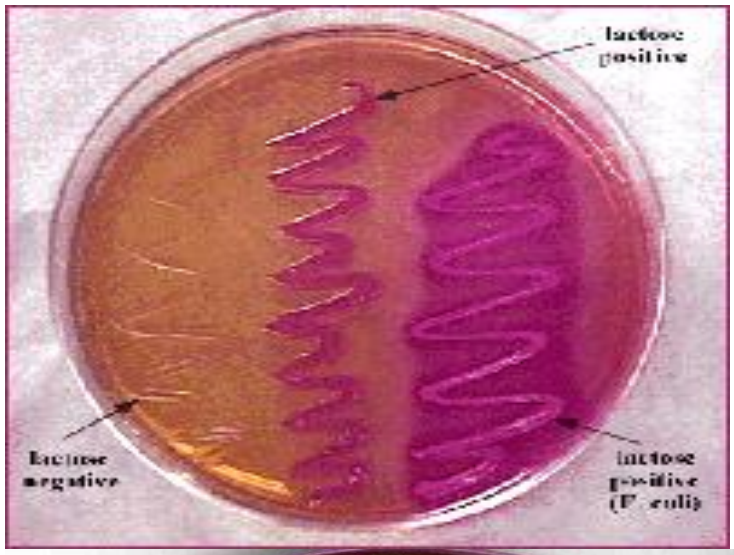
- Selective Medium - contains inhibitors that discourage the growth of certain organisms & enhances the growth of the microbe sought (e.g. SSA, Mannitol Salt Agar)

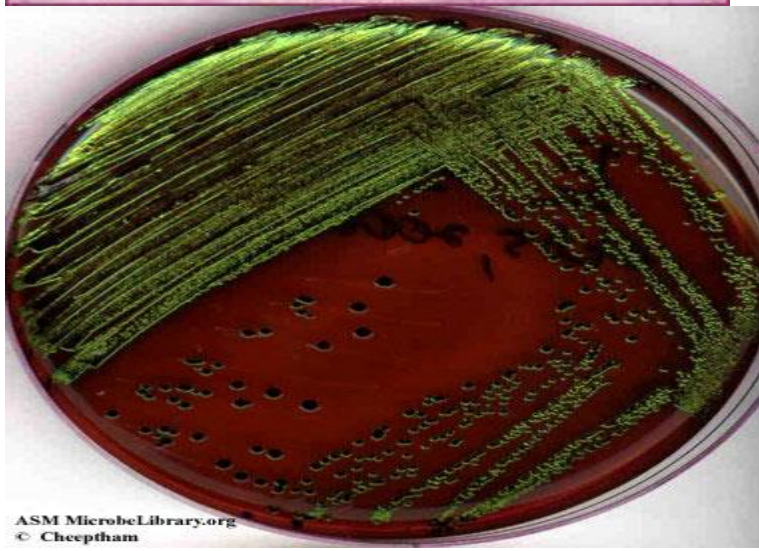




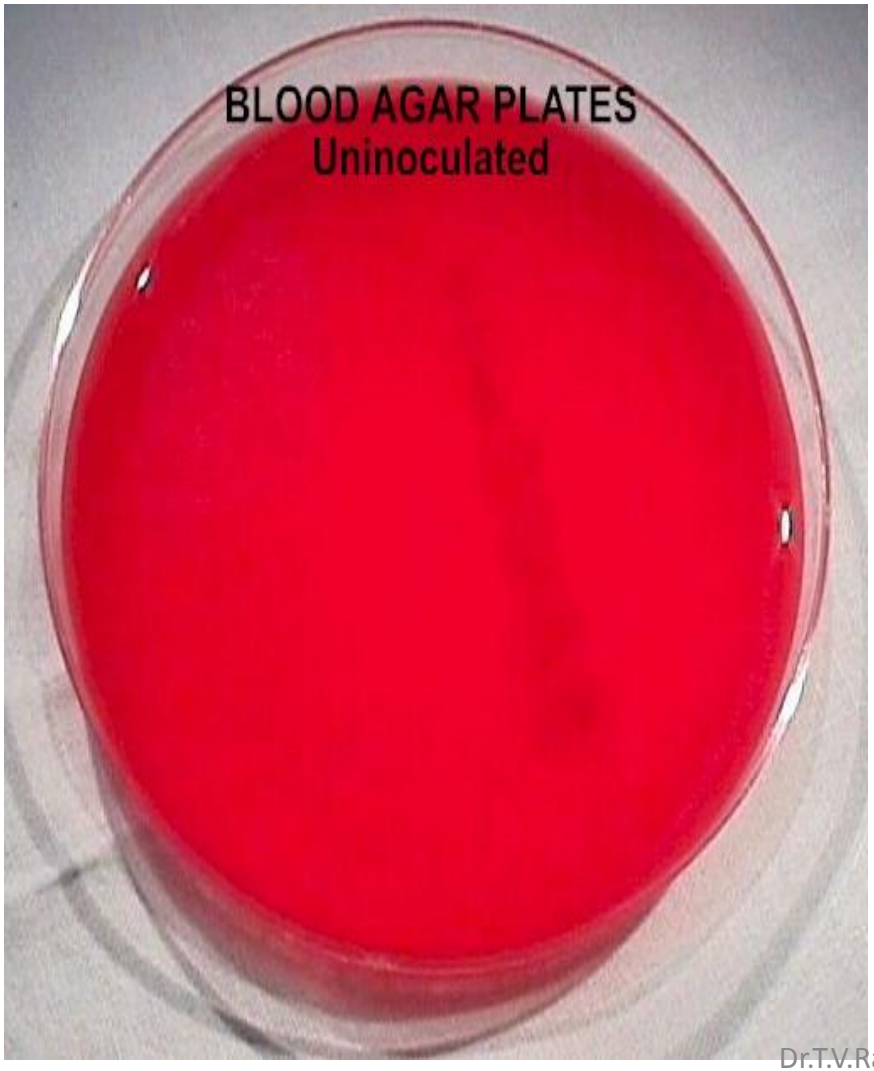
According to Use

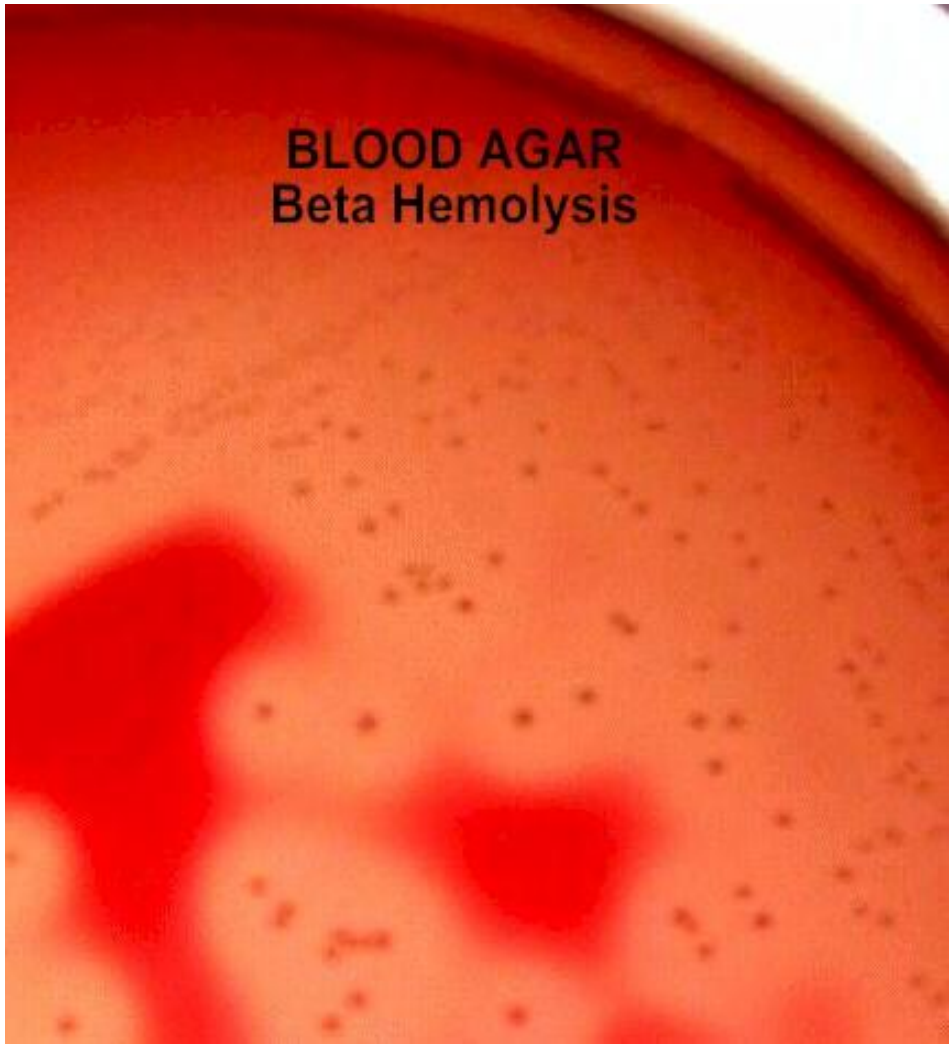
- Differential Medium - contains dyes, indicators or other constituents that give colonies of particular organisms distinctive and easily recognizable characteristics (e.g. McConkey Agar)



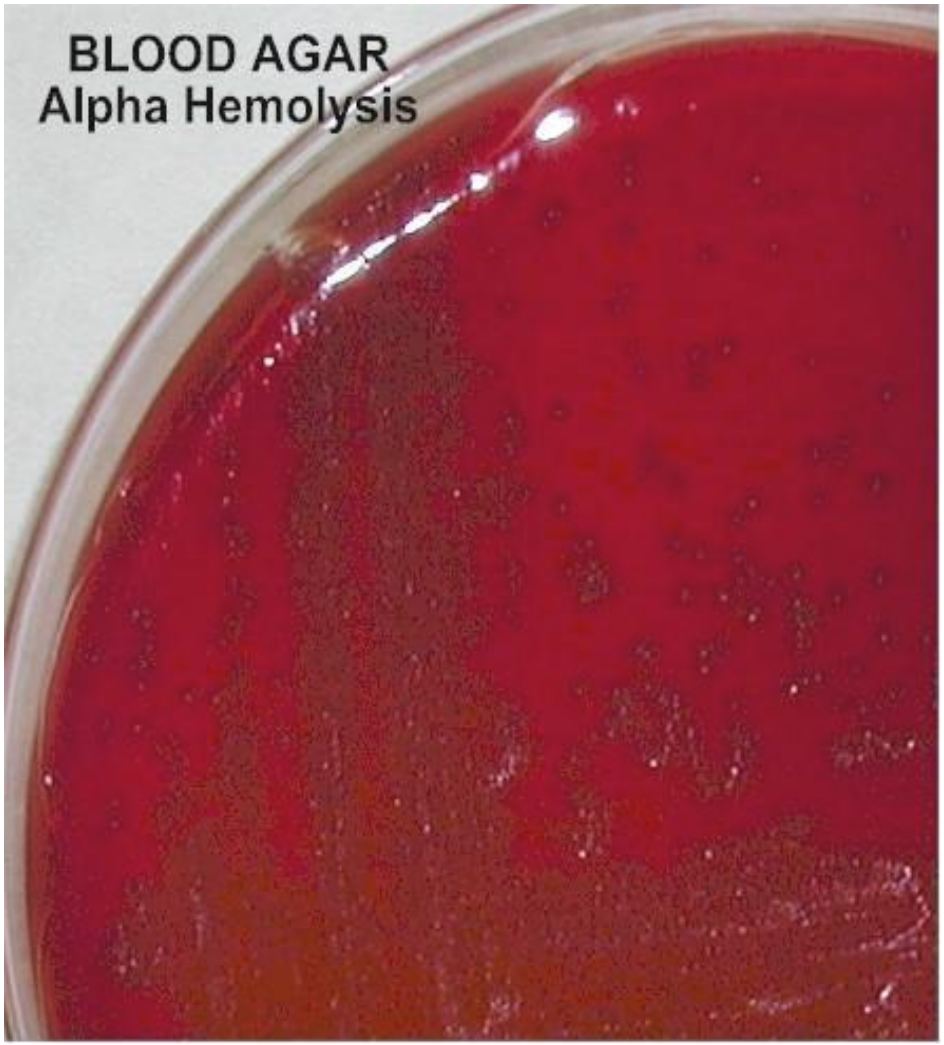


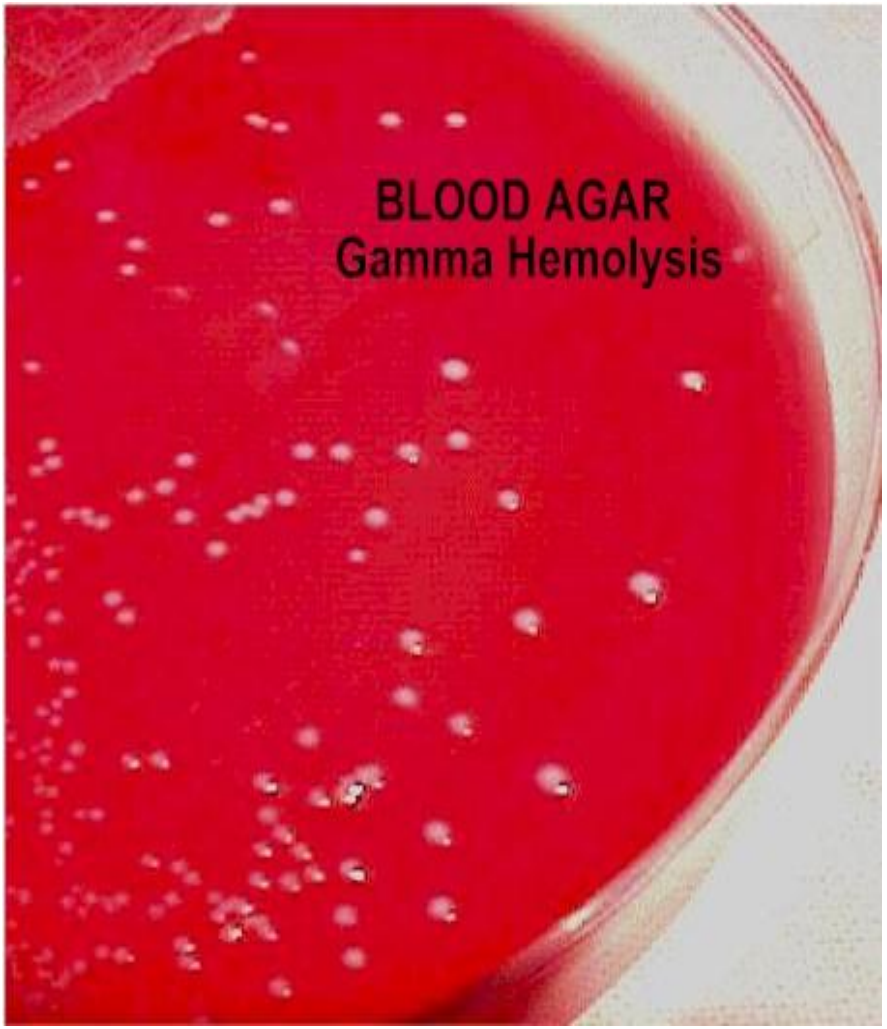
Hemolysis a guiding factor



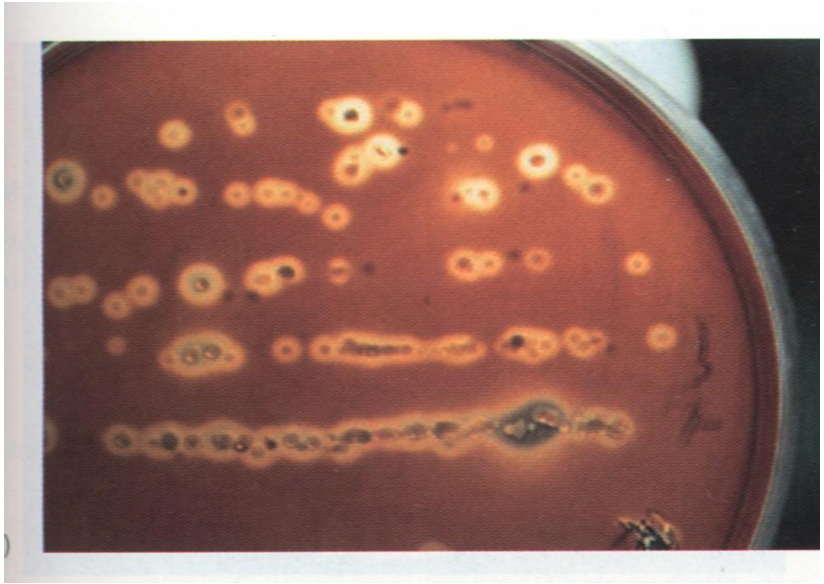


Hemolysis a guiding factor



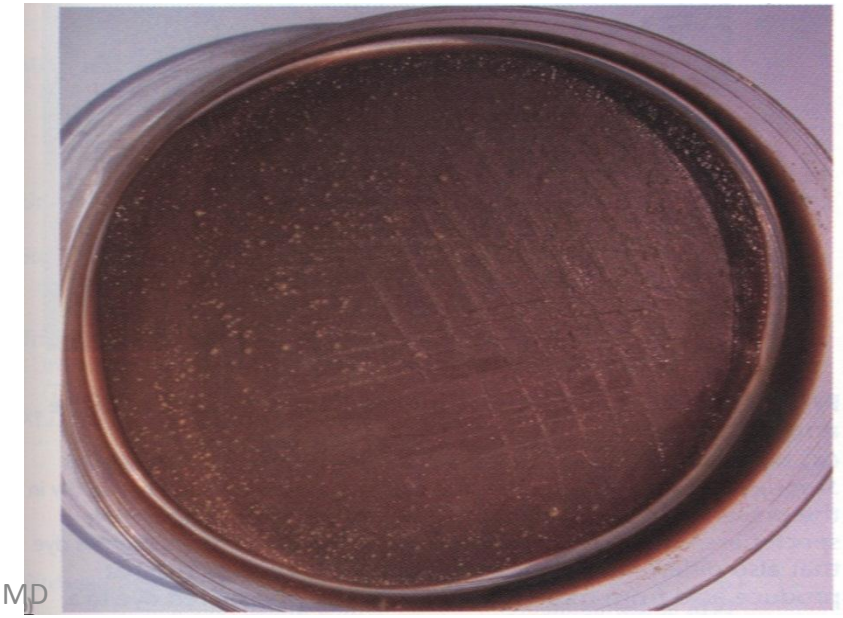


Enriched Media

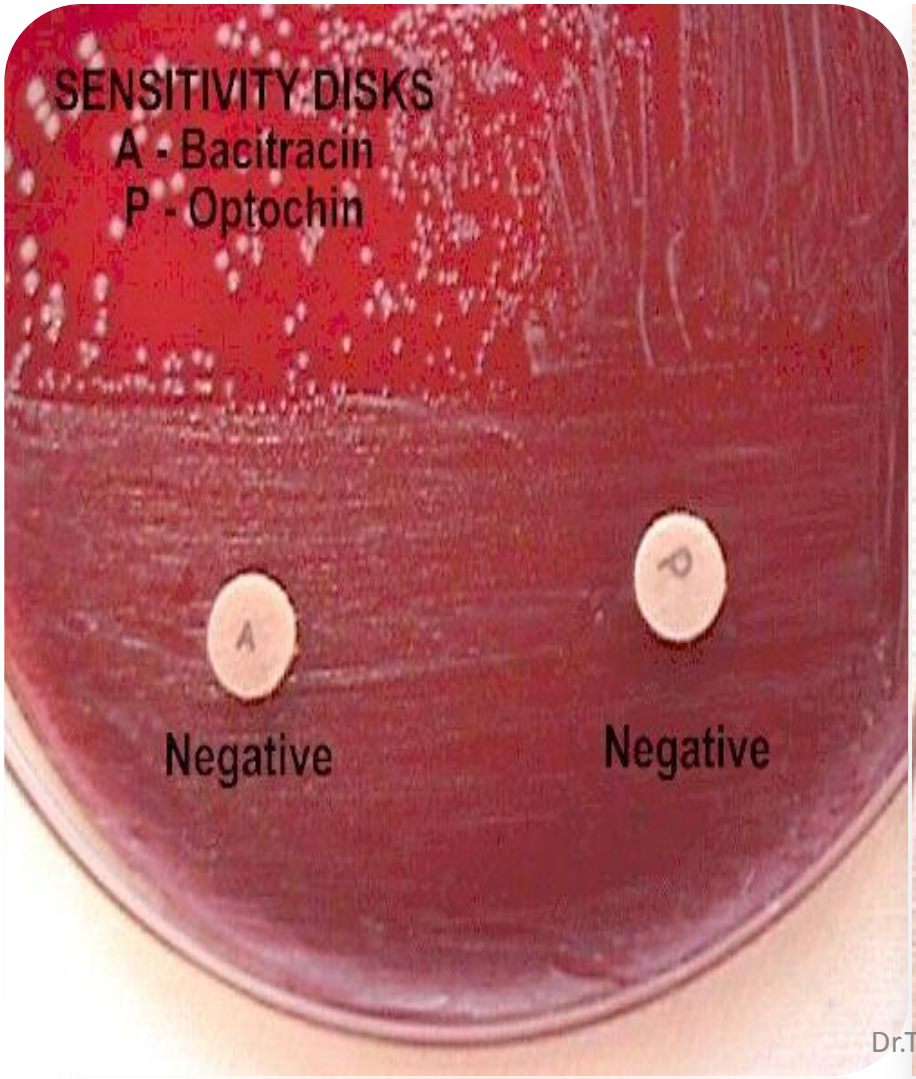


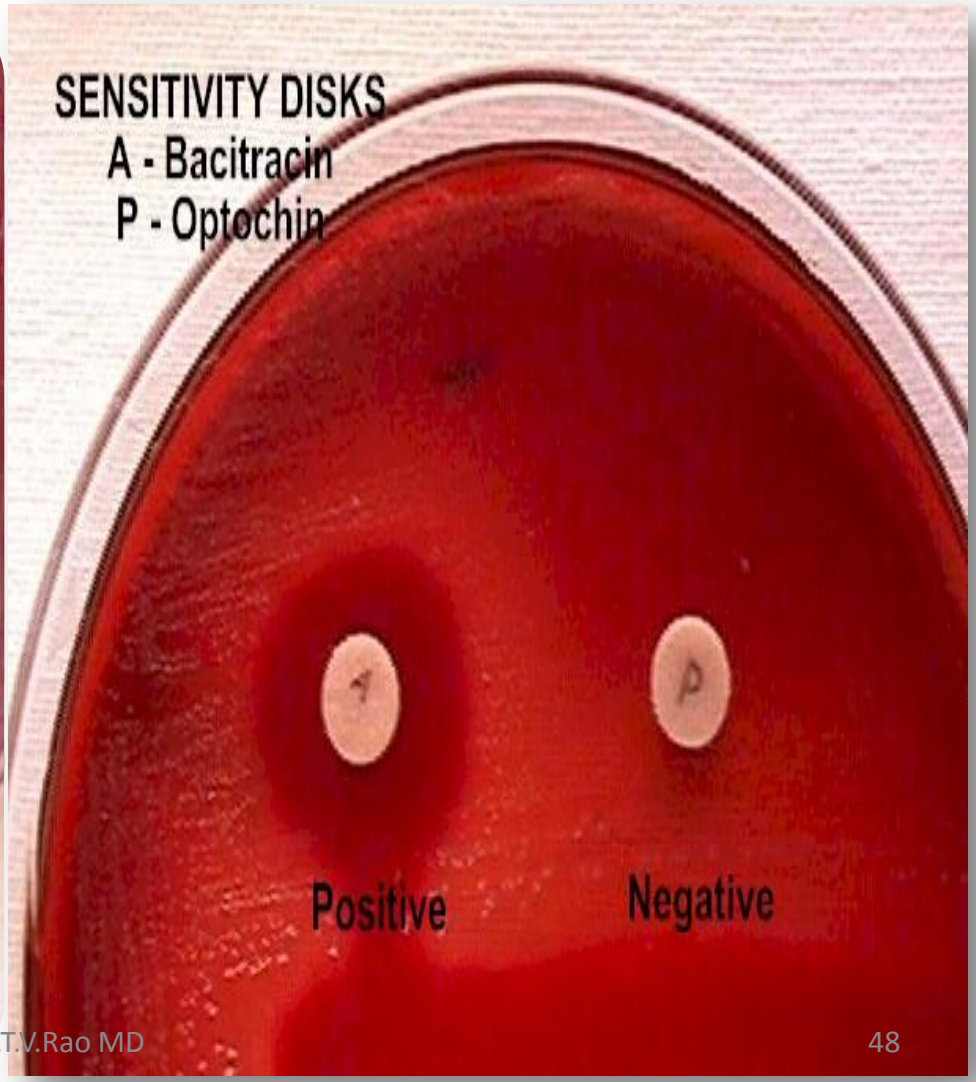
Blood agar plate with bacteria from human throat. This media differentiates among different colonies by appearance

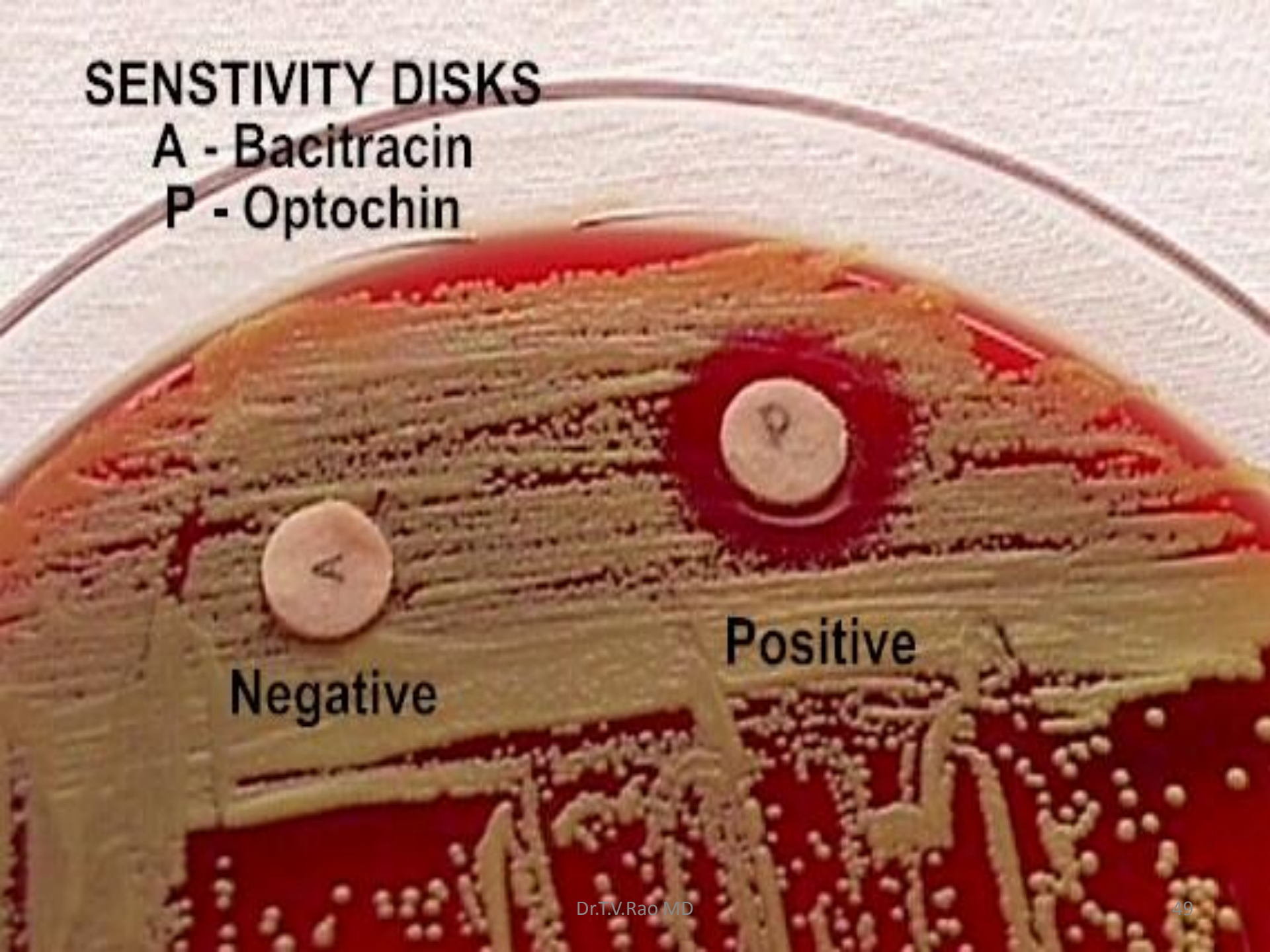
Chocolate agar, a medium that gets brown from heated blood. Used for isolation of *N. gonorrhea*.



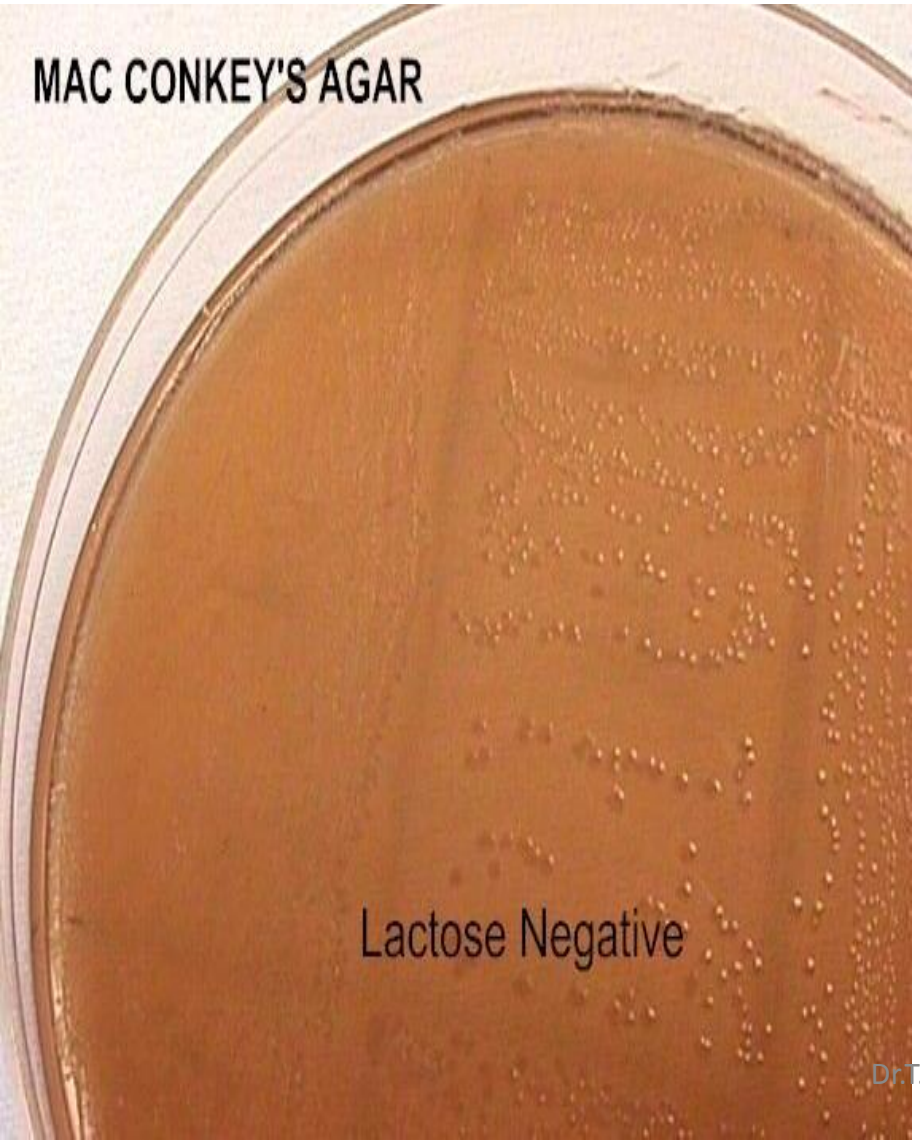
Testing for Bacitracin and Optochin Sensitivity

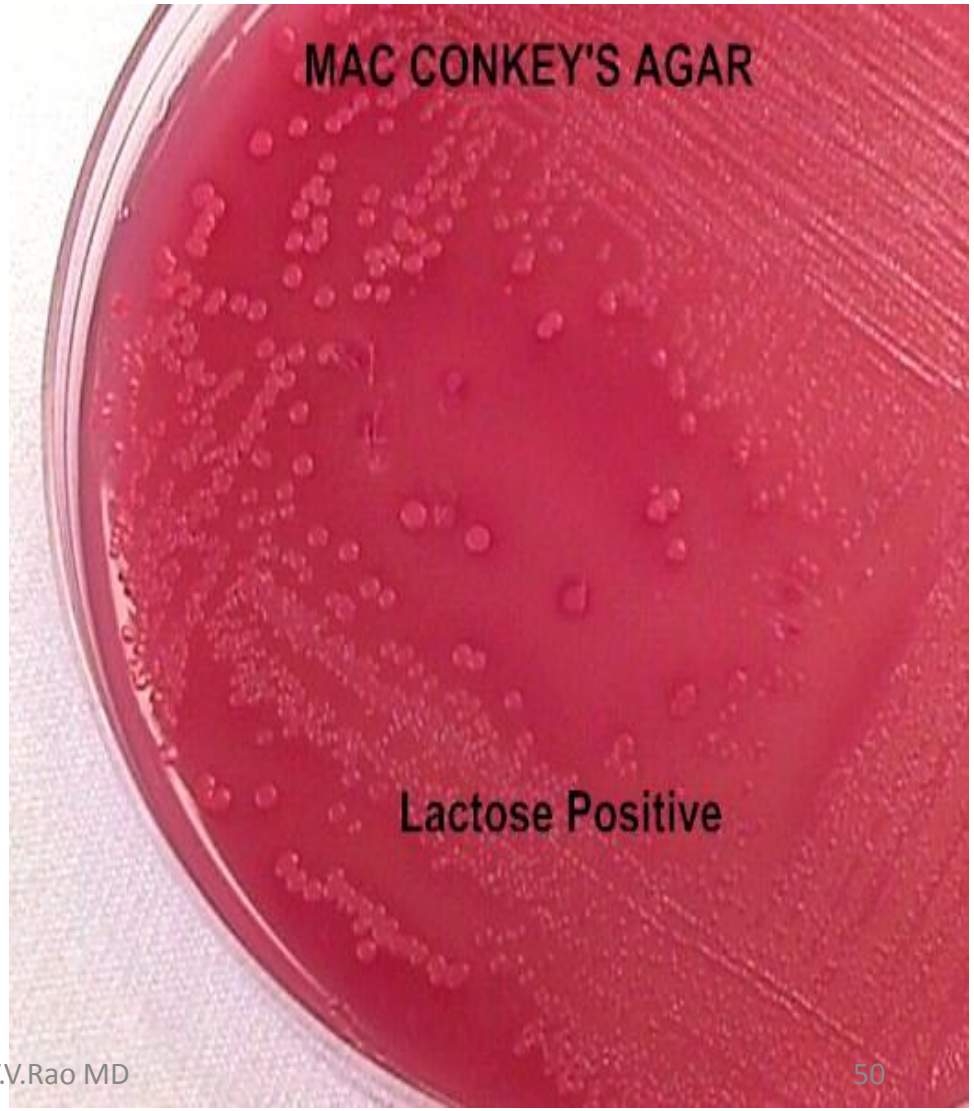





SENSTIVITY DISKS
A - Bacitracin
P - Optochin
A
P
Negative
Positive

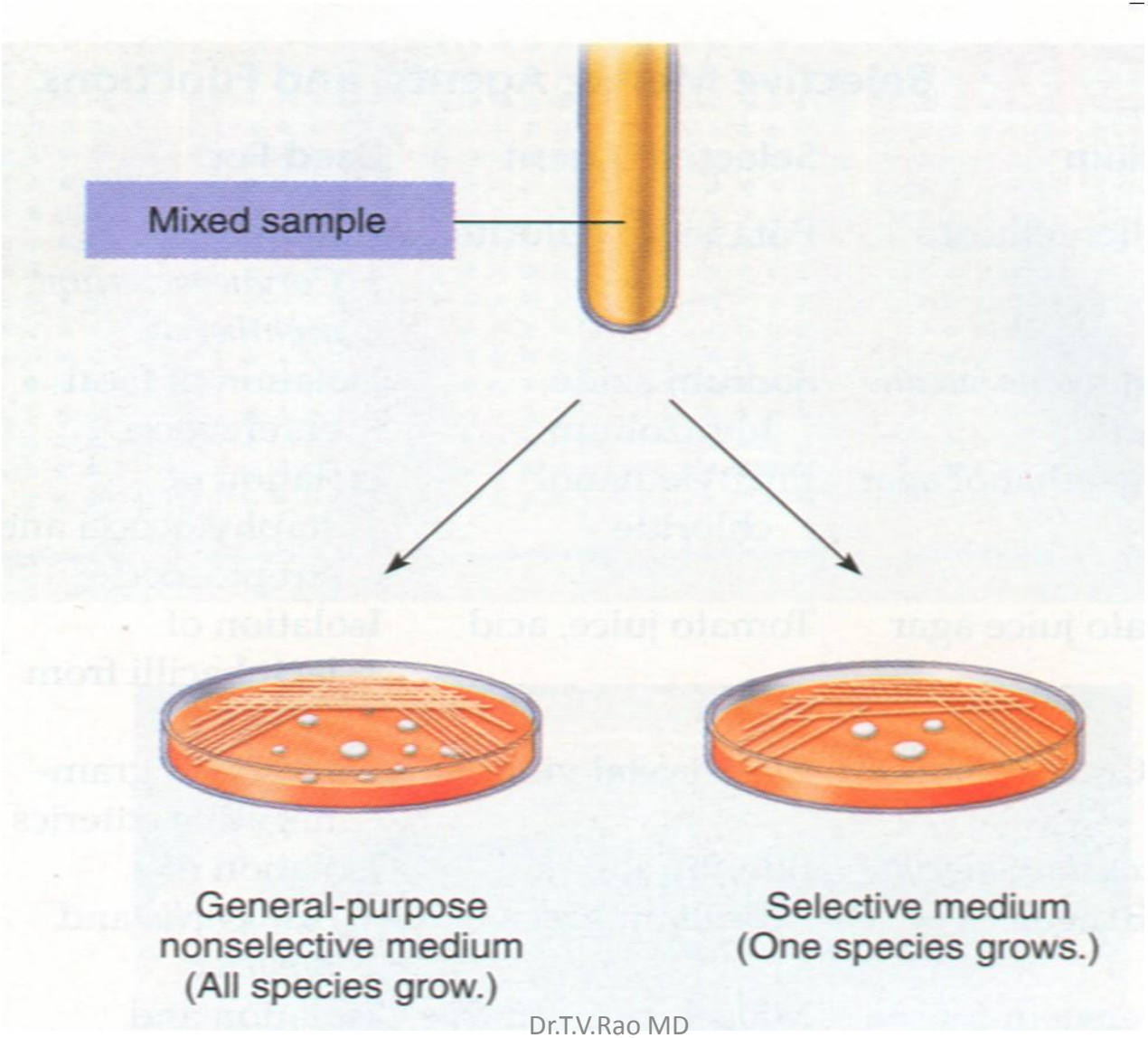
Mac Conkey Agar a Minimal differentiating Medium



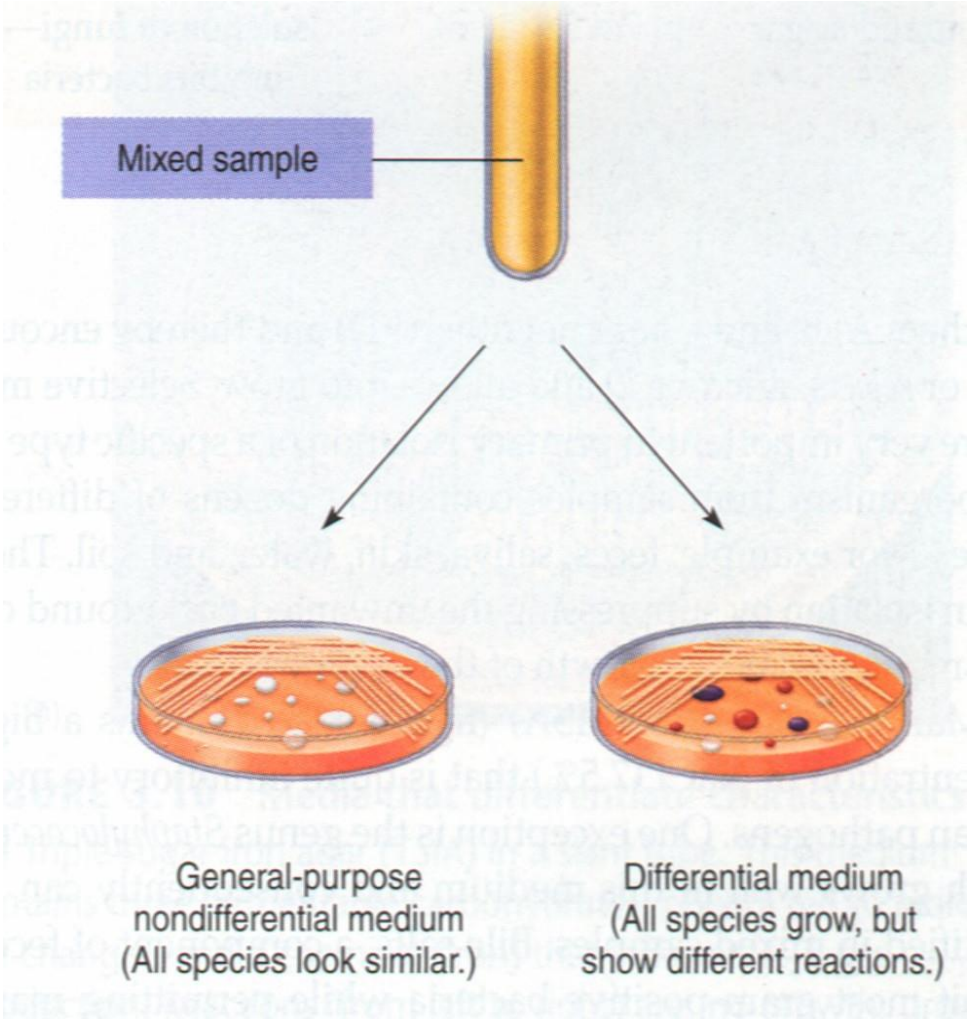


MacConkey Agar

General vs Selective Media

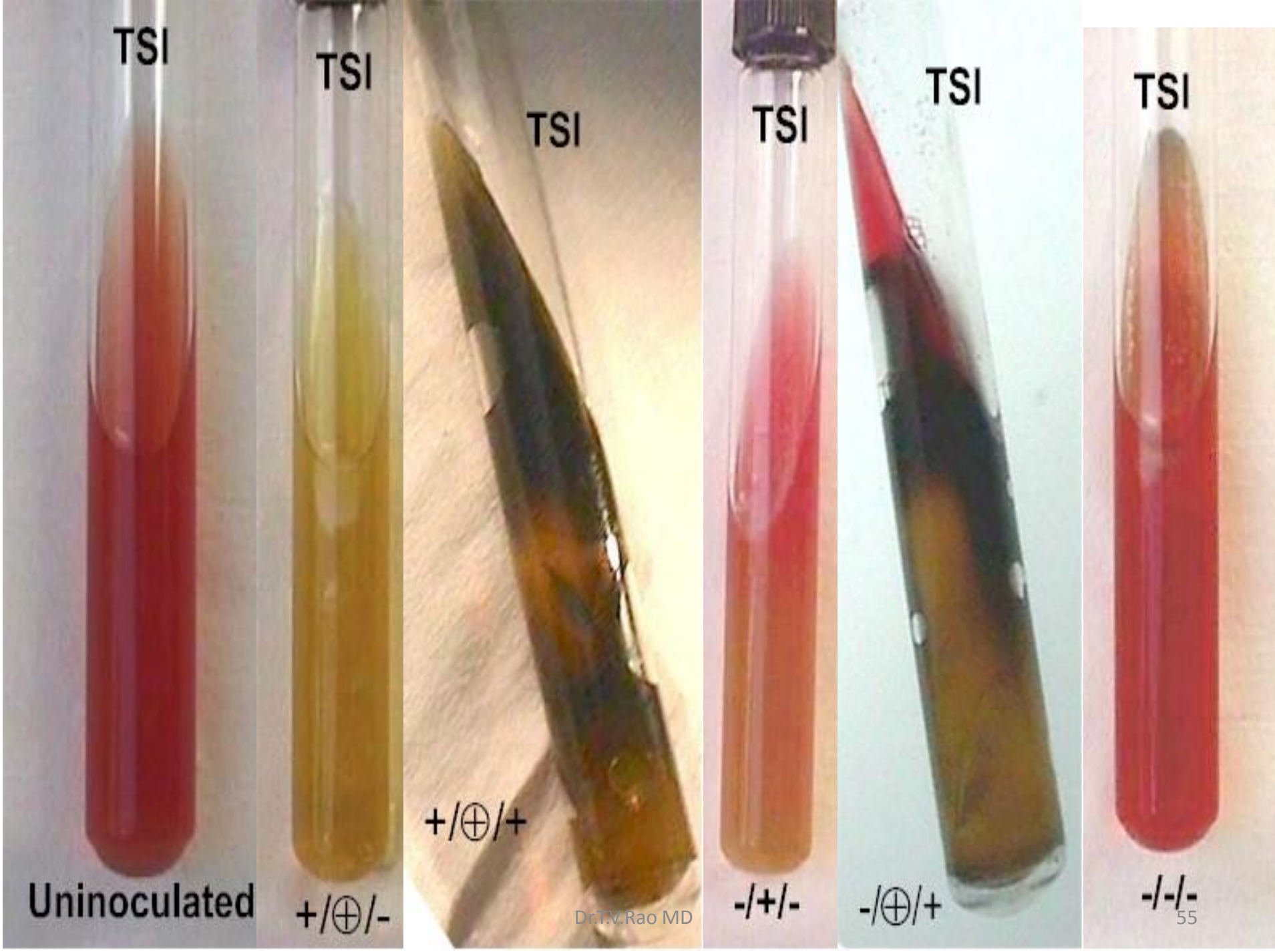


Differential Media



- **Differential media** grow several types of organisms and **display visible differences** among organisms.
- Differences may show up as colony size, media colour, gas bubble formation and precipitate formation.

Salmonella-Shigella Agar


TSI
Uninoculated
TSI
+/⊕/-
TSI
+/⊕/+
TSI
-/+/-
TSI
-/⊕/+
TSI
-/-/-

• Types of artificial media

– Hektoen enteric agar
  - contains bile salts and dyes (bromothymol blue and acid fuchsin) to inhibit non-pathogenic GNRs; non pathogens ferment lactose changing BTB to orange; pathogens *Salmonella* and *Shigella* are clear; ferric ammonium citrate detects H_2S production of *Salmonella* (black colonies)

– Columbia colistin-nalidixic acid (CNA) agar
  - Columbia agar base, sheep blood, colistin and nalidixic acid; selective isolation of gram-positive cocci

• Types of artificial media

– Hektoen enteric agar
  - contains bile salts and dyes (bromothymol blue and acid fuchsin) to inhibit non-pathogenic GNRs; non pathogens ferment lactose changing BTB to orange; pathogens *Salmonella* and *Shigella* are clear; ferric ammonium citrate detects H_2S production of *Salmonella* (black colonies)

– Columbia colistin-nalidixic acid (CNA) agar
  - Columbia agar base, sheep blood, colistin and nalidixic acid; selective isolation of gram-positive cocci

Types of artificial media

- Thayer-Martin agar
  - CAP with antibiotics (colistin inhibits gram neg, vancomycin inhibits gram pos, nystatin inhibits yeast); for *N. gonorrhoeae* and *N. meningitidis*; Martin-Lewis has similar function but different antibiotics

- Preparation of artificial media
  - Sterilization
    - autoclave: pressurized steam at 121ºC for 15-30 min.

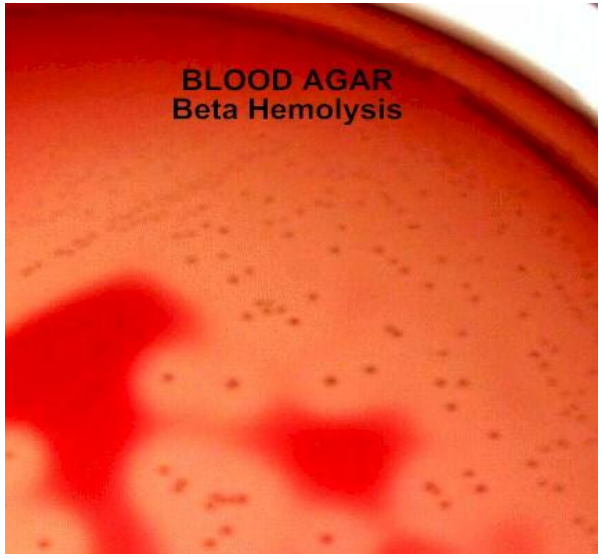
- Environmental requirements

– Oxygen and Carbon dioxide availability
  - aerobic: room air
  - facultative: aerobic or anaerobic
  - microaerophilic: reduced oxygen tension
  - anaerobic
    – strict or aerotolerant
  - capnophilic: increased $C0_2$ (5-10%)

– Temperature
  - 35-37ºC
  - 30ºC
  - cold
  - 42ºC

• Bacterial Cultivation

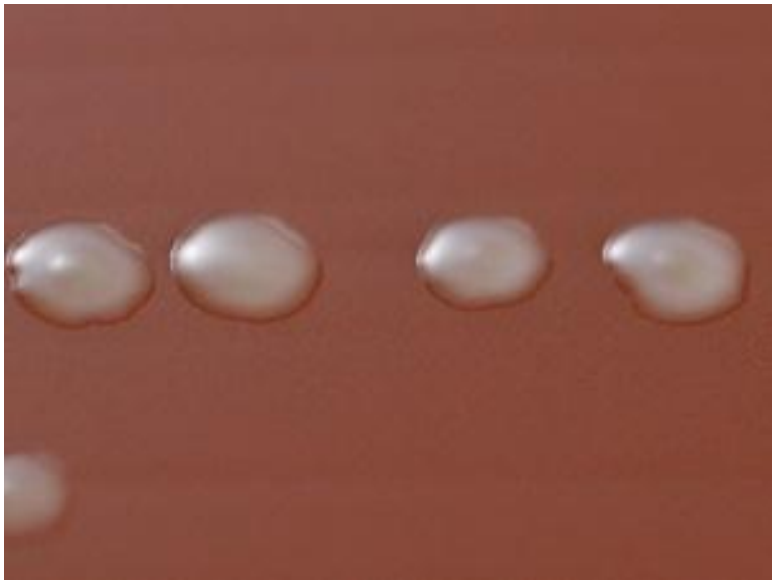
- Isolation of bacteria from specimens
  - streaking for isolation
  - streaking for quantitation
- Evaluation of colony morphologies
  - Type of media supporting growth
  - Relative quantities of each colony type
  - Colony characteristics
    - colony form: pinpoint, circular, filamentous, irregular
    - colony elevation: flat, raised, convex
    - colony margin: smooth, irregular
  - Gram stain and subcultures
    - sterile loop, isolated colonies



http://science.nhmccd.edu/biol/wellmeyer/media/media.htm

Chocolate agar

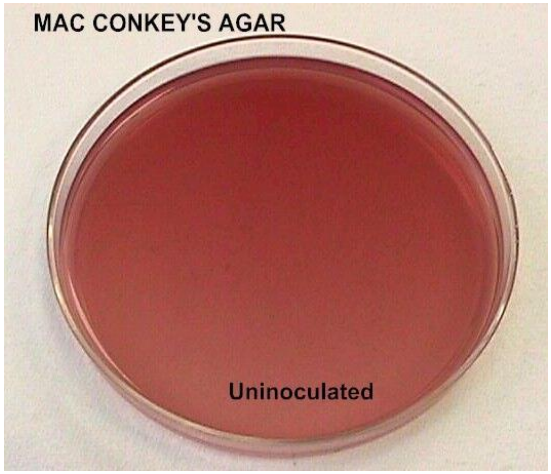
Uninoculated

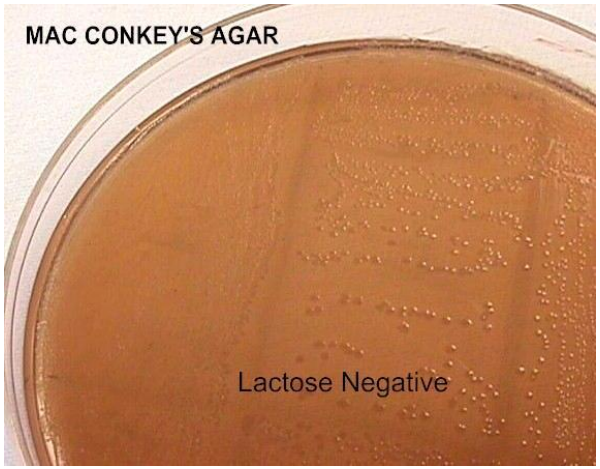


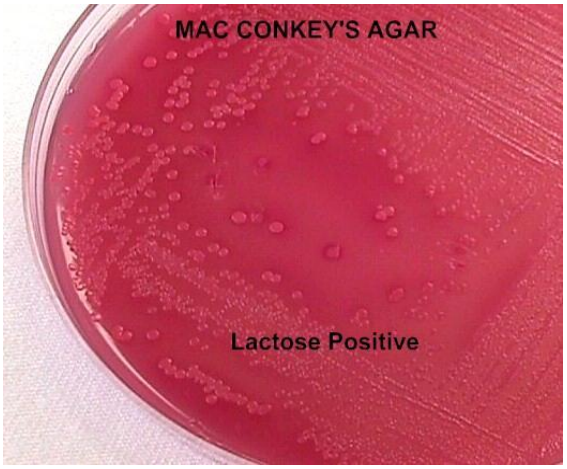
Haemophilus

http://www.hardydiagnostics.com/catalog2/hugo/ChocolateAgar.htm

MacConkey agar







http://science.nhmccd.edu/biol/wellmeyer/media/media.htm

Hektoen agar

Enterobacter produces acid on HE agar and turns the medium orange.

Salmonella growing on HE agar produces colonies with black centers (produces hydrogen sulfide).

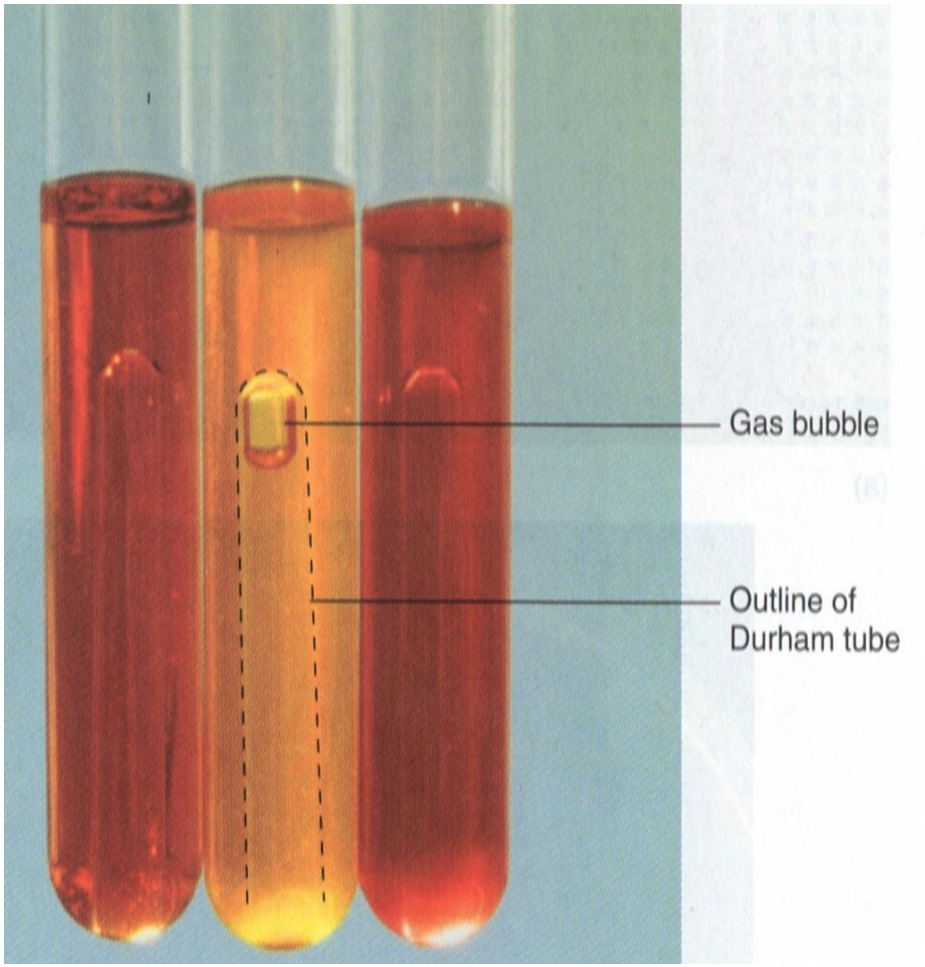
http://medic.med.uth.tmc.edu/path/hekto.htm

- Nutritional requirements and metabolic capabilities
  - Single enzyme tests
    - Catalase: H_2O_2 + catalase = O_2 and H_20; differentiates *Staphylococcus* v. *Streptococcus*, *Listeria* and Corynebacterium v. other non spore forming gram-positive bacilli
    - Oxidase: detection of cytochrome oxidase that participates in nitrate metabolism; *Pseudomonas*, *Aeromonas*, *Neisseria*
    - Indole: tryptophanase degrades tryptophan into pyruvic acid, ammonia, and indole; indole is detected by aldehyde indicator; presumptive id for *E. coli*
    - Urease: hydrolyzes urea into ammonia, water and CO_2; increase pH changes causes bright pink color of indicator
    - PYR: hydrolysis of PYR, indicator turns pink; Group A Strep and enterococci are +

Biochemical Tests

- The microbe is cultured in a media with a **special substrate and tested for an end product**.
- Prominent biochemical tests include **carbohydrate fermentation, acid or gas production and the hydrolysis of gelatin or starch.**
- Many of these test in rapid miniaturized system that can detect for 23 characteristics in small cups called **Rapid test**.
- The info from the rapid test are input into a computer to help in identification of the organisms.

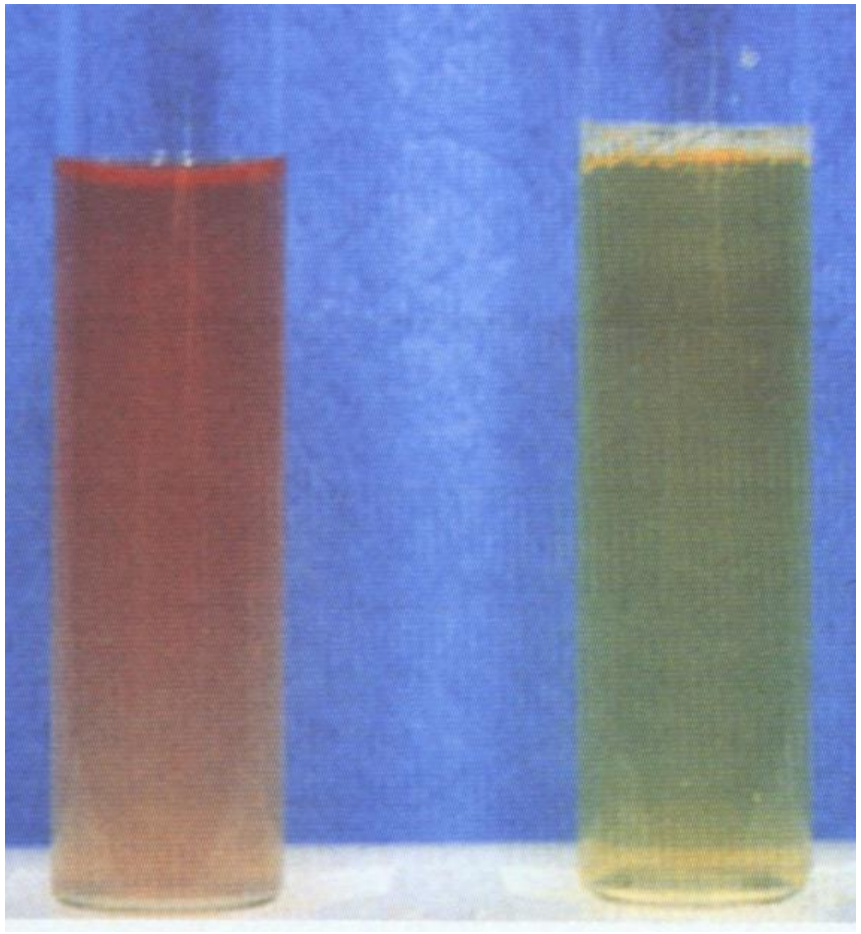
Carbohydrate Fermentation



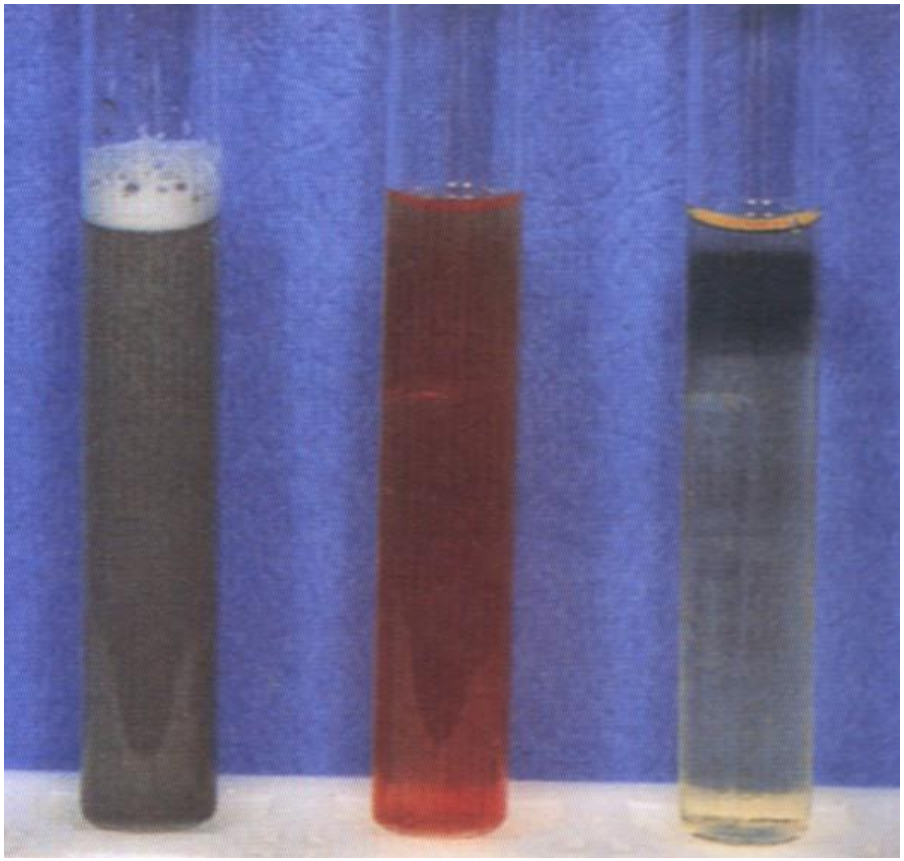
- . This medium show **fermentation** (**acid production**) and **gas formation**.
- The small **Durham tube** for collecting gas bubbles.
- Left- right:
  - Uninoculated negative control
  - Centre, positive for acid (yellow) and gas (open space).
  - Growth but no gas or acid.

Methyl Red Test

- This is a qualitative test for **acid production**.
- The bacteria is grown in MR-VP broth.
- After addition of several drops of **methyl red solution** a bright red colour is positive and yellow-orange negative.



Nitrate Reduction

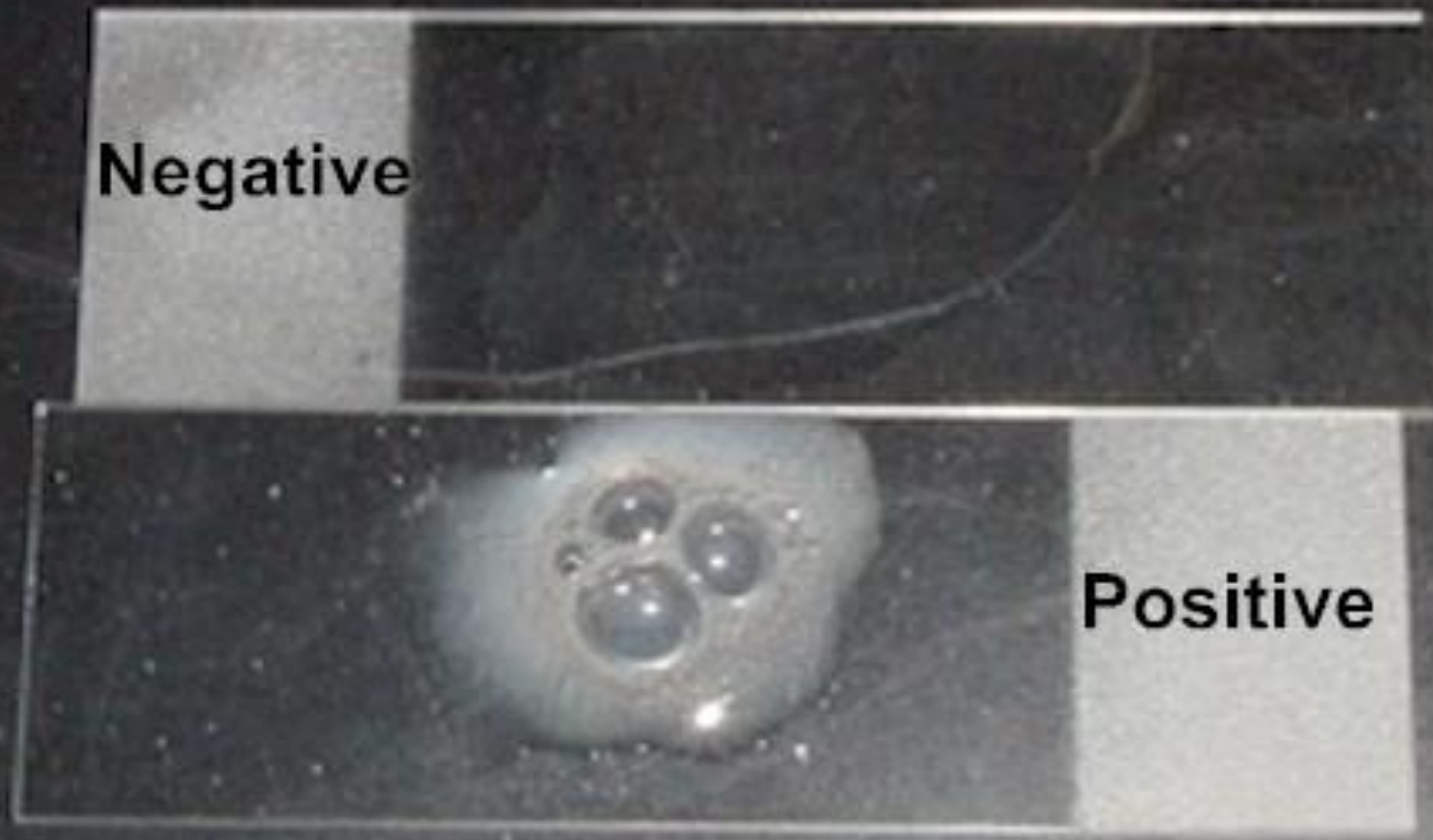


- After 24-48 hrs of incubation, **nitrate reagents** are added.
- Left to right:
  - Gas formation (positive for nitrate reduction).
  - positive for nitrate reduction to nitrite (red colour).
  - Negative control

Biochemical Tests

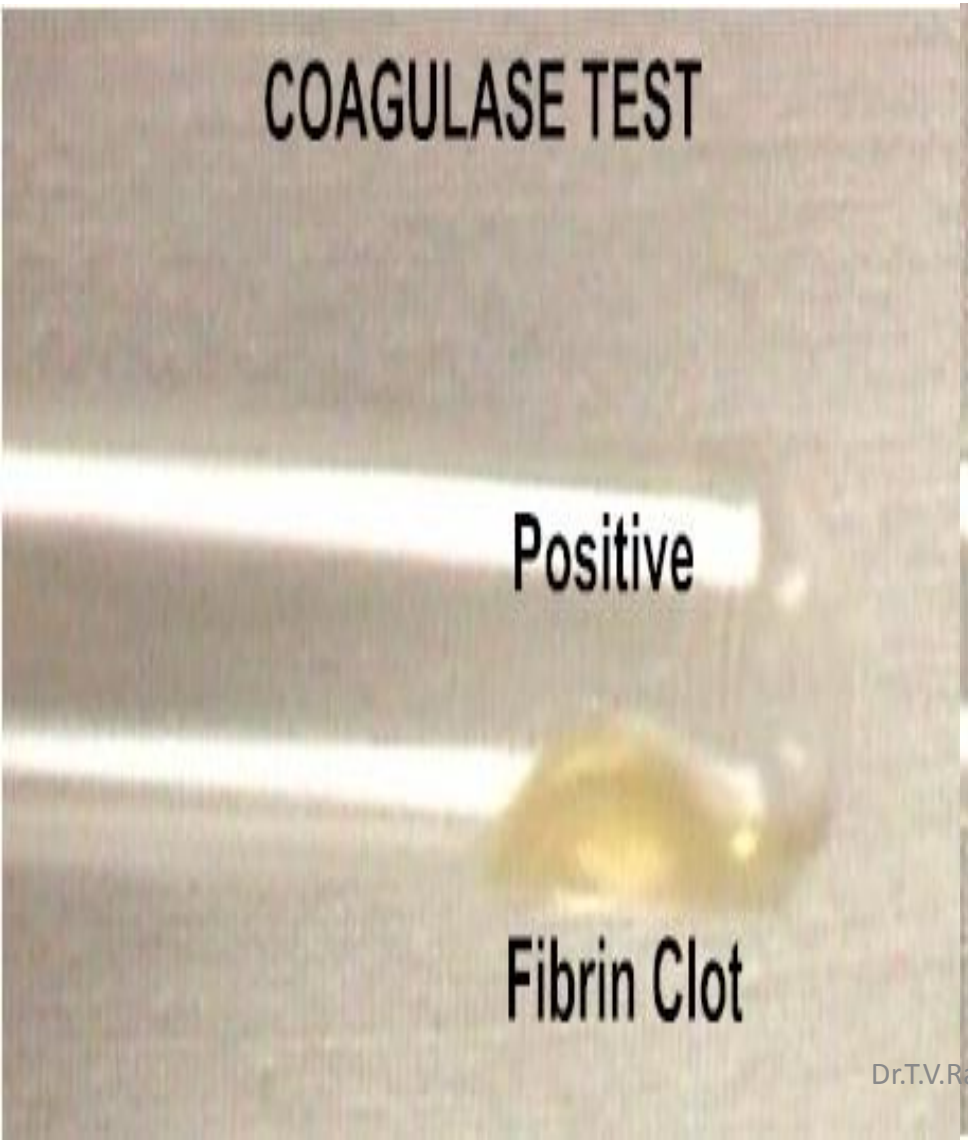
- Other biochemical tests of interest include:
  - H_2S production
  - Indole test
  - Oxidase test
  - Oxidation fermentation
  - Phenylalanine deaminase test
  - Antibiotic susceptibility tests
- Principle, procedure, most common use.

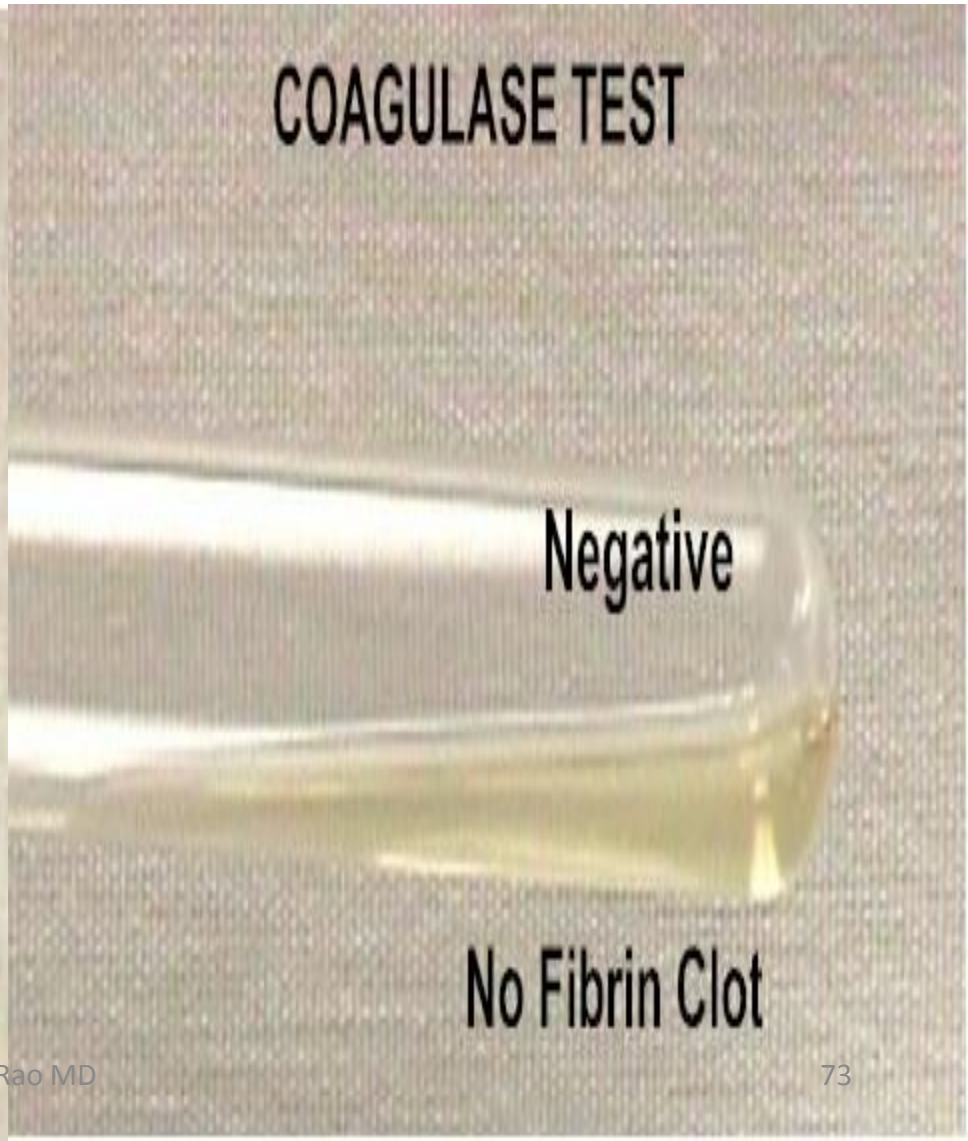
CATALASE TEST




COAGULASE TEST
Rabbit Plasma
BBL®
COAGULASE PLASMA
Nutrient Broth
Pipette

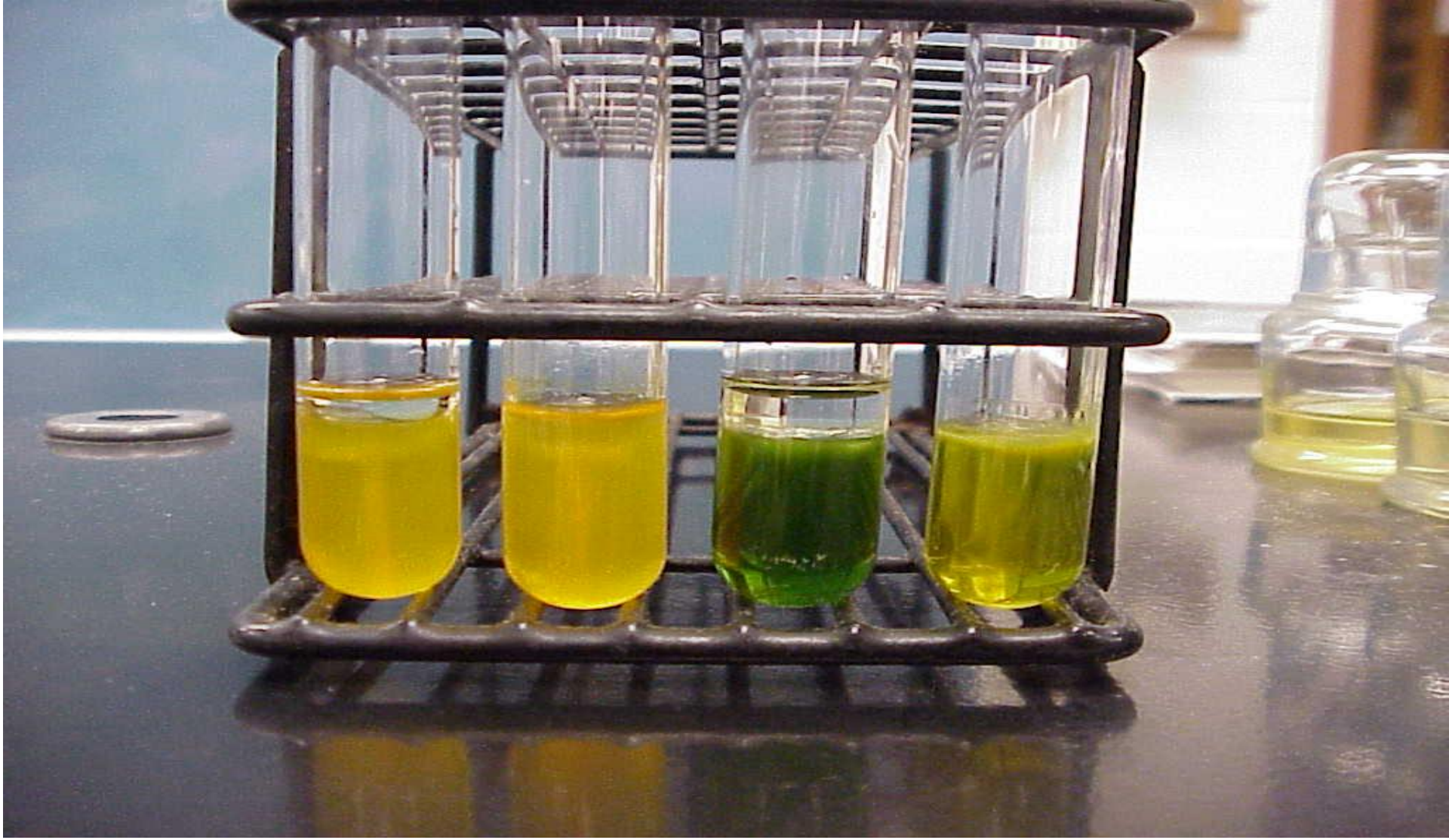
Coagulase Test





–Tests for presence of metabolic pathways

- Oxidation and fermentation: oxidation of glucose requires oxygen, fermentation does not; pH decreases causing yellow color
- Amino acid degradation: detection of amino acid decarboxylase enzymes



O-F glucose media
http://academic.mwsc.edu/jcbaker/bio390sec01/bio390_laboratory_study_images.htm

Differential Media Chromagar

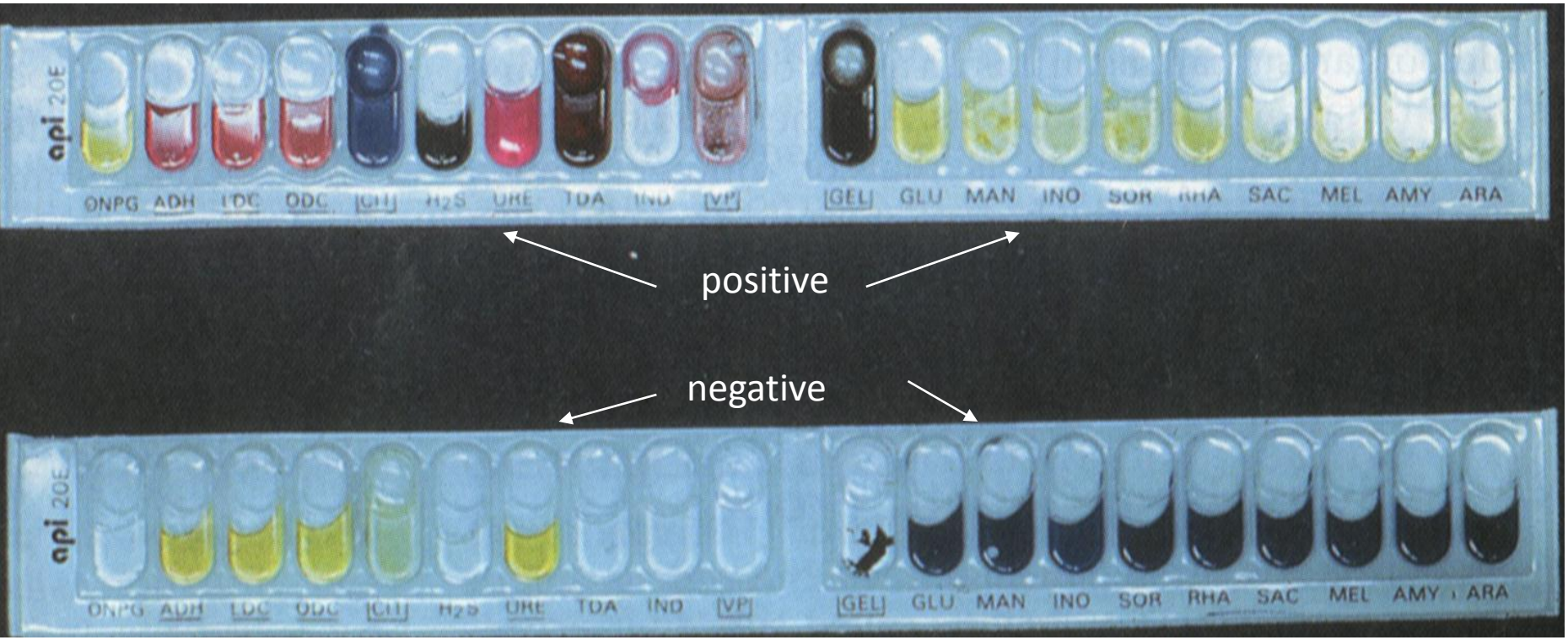
- Chromagar orientation uses colour-formation to distinguish at least 7 common urinary pathogens.
- This allow for rapid identification and treatment.
- The bacteria were streaked as to spell their names.

- Principles of Phenotype-based ID schemes
  - **Selection and inoculation of ID test battery**
    - Type of bacteria to be identified
    - Clinical significance of isolate
    - Availability of reliable testing methods
  - Incubation for substrate utilization
    - Conventional ID
    - Rapid ID
  - Detection of metabolic activity
    - Colorimetry: pH change of indicators
    - Fluorescence: release of fluorophore from substrate or changes in fluorescence due to pH changes
    - Turbiditiy: growth or no-growth
  - Analysis of metabolic profiles
    - ID databases
    - Use of databases to ID unknowns
      - Confidence in ID

Rapid Tests

- **Rapid test**: a biochemical system for the identification of ***Enterobacteriaceae*** and other Gram –ve bacteria.
- It consist of **plastic strips with 20 µl of dehydrated biochemical substrates** used to detect biochemical characteristics.
- The biochemical **substrates are inoculated** with pure cultures and suspended in physiological saline.
- After 5 hrs-overnight the 20 tests are converted to **7-9 digital profile.**

Rapid Tests

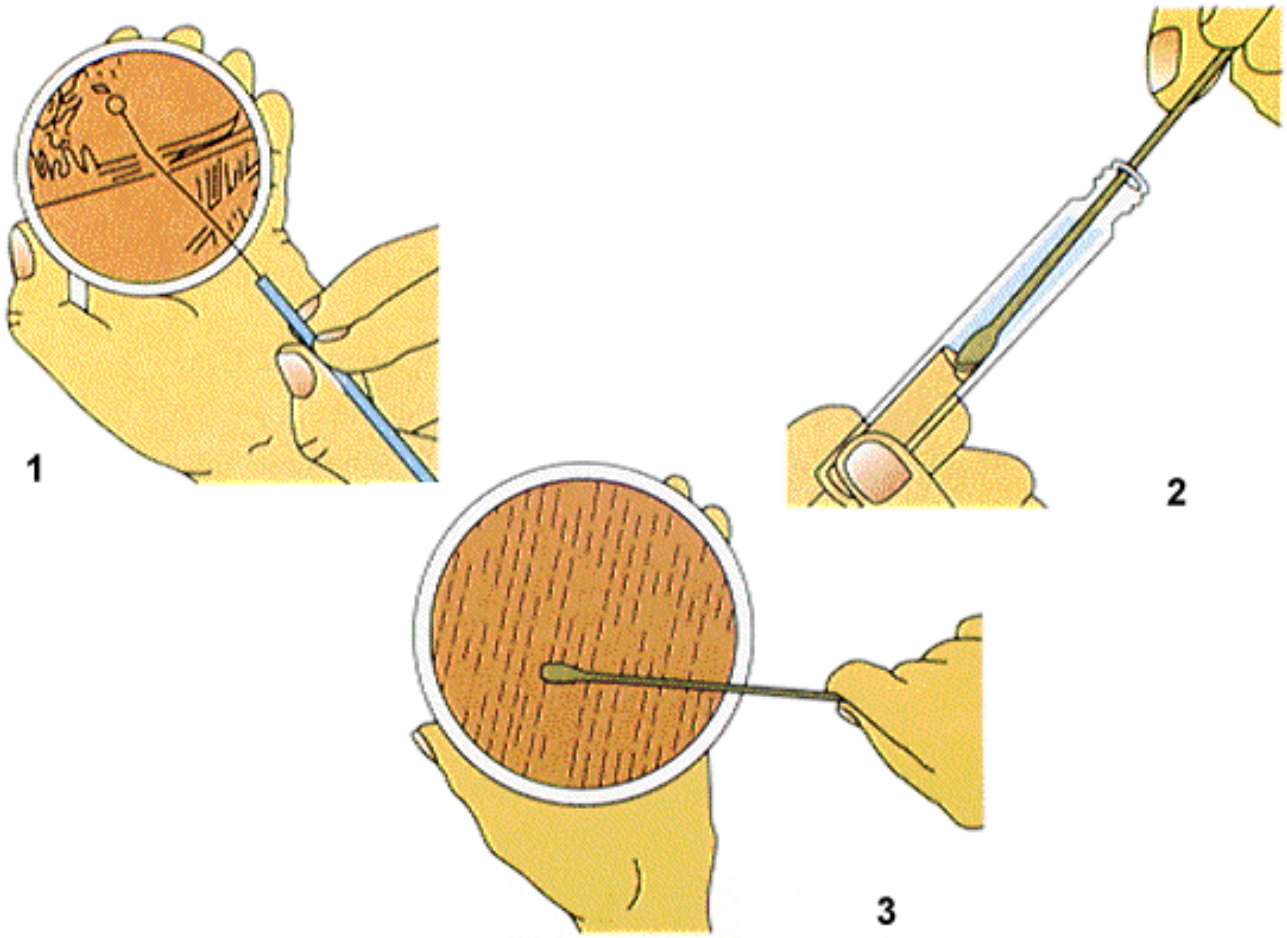


ONPG (β galactosidase); ADH (arginine dihydrolase); LDC (lysine decarboxylase); ODC (ornithine decarboxylase); CIT (citrate utilization); H_2S (hydrogen disulphide production); URE (urease); TDA (tryptophan deaminase); IND (indole production); VP (Voges Proskauer test for acetoin); GEL (gelatin liquefaction); the fermentation of glucose (GLU), mannitol (MAN), inositol (INO), sorbitol (SOR), rhamnose (RHA), sucrose (SAC); Melibiose (MEL), amygdalin (AMY), and arabinose (ARA); and OXI (oxidase).

Bacteriophage Typing

- **What are bacteriophages?**
- Bacteriophage typing is based on the **specificity of phage surface receptor** for the cell surface receptor.
- Only those phages that can **attach to the surface receptors** can cause lysis.
- The procedure involves:
- A plate is **heavily inoculated** so that there is no uninoculated areas.
- The plate is **marked off** in squares (15-20 mm) and each square **inoculated** with a drop of suspension for **different phages**.

Heavily Inoculated Plate

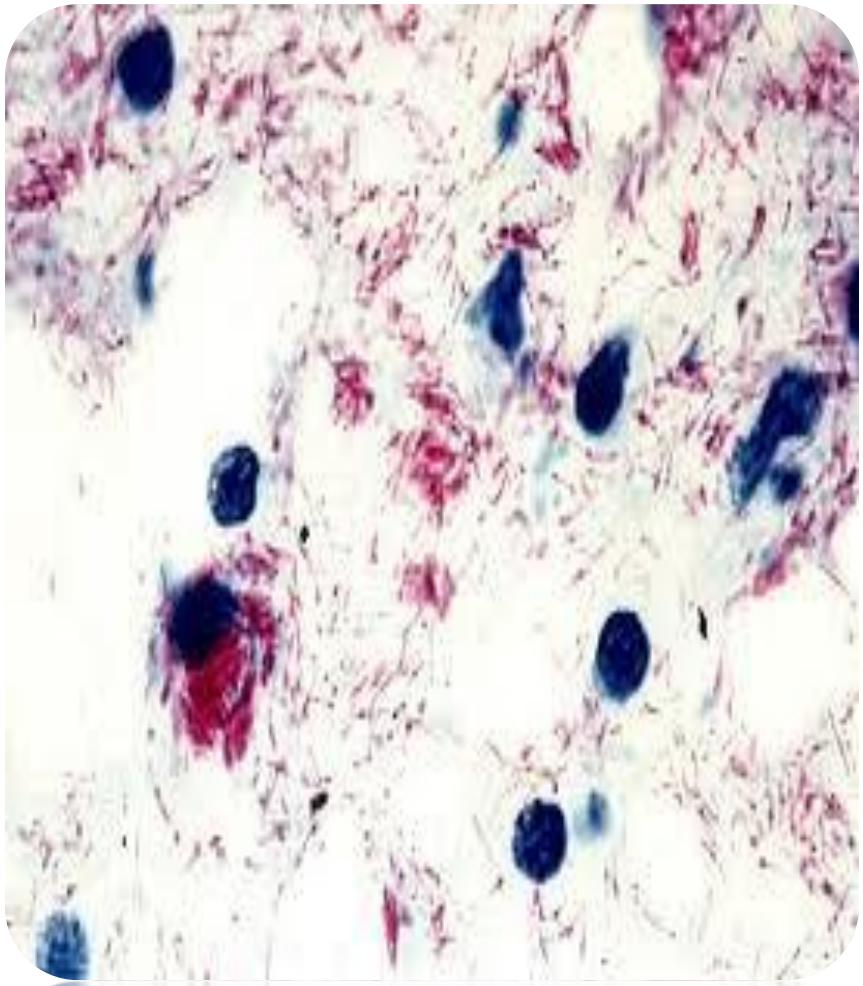


Bacteriophage Typing

- The plate is incubated for 24 hrs then observed for plaques.
- The phage type is reported as a specific **genus and species** followed by **the types** that can infect the bacterium.
- E.g. 10/16/24 means that the bacteria is sensitive to phages 10, 16 and 24.
- Phage tying remain a **tool for research** and **reference labs**.

Uncultivable Organisms

- Environmental researchers estimate that **< 1%** of microorganisms **are culturable** and therefore it is not possible to use phenotypic methods of identification.
- These microorganisms are called viable nonculturable (VNC).



Flow Cytometry

- Classical techniques are not successful in identification of those microorganisms that cannot be cultured.
- What do is the name of microorganisms that cannot be cultured?
- **Flow cytometry** allows single or multiple microorganisms detection an easy, reliable and fast way.
- Inflow cytometry microorganisms are identified on the basis of the **cytometry parameters** or by means of certain dyes called **fluorochromes** that can be used independently or bound to specific antibodies.

Flow Cytometry

- The cytometer forces a suspension of cells through a **laser beam** and measures the light they scatter or the fluorescence the cell emits as they pass through the beam.
- The cytometer also can measure the cell's shape, size and the content of the DNA or RNA.

Recent Advances in Flowcytometry

- Flow cytometry (FCM) allows single- or multiple-microbe detection in clinical samples in an easy, reliable, and fast way. Microbes can be identified on the basis of their peculiar cytometry parameters or by means of certain fluorochromes that can be used either independently or bound to specific antibodies or oligonucleotides. FCM has permitted the development of quantitative procedures to assess antimicrobial susceptibility and drug cytotoxicity in a rapid, accurate, and highly reproducible way

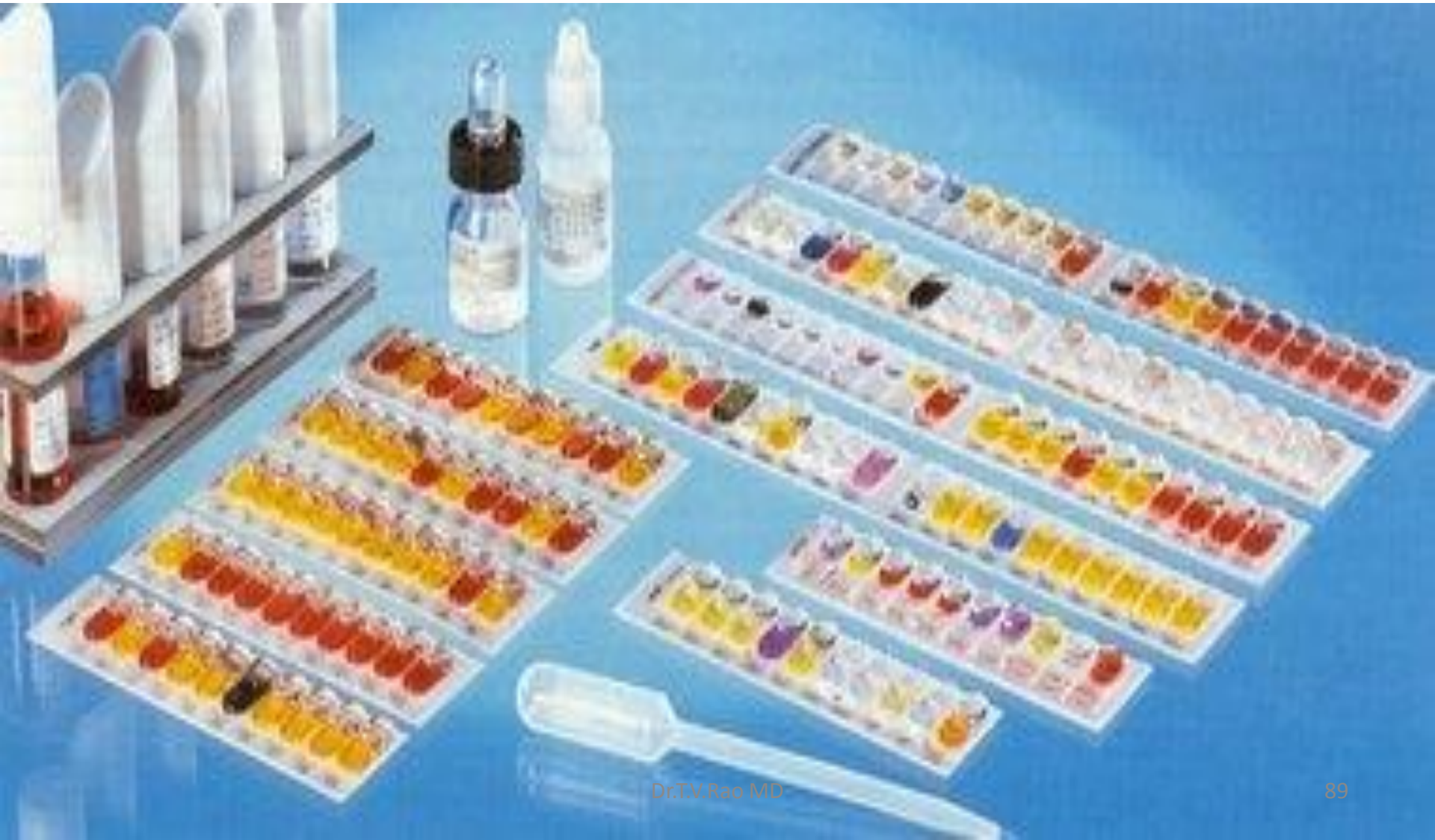
Immunological Methods

- Immunological methods involve the interaction of a microbial **antigen with an antibody** (produced by the host immune system).
- Testing for microbial antigen or the production of antibodies is **often easier** than test for the microbe itself.
- **Lab kits** based on this technique is available for the identification of many microorganisms.

Principles of Serologic Test Methods

- Methods for antibody detection
  - Direct whole pathogen agglutination assays
    - pos patient sera causes organism to clump
  - Particle agglutination tests
    - latex beads or RBCs coated with Ag
  - Flocculation tests
    - RPR – precipitation of soluble Ag with Ab
      - » charcoal particles coated with cardiolipin-lecithin binds reagin
  - ELISAs
  - IFAs
    - organism/antigen on slides; patient Ab detected with fluorescent secondary Ab
  - Western blots

API strips – bioMerieux

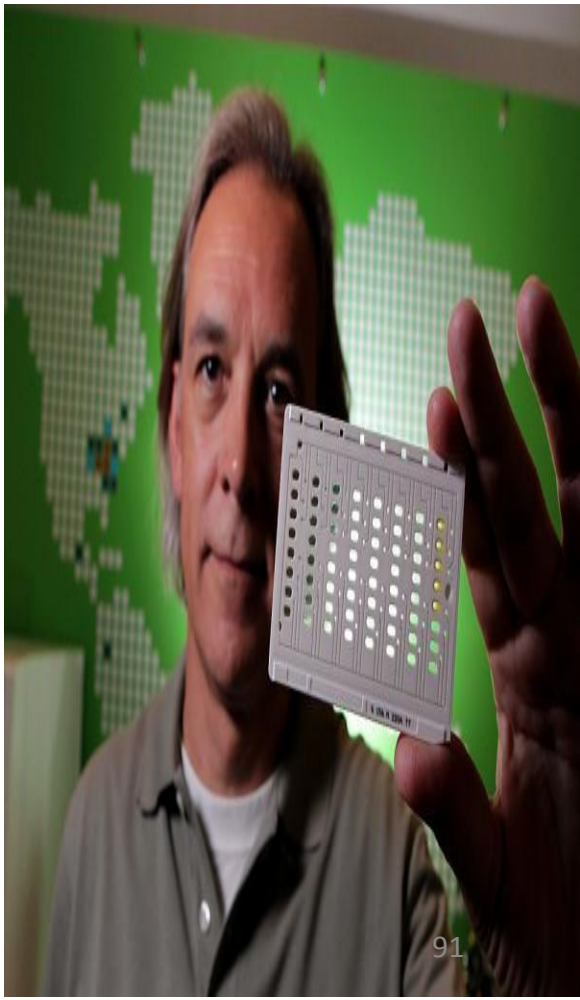


Commercial ID systems

- Advantages and examples of commercial systems
  - ARIS (Trek)
  - MicroScan (Dade-Behring/Seimens)
  - Phoenix (Becton-Dickinson)
  - **Vitek (bioMérieux)**

Immunochemical methods for ID

- Principles of immunochemical methods
  - Particle agglutination
    - Latex agglutination
  - Immunofloresecent assays
    - Direct immunofluorescence assay (DFA)
    - Indirect immunofluorescence assay (IFA)
    - Enzyme immunoassays
  - Solid-phase immunoassays
    - Membrane bound immunoassays
    - Immunochromatographic assays
  - Optical immunoassays



Serologic methods for diagnosis

- Features of the Immune Response
  - Characteristics of antibodies
    - Features of immune response useful in diagnostic testing
      - Acute v. anamnestic response
      - IgM v. IgG
      - IgM can’t cross placenta
      - immunocompetent v. immunocompromised
    - Interpretation of serologic tests
      - single v. paired sera; rare pathogen
      - 4-fold rise in titer v. qualitative testing
      - cross reactivity (herpes viruses, heterophile Abs, pregnancy)

Principles of Serologic Test Methods

- Methods for antibody detection
  - Direct whole pathogen agglutination assays
    - pos patient sera causes organism to clump
  - Particle agglutination tests
    - latex beads or RBCs coated with Ag
  - Flocculation tests
    - RPR – precipitation of soluble Ag with Ab
      - » charcoal particles coated with cardiolipin-lecithin binds reagin
  - ELISAs
  - IFAs
    - organism/antigen on slides; patient Ab detected with fluorescent secondary Ab
  - Western blots

Genotypic methods

- Genotypic methods of microbe identification include the use of :
  - ✓Nucleic acid probes
  - ✓PCR (RT-PCR, RAPD-PCR)
  - ✓Nucleic acid sequence analysis
  - ✓rRNA analysis
  - ✓RFLP
  - ✓Plasmid fingerprinting.

Genotypic Methods

- Genotypic methods involve examining the genetic material of the organisms and has **revolutionized bacterial identification** and classification.
- Genotypic methods include PCR (RT-PCR, RAPD-PCR),use of nucleic acid probes, RFLP and plasmid fingerprinting.
- Increasingly genotypic techniques are becoming the **sole means of identifying** many microorganisms because of its **speed and accuracy**.

Real Time PCR and RT-PCR

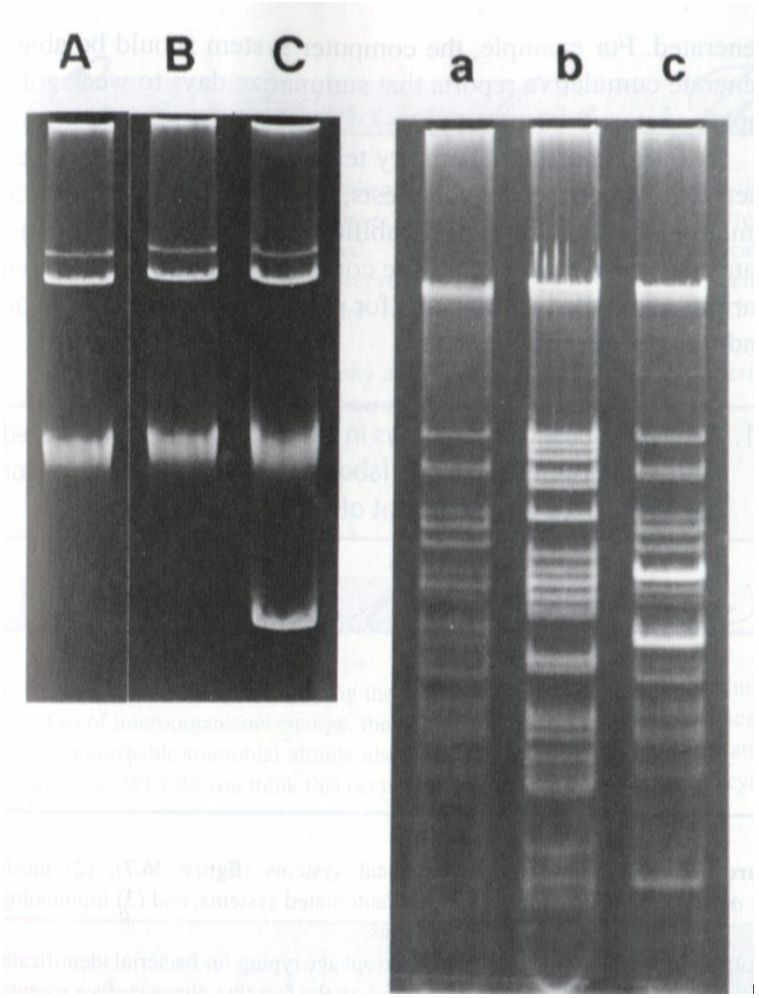
- Currently many PCR tests employ **real time PCR**.
- This involves the use of **fluorescent primers**.
- The PCR machine **monitors the incorporation of the primers** and display an **amplification plot** which can be viewed continuously thru the PCR cycle.
- Real time PCR **yields immediate results**.
- Another application of PCR is **RT-PCR** (reverse trancriptase PCR).
- During RT-PCR an RNA template is used to **generate cDNA and from this dsDNA** is generated.
- The enzyme used is **reverse transciptase**.
- RT-PCR is used to detect for HIV and to monitor the progress of the disease.

RAPD-PCR

- Random amplified polymorphic DNA PCR uses **a random primer** (10-mer) to generate a DNA profile.
- What are random primers?
- **The primer** anneals to several places on the DNA template and generate a DNA profile which is used for microbe identification.
- RAPD has many advantages:
  - Pure DNA is not needed
  - Less labour intensive than RFLP.
  - There is not need for prior DNA sequence data.
- RAPD has been used to fingerprint the outbreak of *Listeria monocytogenes* from milk.

Plasmid fingerprinting

- The procedure involves:
- The bacterial strains are **grown**, the cells lysed and harvested.
- The plasmids are **separated** by agarose gel electrophoresis
- The gels are **stained with EtBr** and the plasmids located and compared.



Computer and Bacteria Identification

- Computers improve the efficiency of the lab operations and increase the speed and clarity with which results can be reported.
- Computers are also important for the result entry, analysis and preparation.

- **Programme created by Dr.T.V.Rao MD for Medical Microbiologists in Developing world**
- **Email**
- doctortvrao@gmail.com