1. Neisseria

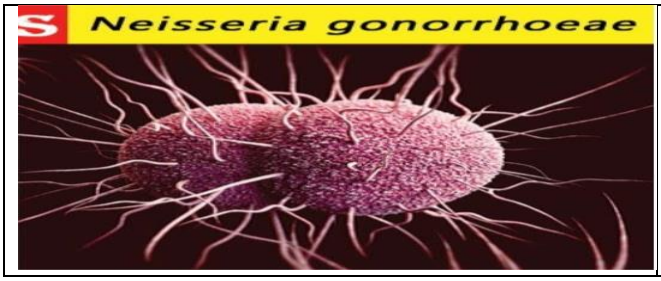
1 .1 Neisseria gonorrhea

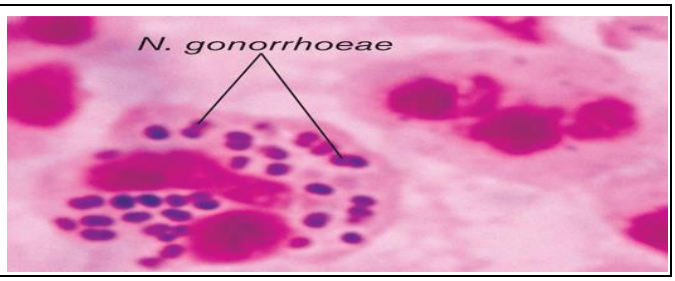
A. Specimens:

Pus and secretions are taken from the urethra, cervix, rectum, conjunctiva, throat, or synovial fluid for culture and smear

B. Microscopy (Gram stain):

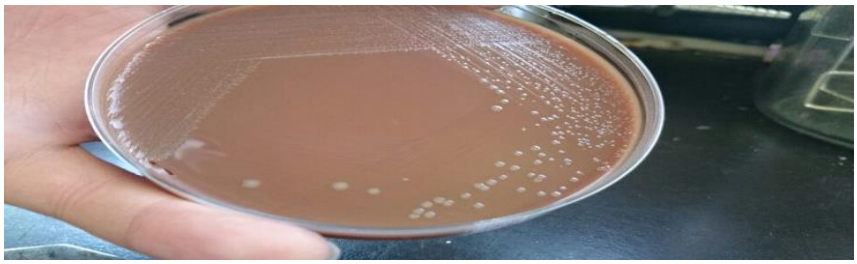
- Gram-stained smears of urethral or endocervical exudates typically reveal many diplococci within PMNs, therefore providing a presumptive diagnosis.





C. Culture/ Colony appearance:

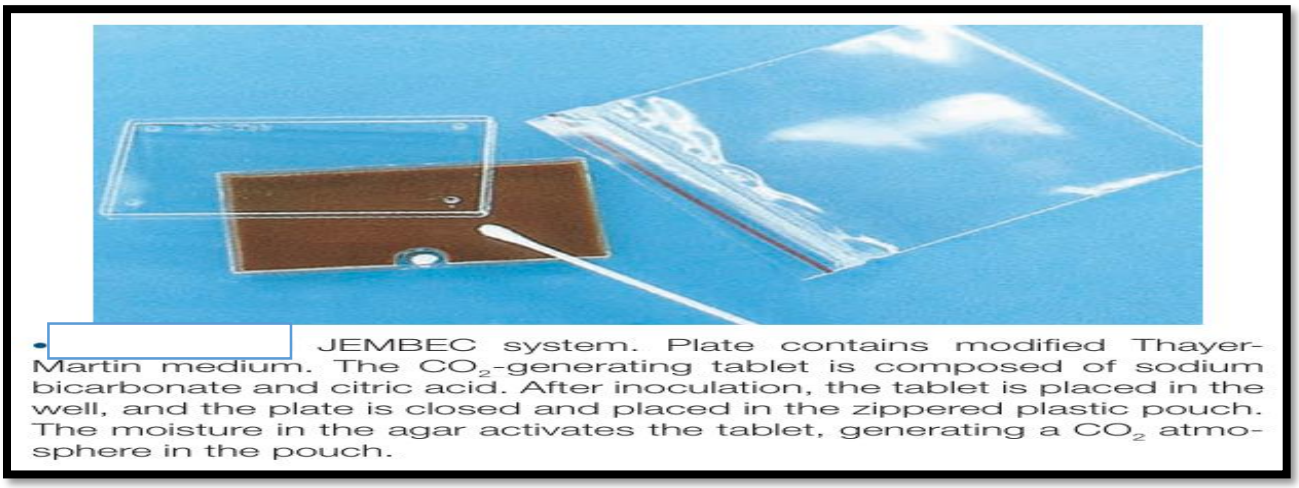
Culture	Colony characters
Chocolate agar	Small, grayish white, convex, trans lucent, shiny colonies with either smooth or irregular margins; may be up to five different colony types on primary plates.



D. Incubation conditions

- Incubated in an atmosphere containing 5% CO2 at 37°C.

- If immediate incubation is not possible, the specimen should be placed in a CO2 - containing transport-culture system e.g JEMBEC system



JEMBEC system. Plate contains modified Thayer-Martin medium. The CO_2-generating tablet is composed of sodium bicarbonate and citric acid. After inoculation, the tablet is placed in the well, and the plate is closed and placed in the zippered plastic pouch. The moisture in the agar activates the tablet, generating a CO_2 atmosphere in the pouch.

E. Biochemical test :

- Catalase Positive
- Oxidase positive.
- Oxidation for glucose only

1.2 *Neisseria meningitidis*

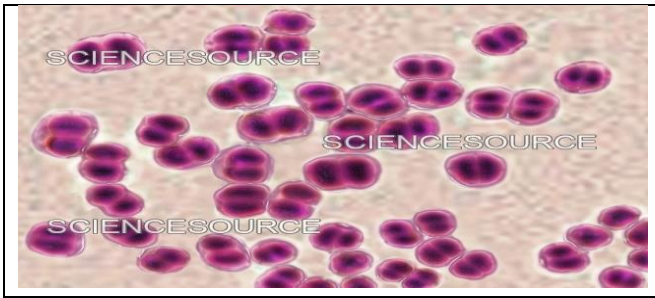
Diagnostic Laboratory Tests:

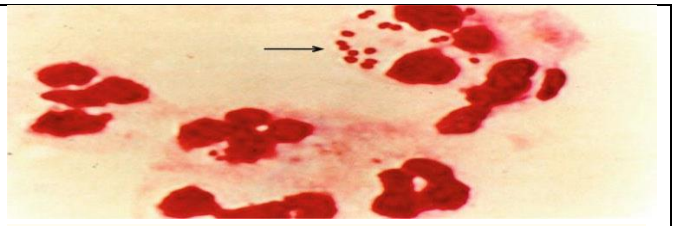
A. Specimens:

Blood, Cerebrospinal fluid (CSF)**,** Puncture material or biopsies from petechial lesions **,** Nasopharyngeal swabs

B. Microscopy (Gram stain):

Gram-stained smears of the sediment of centrifuged spinal fluid or of petechial aspirate often show typical neisseriae within polymorphonuclear leukocytes or extracellularly.





Intracellular gram-negative diplococci and leukocytes in the CSF from a patient with meningococcal meningitis.

C. Culture/ Colony appearance:

Culture	Colony characters
Blood agar	grayish, nonhemolytic, round, convex, smooth, moist, and glistening with a clearly defined edge.
Chocolate agar	Medium, smooth, round, moist, gray to white; encapsulated strains are mucoid; may be greenish cast in agar underneath colonies.
Modified Thayer-Martin medium (MTM)	Gray, convex, and glistening, with entire edges

Blood agar

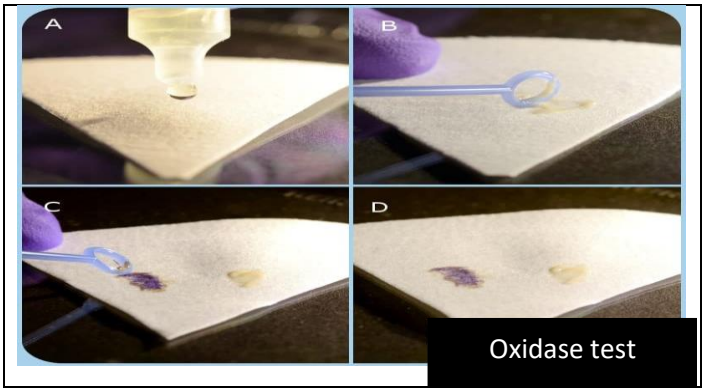
Chocolate agar

D. Incubation Conditions

- **Temperature:** 35–37 °C
- **Atmosphere:** CO_2-enriched environment (3–7%)
- **Duration:** Incubate for **24-48 hours**

E. Biochemical test :

- Catalase Positive
- Oxidase positive.
- Oxidation of glucose and maltose

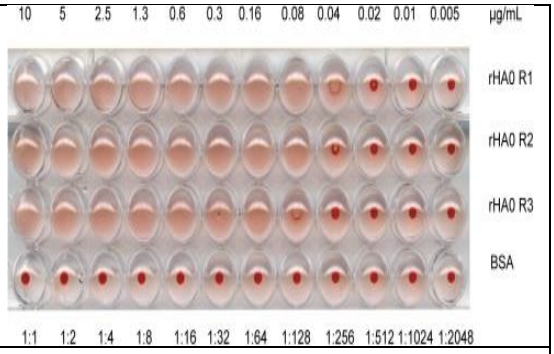


Oxidase test

F. Serologic test

Antibodies to meningococcal polysaccharides can be measured by latex agglutination or hemagglutination tests or by their bactericidal activity.

Latex test



Hemagglutination tests

2. Enteric Gram-negative rods: *Escherichia coli*

Escherichia coli

Diagnostic Laboratory Tests:

A-Specimens: Urine (UTI), **Stool** (diarrhea), **Blood** (septicemia)

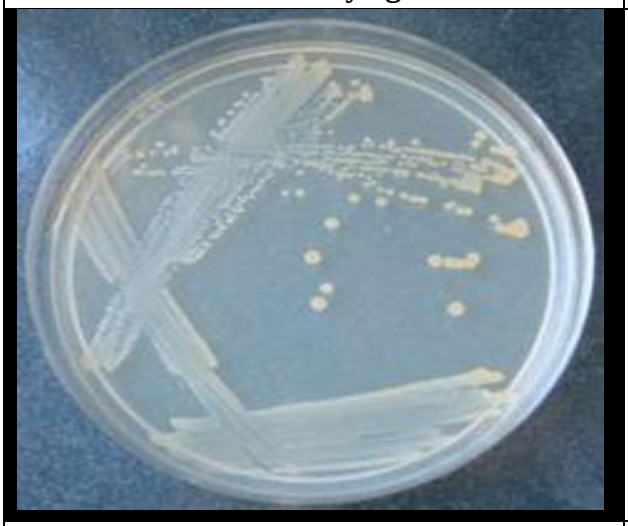
B- Microscopy (Gram stain): Gram-negative bacilli (Short rods)

C. Culture/ Colony appearance:

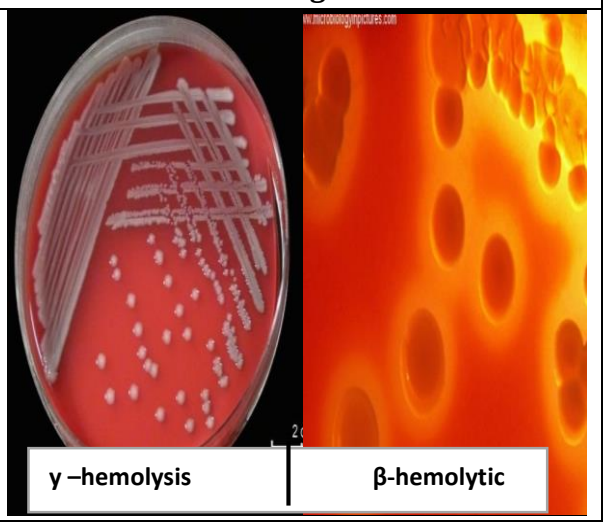
Culture	Colony characters
Blood agar	Colonies are big, circular, gray, and moist. Non-hemolytic colonies **(gamma-hemolysis)** OR **Beta (β)hemolytic** colonies are formed. Many pathogenic strains are haemolytic on blood agar.
MacConkey agar	Colonies are circular, moist, smooth with entire margins; they appear flat and pink, and are **lactose-fermenting**
Nutrient agar	form large, thick, greyish white, moist, smooth, opaque, or translucent discs like colonies
EMB agar	Green Metallic sheen colonies are formed

MacConkey agar

EMB agar



Blood agar- Beta (β)hemolytic



Blood agar

D. Incubation :

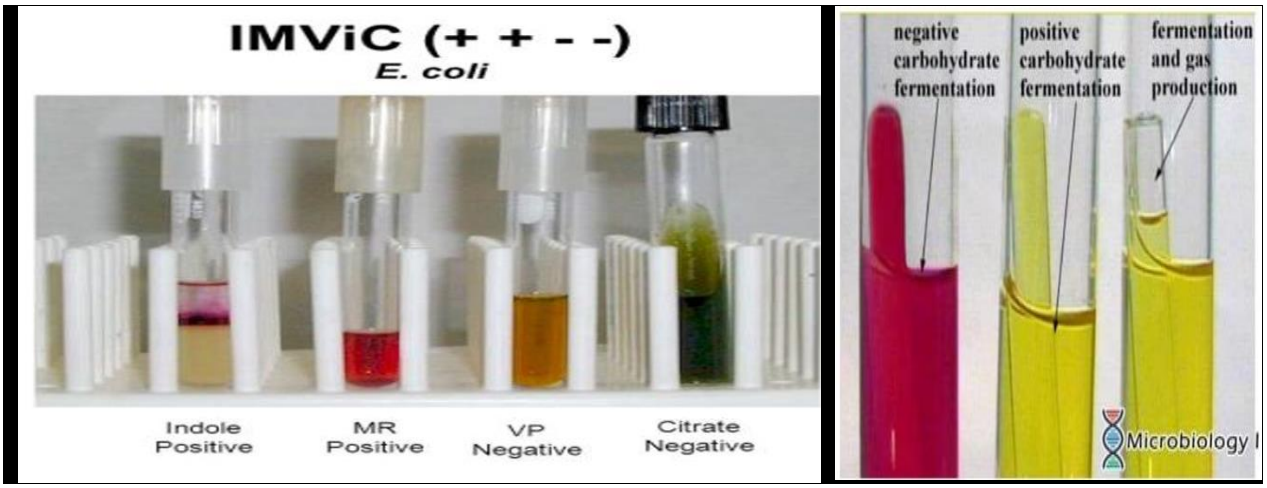
- 35–37 °C
- Aerobic
- 18–24 hours

E.1. Biochemical identification

Test	Result
Indole	+
Methyl red	+
Voges-Proskauer	–
Citrate	–
Urease	–
TSI	**A/A, gas +, H_2S –**
Motility test	+
Oxidase	–
Catalase	+

E.2. Sugar Fermentation

Glucose, lactose, mannitol, and maltose are fermented with the production of acid and gas.



F- Serologic & Molecular tests

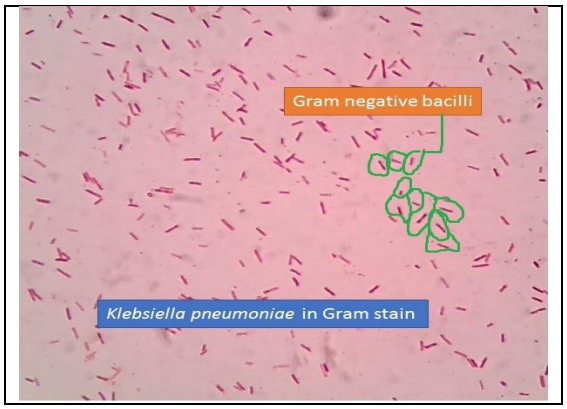
- **Serotyping** (O, H antigens)
- Important strain ⟹ ***E. coli* O157:H7**

3-Klebsiella

Diagnostic Laboratory Tests:

A- Specimens:

- **Respiratory secretions** (pneumonia)
- **Urine** (UTIs)
- **Blood** (septicemia)
- **Wound swab** (abscesses)



B- Microscopy (Gram stain):

- **Gram-negative bacilli**
- Often **encapsulated**, appear as **large, mucoid rods**
- Non-motile

C-Culture/ Colonial appearance

Table 3.1 Cultural Characteristics of *Klebsiella pneumoniae* on some laboratory media

Cultural Characteristics	Nutrient Agar Medium (NAM)	MacConkey Agar medium	Blood Agar Medium	EMB Agar medium
Shape	Circular	Circular	Circular	Circular
Size	2-3 mm	2-3 mm	2-3 mm	2-3 mm
Elevation	Dome-shaped	Convex	Dome-shaped	Convex
Surface	Mucoid	Mucoid	Mucoid	Mucoid
Color	Greyish white	Pink – Red	Greyish white	Pink – Purple
Structure	Translucent–Opaque	Opaque	Translucent–Opaque	Translucent–Opaque
Hemolysis	-----	-----	γ-Hemolysis (Non-hemolytic)	-----

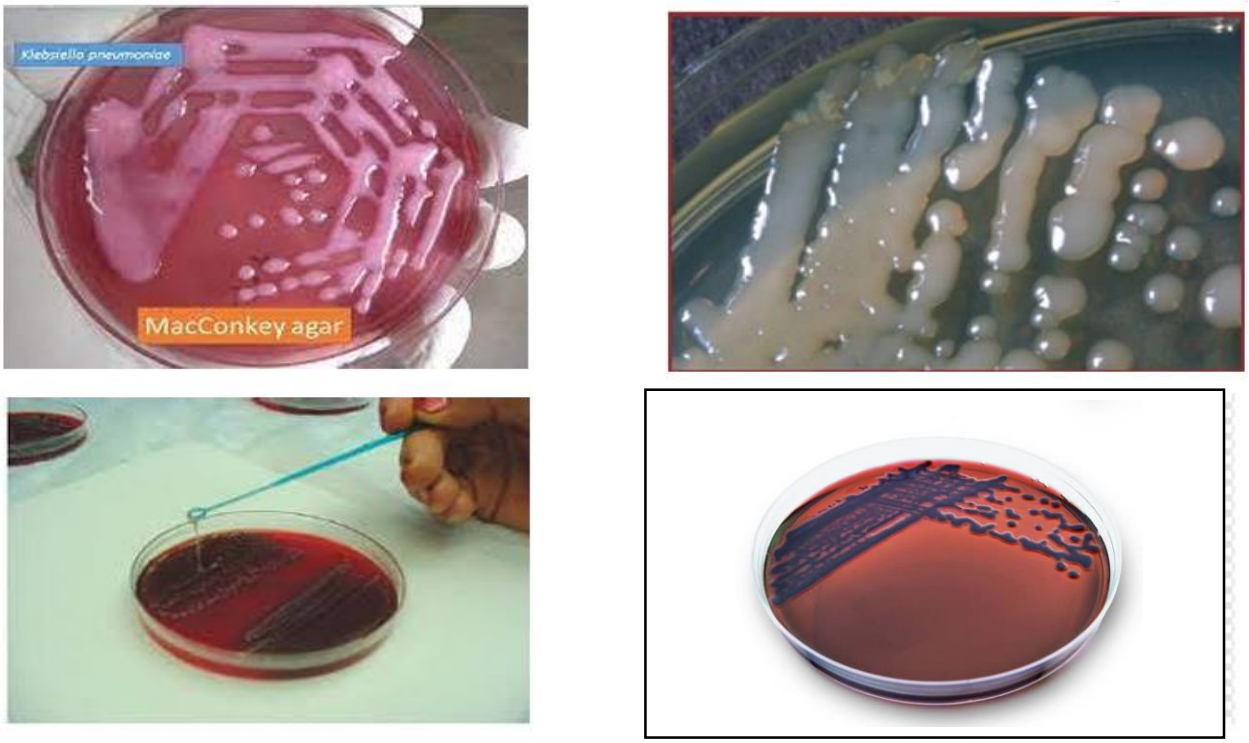


Figure 3.1 *Klebsiella pneumoniae* Growing on MacConkey, Nutrient, Blood and Eiosin Methylene Blue agar plates respectively.

D- Incubation conditions

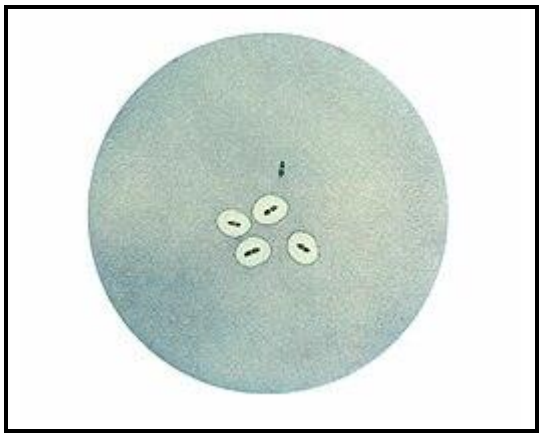
- **35–37 °C**
- **Aerobic**, **18–24 hours**

E-Biochemical identification

Table 3.2 Biochemical tests of *Klebsiella pneumoniae*

Characteristics	*Klebsiella pneumoniae*
Capsule	+ve
Catalase	+ve
Citrate	+ve
Gelatin Hydrolysis	-ve
Gram Staining	-ve
H_2S	-ve
Indole	-ve
Motility	-ve
MR (Methyl Red)	-ve
Nitrate Reduction	+ve
Oxidase	-ve
Pigment	-ve
Shape	Rod
Spore	-ve
TSIA (Triple Sugar Iron Agar)	A/A
Urease	+ve
VP (Voges Proskauer)	+ve

7-Serologic tests



- Uses **specific antisera** against known K antigens.
- **Capsular swelling (Quellung) test:**
  - A drop of **specific anti-K serum** is mixed with a bacterial suspension on a slide.
  - If the bacteria have the corresponding capsule type, the **capsule swells**, becoming visible under a microscope.

Proteus

Identification of *Proteus* spp.

A-Specimen collection

- **Urine** (most common – UTIs)
- **Wound swabs** (ulcers, surgical wounds)
- **Blood** (septicemia in severe cases)
- **Stool** (gastrointestinal infections)

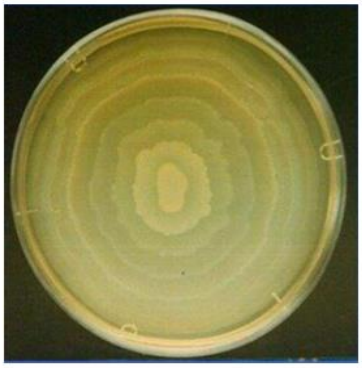
B- Microscop (Gram stain)

- **Gram-negative bacilli**
- Motile: may show **swarming on smear**

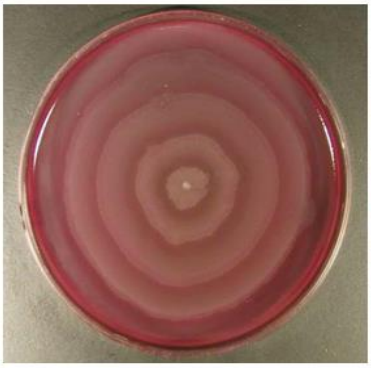
C- Culture / Colony appearance

Table 3.3: Culture Characteristics of *Proteus vulgaris*

Cultural Characteristics	Nutrient Agar Medium (NAM)	MacConkey Agar medium	Blood Agar Medium	EMB Agar medium
Shape	Irregular (due to swarming)	Circular	Irregular (due to swarming)	Circular
Size	1-2 mm	2-3 mm	1-2 mm	2-3 mm
Elevation	Effuse	Low Convex	Effuse	Effuse
Surface	Glistening	Smooth	Glistening	Glistening
Color	Greyish white	Colorless or Pale colored	Greyish white	Colorless
Structure	Translucent	Transparent	Translucent – Opaque	Transparent
Hemolysis	-----	-----	γ-Hemolysis (Non-hemolytic)	-----

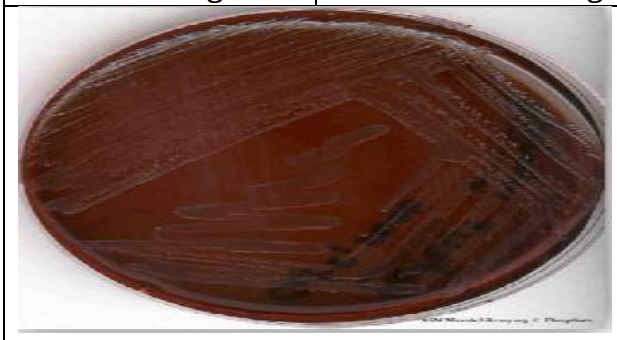


Nutrient agar



Blood agar

MacConkey agar



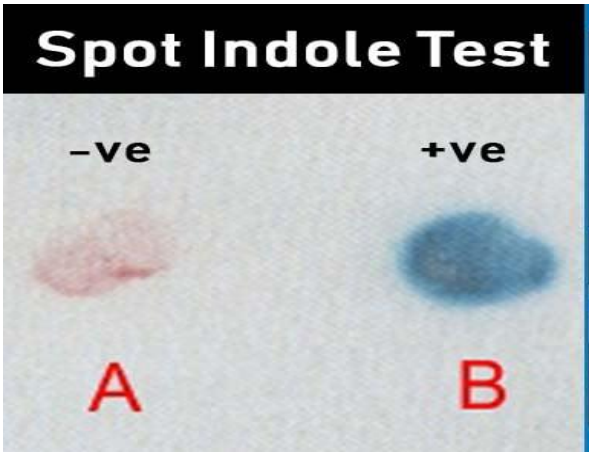
EMB

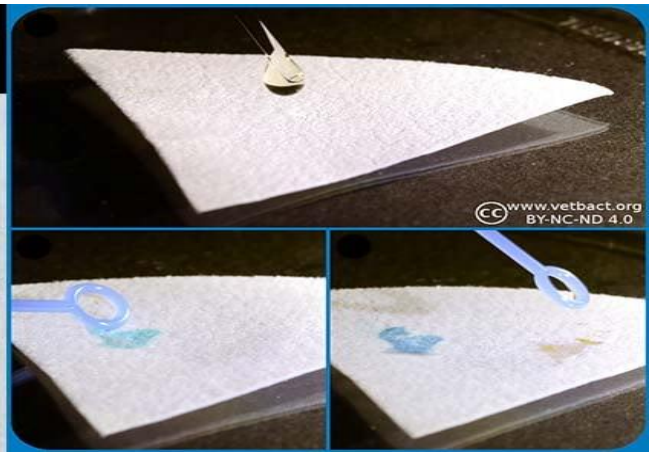
D-Incubation conditions

- **35–37 °C**
- **Aerobic**,
- **18–24 hours**

E. Biochemical identification

The spot-indole test is useful for differentiation between the two most common Proteus species: is *P. vulgaris* indole positive, whereas *P. mirabilis is negative*.





Basic Characteristics	Properties (Proteus mirabilis)
Flagella	Positive (+ve)
Gas from Glucose	Positive (+ve)
Gelatin Hydrolysis	Positive (+ve)
Gram Staining	Negative (-ve)
H_2S	Positive (+ve)
Indole	Negative (-ve)
Motility	Positive (+ve)
MR (Methyl Red)	Positive (+ve)
Nitrate Reduction	Positive (+ve)
Oxidase	Negative (-ve)
Pigment	Negative (-ve)
Shape	Rods
Spore	Negative (-ve)
Urease	Positive (+ve)
VP (Voges Proskauer)	Negative (-ve)
Fermentation of	
Glucose	Positive (+ve)
Lactose	Negative (-ve)
Enzymatic Reactions	
Acetate Utilization	Negative (-ve)
Esculin Hydrolysis	Negative (-ve)
Lipase	Positive (+ve)
Lysine decarboxylases	Negative (-ve)
Phenylalanine Deaminase	Positive (+ve)
Tryptophan Deaminase	Negative (-ve)

Biochemical tests and identification

Basic Characteristics	Properties (Proteus mirabilis)
Capsule	Negative (-ve)
Catalase	Positive (+ve)
Citrate	Positive (+ve)

4- *Pseudomonas and Acinetobacter*

Identification of *Pseudomonas*

A. Specimens

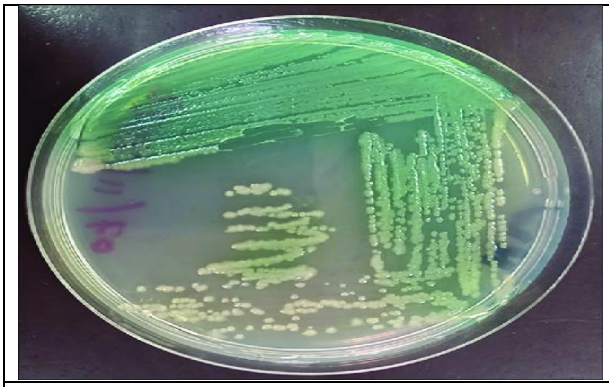
- ***Pseudomonas* species may be isolated from**: Pus, Urine, Sputum, Blood, Eye swabs, Surface swabs
- ***Pseudomonas pseudomallei*** is usually isolated from: Sputum, Blood, Pus from abscesses

B. Microscopic Examination (Gram stain)

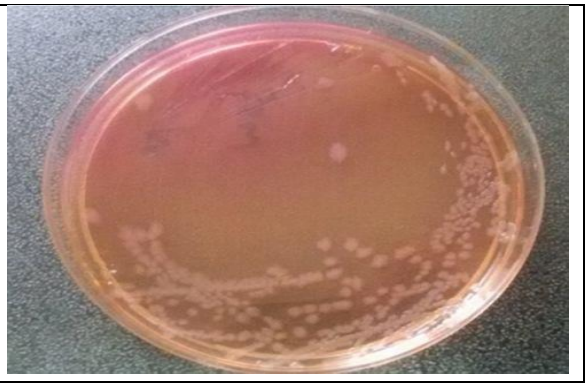
- **Gram stain:** Gram-negative rods

C. Culture Characteristics

Culture Medium	Colony Characteristics
Nutrient Agar	Large colonies, smooth or rough; often bluish-green pigmented
Blood Agar	Large colonies surrounded by a wide zone of **β-hemolysis**
MacConkey Agar	Good growth; **non-lactose fermenting** (pale colonies)
Cetrimide Agar	Medium-sized, greenish-blue colonies with irregular growth



Nutrient agar



MacConkey Agar

Blood agar

Cetrimide agar

- **Selective Media**

Media can be made selective for *Pseudomonas* by adding agents to which it is naturally resistant, such as:

- Cetrimide
- Irgasan
- Nalidixic acid

D-Incubation conditions

- Wide range temperature (5–42 °C)
- Aerobic
- 24-48 hr

E. Biochemical identification

Biochemical Test	Result
Oxidase test	Positive
Catalase test	Positive
Citrate utilization	Positive
Indole test	Negative
Carbohydrate metabolism	Acid production by **oxidation only** (no fermentation)

Examples of biochemical tests used in the identification of Pseudomonas spp.

Species	*Oxidase*	*Lactose*	*ADH*	*ODC*	*Gelatin*
P. aeruginosa	+	–	+	–	+
P. pseudomallei	+	+	+	–	+

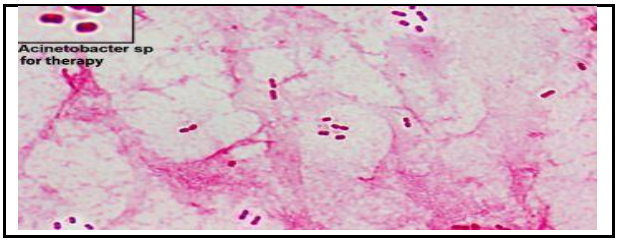
ADH: Arginine dehydrolase, ODC: Ornithine decarboxylase

Identification of *Acinetobacter* spp.

A. Specimens:

***Acinetobacter* species may be isolated from:** Sputum, Blood, Urine, Wound swabs, Pus, CSF

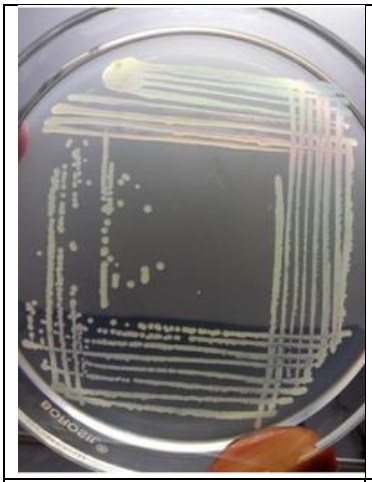
B. Microscopic Examination (Gram stain)



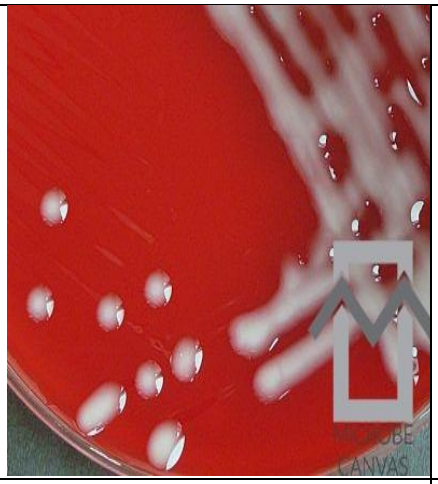
- **Gram stain:** Gram-negative coccobacilli

C. Culture Media and Colony Characteristics

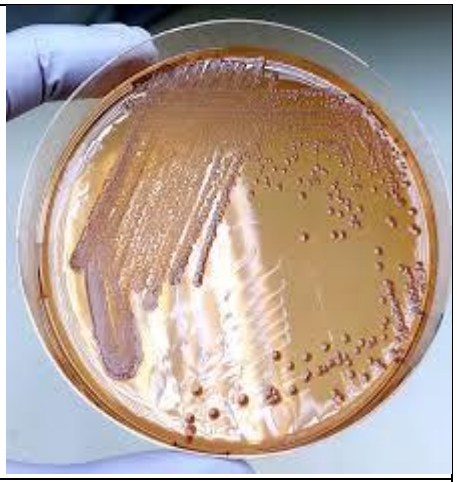
Culture Medium	Colony Characteristics
Nutrient Agar	Smooth, opaque, convex colonies
Blood Agar	Small to medium, gray-white colonies; usually **non-hemolytic**
MacConkey Agar	**Non-lactose fermenting** colonies (pale)



Nutrient Agar



Blood Agar



MacConkey Agar

D. Incubation: **incubate at** 35–37°C for 24–48 hours

E. Biochemical Reactions

Biochemical Test	Result
Oxidase test	**Negative**
Catalase test	**Positive**
Motility	**Negative**
Indole test	**Negative**
Citrate utilization	**Positive** (most species)
Carbohydrate metabolism	**Non-fermenter** (oxidative or inert)
Nitrate reduction	Negative

5- *Shigella and Salmonella*

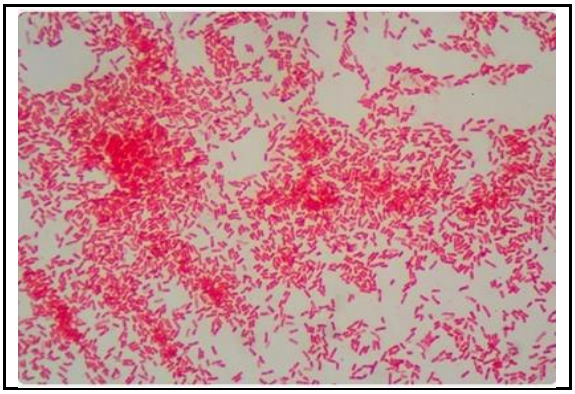
1- Identification of *Shigella* spp

Laboratory Diagnosis test:

A. Specimens

- Fresh stool
- Mucus flecks in stool

- Rectal swabs (for culture)



B. Microscopic examination:

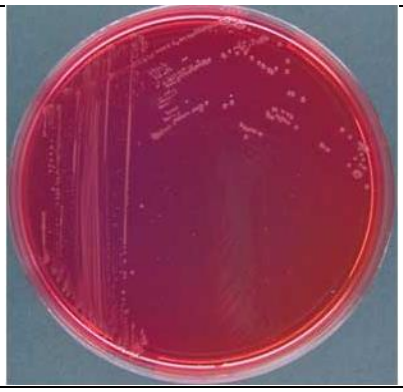
- Large numbers of **fecal leukocytes**
- Presence of **red blood cells**

- Gram-negative bacilli
- Short rods
- Non-motile

C. Culture Media and Colony Characteristics

Culture Medium	Colony Characteristics
MacConkey Agar	Colorless colonies (non-lactose fermenter)
EMB Agar	Colorless or pale colonies
Salmonella–Shigella (SS) Agar	Clear, colorless, transparent colonies
XLD Agar	Red colonies (*Shigella flexneri*)
Hektoen Enteric Agar	Green colonies without black centers

SS agar



XLD Agar

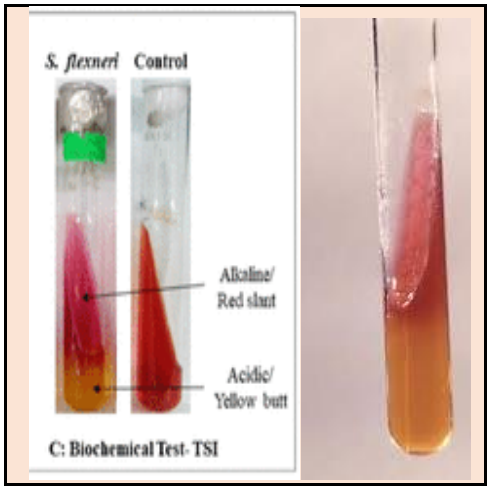
Hektoen Enteric Agar

D. Incubation

- **35–37 °C**, 18–24 h
- Aerobic

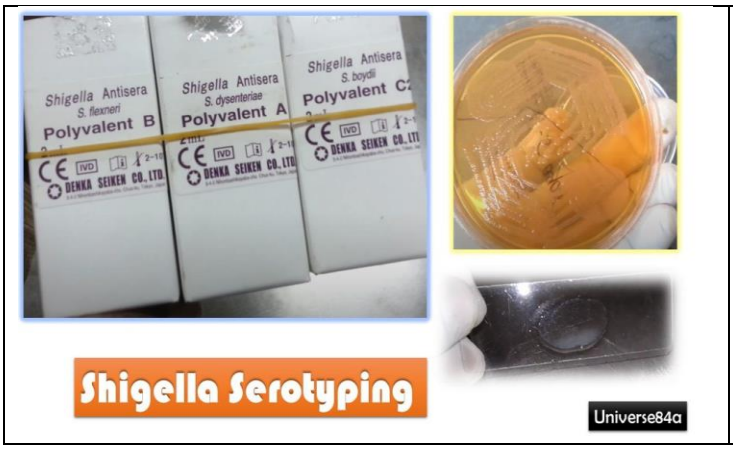
E-Biochemical Identification

Test	Result
Oxidase	–
Catalase	+
Indole	Variable (depends on species)
Methyl Red	+
Voges-Proskauer	–
Citrate	– (except *S. sonnei* can be +)
Urease	–
TSI	K/A, no gas, H_2S –
Lysine decarboxylase	–
Motility	–
Phenylalanine deaminase	–



F-Serologic Identification

- Slide agglutination with **O-antigen specific antisera**
- Four main groups: A (*S. dysenteriae*), B (*S. flexneri*), C (*S. boydii*), D (*S. sonnei*)



2. Identification of *Salmonella* spp

Laboratory diagnosis test:

A. Specimens

- Stool (especially in **chronic carriers)**
- Blood (especially during the **first week** of typhoid fever)
- Urine (usually positive **after the second week of typhoid fever**)

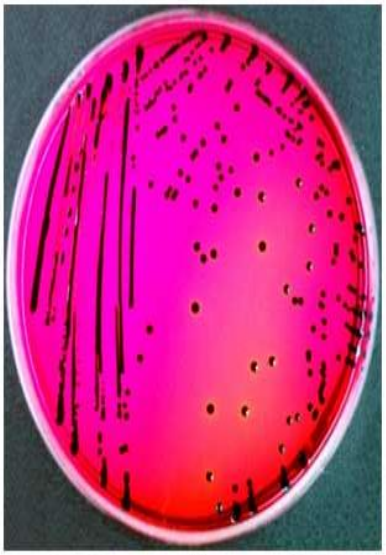
B. Microscopic Examination

- **Gram stain:** Gram-negative rods

C. Culture Media and Colony Characteristics

Culture Medium	Colony Characteristics
MacConkey Agar	Colorless colonies (non-lactose fermenter)
EMB Agar	Pale or colorless colonies
SS Agar	Colorless colonies with **black center** (H_2S)
XLD Agar	Red colonies with **black center**
Hektoen Enteric Agar	Blue-green colonies with **black center**

SS Agar



XLD Agar

Hektoen Enteric Agar

D. Incubation condition

- **35–37 °C**, 18–24 h
- Aerobic

E. Biochemical Reactions

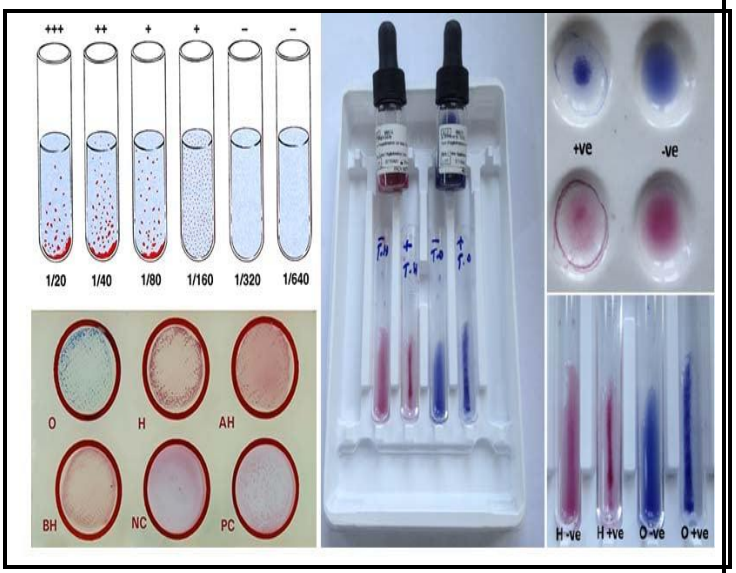
Biochemical Test	Result
Glucose fermentation	Positive (acid, no gas)
Lactose fermentation	Negative
Sucrose fermentation	Negative
Mannitol fermentation	Positive
Oxidase	Negative
Catalase	Positive
Urease	Negative
Indole	Negative
Methyl Red (MR)	Positive
Voges–Proskauer (VP)	Negative
Citrate utilization	Positive
Motility	Positive
H_2S production	Positive
TSI	K/A + H_2S (no gas)

TSI test

K/A + H_2S (no gas)

F. Serologic Identification

- I. Agglutination test
- II. Tube dilution agglutination test (Widal test): Serum agglutinins rise sharply during the second and third weeks of S serotype Typhi infection.



The Widal test is a serological blood test:

- **Prepare serum:** Bring to room temperature.
- **Dilute serum:** Serial dilutions (1:20 → 1:320).
- **Add antigens:** O, H, (Vi if needed).
- **Controls:** Positive and negative controls.
- **Incubate:** 37°C, 16–20 hours.
- **Read titer:** Highest dilution showing agglutination.
- **Interpret:**
  - O ≥ 1:160 → active infection
  - H ≥ 1:160 → recent/past infection
  - Vi ≥ 1:80 → carrier/vaccination
- **Timing:** The test is most effective when conducted 7–14 days after the onset of fever, as it takes time for antibodies to rise.

6-Yersinia spp

Laboratory Diagnosis of *Yersinia pestis*

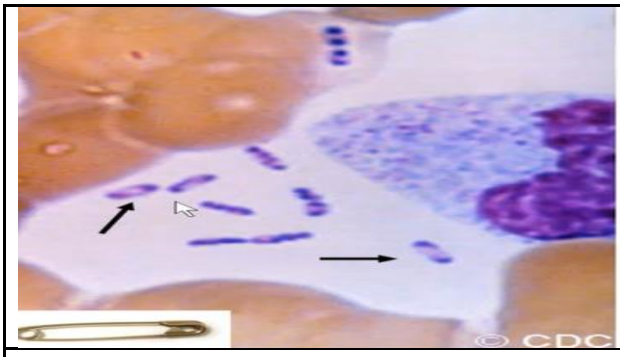
A. Specimen Collection

- **Sputum** → Pneumonic plague
- **Blood for culture** → Septicemic plague
- **Aspirate from enlarged lymph nodes (buboes)** → Bubonic plague
- **Cerebrospinal fluid (CSF)** → Meningeal involvement (rare)

B. Direct Microscopic Examination

- **Stained with Gram stain**, appearing as **Gram-negative rods**.

- **Smears may also be stained with special stains**, including:
  - Wayson stain
  - Giemsa stain
  - Wright stain



***Yersinia* stained with Giemsa stain**

- These special stains demonstrate the **characteristic bipolar (safety-pin) appearance** of the organism.

Procedure of stains:

1. Make thin smear and air-dry.
2. Fix with methanol (Wright/Giemsa) or without fixation (Wayson).
3. Stain:
   - **Wright**: 1–3 min then Add a few drops of buffered water Let stand 5–10 minutes.
   - **Giemsa**: Dilute with buffered water (1:20–1:50) and cover the smear for 15–30 minutes.
   - **Wayson**: 3–5 min
4. Rinse & air-dry.
5. Examine under oil immersion (1000×) → **Yersinia appears bipolar (safety-pin)**

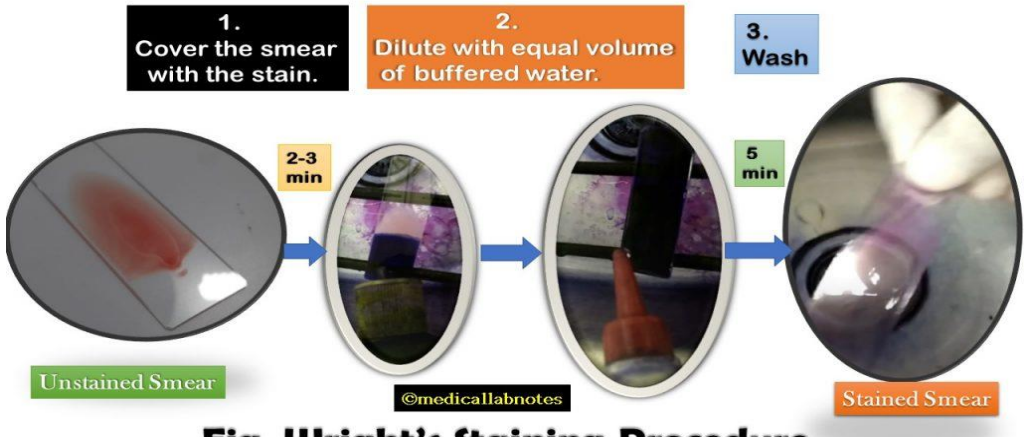


Fig. Wright's Staining Procedure

C. Culture media and colony characters

Culture Medium	Colony Characteristics
MacConkey Agar	Colorless colonies (non-lactose fermenter)
Blood Agar	Gray-white, sometimes opaque colonies with irregular edges; non-hemolytic.

Blood agar

D. Incubation condition:

Grows better at 28°C than at 37°C.

⚠ Culture must be handled in **biosafety level 3 (BSL-3)** laboratories.

-Definite identification of cultures is best done by immunofluorescence or by lysis by a specific *Y. pestis* bacteriophage

E. Biochemical Identification

Biochemical Test	Result
Oxidase	Negative
Catalase	Positive
Urease	Negative
Motility	Positive

F. Serological Tests

F1 antigen detection uses fluorescent antibodies for rapid, specific identification of *Y. pestis*, while antibodies against F1 can be detected by ELISA.

7-*Vibrio*

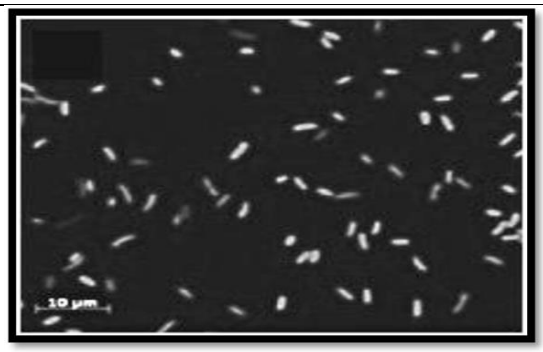
Laboratory diagnostic test of *Vibrio cholerae*:

A-Specimen

- **Type:** Stool
- **Collection:** Early in diarrheal illness for optimal recovery
- **Handling:**
  - Inoculate onto appropriate agar within 2–4 hours.
  - If delay occurs, mix stool with **Cary-Blair transport medium** and refrigerate.

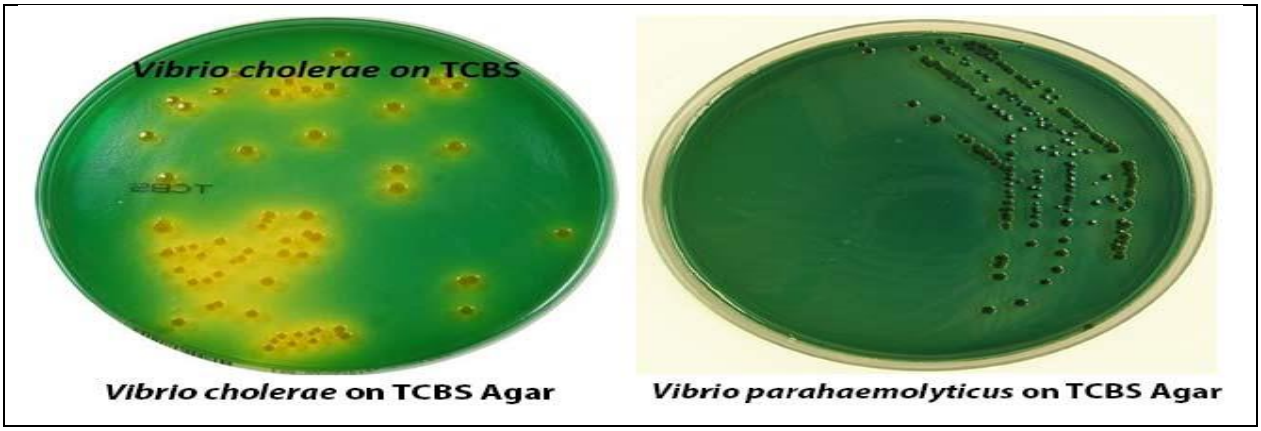
B. Microscopic Detection

- Direct smear detection is **not distinctive**, not routinely recommended.
- **Special microscopy:** Dark-field or phase-contrast microscopy
  - Detects *V. cholerae O1* directly from stool or enrichment broth.
  - **Characteristic motility:** "shooting star" movement
  - **Confirmation:** Motility stops after mixing with **polyvalent O1 antiserum** → confirms *V. cholerae O1*.

	
	Dark-field or phase-contrast microscopy

C. Culture Media and Colony Characteristics

Culture Media	Characteristics
Blood Agar	Convex, smooth, round colonies that are opaque and granular in transmitted light
TCBS Agar **(Thiosulfate-citrate-bile salts-sucrose)**	*V. cholerae* → yellow colonies (sucrose fermentation) *V. parahaemolyticus* / *V. vulnificus* → green colonies (non-sucrose-fermenting)
Alkaline Peptone Water (1% NaCl, pH 8.5)	Promotes rapid growth of Vibrio
Taurocholate Peptone Broth (pH 8–9)	Few drops of stool incubated 6–8 h For subculture or staining



D. Biochemical Reactions

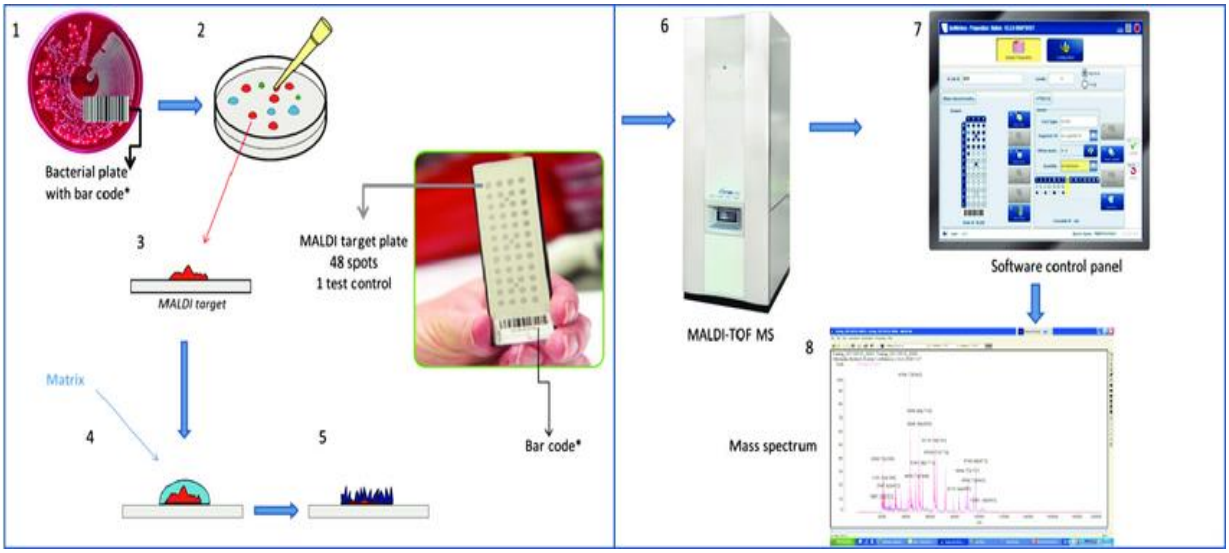
Test	Result for *V. cholerae*
Sucrose fermentation	+
Mannose fermentation	+
Arabinose fermentation	–
Oxidase	+
Voges-Proskauer (VP)	+ in El Tor biotype

E. Serology

- **Slide or tube agglutination:** Detects O1 and O139 antigens in colonies or patient serum; confirms serogroup.
- **Latex agglutination:** Rapid detection of antigens in stool or colonies; useful during outbreaks.
- **ELISA:** Detects IgG or IgM antibodies against *V. cholerae* in patient serum; mainly for research or epidemiology.

F. MALDI-TOF MS:

- **MALDI-TOF MS:** Rapid, accurate identification, especially for *V. parahaemolyticus*



8- Campylobacter

Diagnostic Laboratory Tests:

A. Specimens:

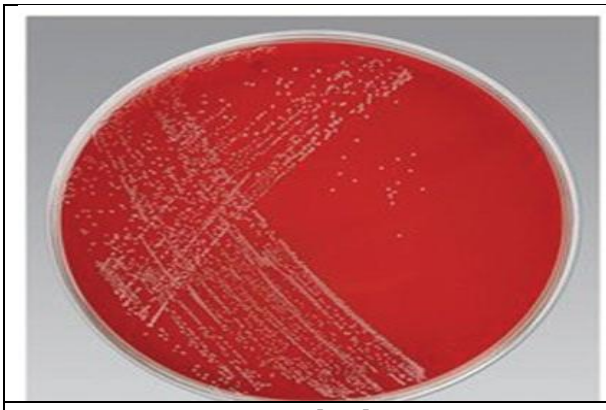
- **Stool sample**: Preferred specimen
- **Rectal swab**: Acceptable specimen
- **Blood culture**: Occasionally positive, especially in elderly or immunocompromised patients

B. Smears

- **Gram stain**: Shows characteristic gull wing–shaped Gram-negative curved rods
- **Dark-field or phase-contrast microscopy**: Demonstrates darting motility

C. Culture and colony characters

Culture Medium	Antibiotics / Components	Notes
Campy Blood Agar	Selective antibiotics	Suitable for *C. jejuni* & *C. coli*
Skirrow's Medium	Vancomycin, Polymyxin B, Trimethoprim	Routine stool isolation
Charcoal-based selective media	Charcoal + cephalosporins	Commonly used in labs



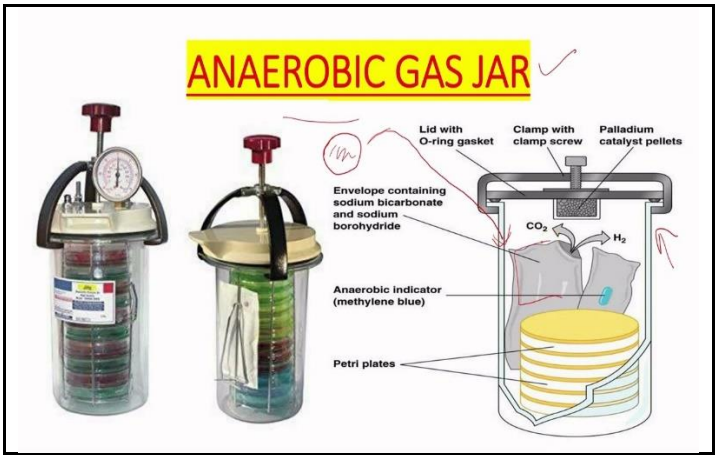
Campy Blood Agar

Skirrow's Medium

Charcoal-based selective media

D. Incubation condition:

- Microaerophilic conditions
- O_2: 5–7%
- CO_2: 10%
- **Achieved by**:
  - Anaerobic jar without catalyst
  - Gas-generating pack



E. Biochemical Tests

Test	Result	Notes
Motility	Positive	Darting motility
Oxidase	Positive	
Catalase	Positive	
Carbohydrate fermentation	Negative	No oxidation or fermentation
Hippurate hydrolysis	**Positive in *C. jejuni***	Key differentiating test
Nitrate reduction	Positive	
H_2S production	Variable	

9- *Helicobater pylori*

A. Specimen, Transport, and Processing:

- **Gastric biopsy:** The main specimen for histologic examination and culture; collected via **endoscopy**.
- **Blood:** Used for the detection of ***H. pylori* antibodies** in serologic tests.
- **Stool:** Can be collected for ***H. pylori* antigen detection**.

-Transport:

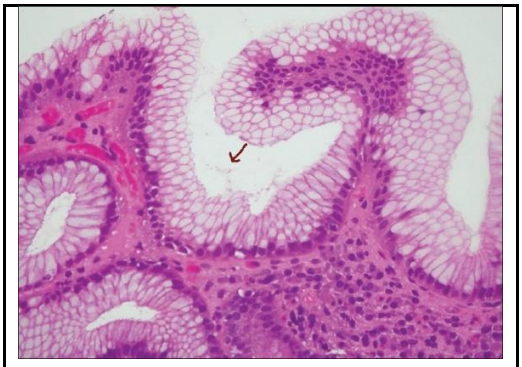
If transport to the laboratory exceeds **2 hours**, **special transport media** (e.g., **Stuart's transport medium**) should be used to maintain viability.

- Processing:

The biopsy specimen may be **homogenized** to increase recovery of the organism

B. Diagnostic Testing Methods for *H. pylori*

1. Invasive Testing



Gastric mucosa showing a number of Helicobacter pylori organisms (H and E)

a- Histology:

- ❖ Based on examination of a **gastric biopsy** obtained during endoscopy.
- ❖ Routine stains such as **hematoxylin and eosin (H&E)** are used to
- ❖ demonstrate **acute or chronic gastritis**.
- ❖ **Special stains** (e.g., *Giemsa, silver stains, or immunohistochemical stains*)
- ❖ are used to visualize ***H. pylori***, which appears as **curved or spiral-shaped bacteria**.
- ❖ This method is **more sensitive than culture** and allows evaluation of both the organism and the degree of tissue damage.

b- Urease detection in tissue:

- ❖ A **gastric biopsy** is placed into a medium containing **urea and a pH indicator**.
- ❖ ***H. pylori* produces urease**, which converts urea into ammonia, causing a **color change** in the medium.
- ❖ Results are usually available within **minutes to 2 hours**.

A

B

c-Microbiologic Culture

- Gastric biopsy specimens are inoculated onto **special enriched media** such as:
  - **Chocolate agar**
  - **Skirrow's medium**
- Plates are incubated at **37°C** in a **microaerophilic atmosphere** (low O_2 and increased CO_2).
- Growth usually appears in **3–6 days**, but plates may be held up to **14 days** before reporting a negative result.
- Colonies are **small (1–2 mm), gray to translucent** in appearance.

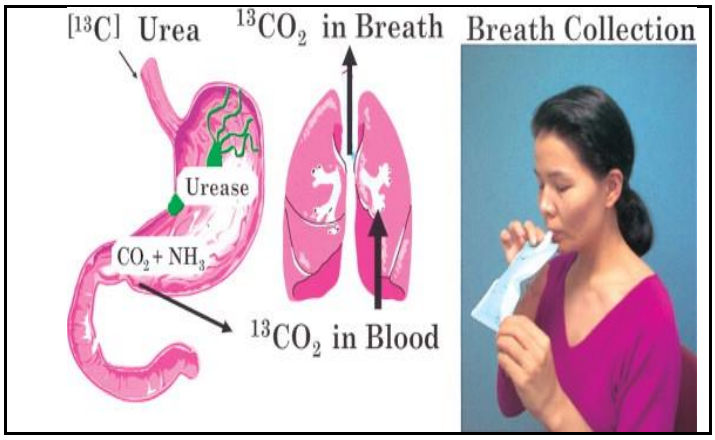
2. Noninvasive Testing

a- Serology

- Detects **serum antibodies (mainly IgG)** against *H. pylori* using blood samples.
- Indicates **exposure to the organism**, not necessarily active infection.
- Antibodies may remain positive **even after successful treatment**.

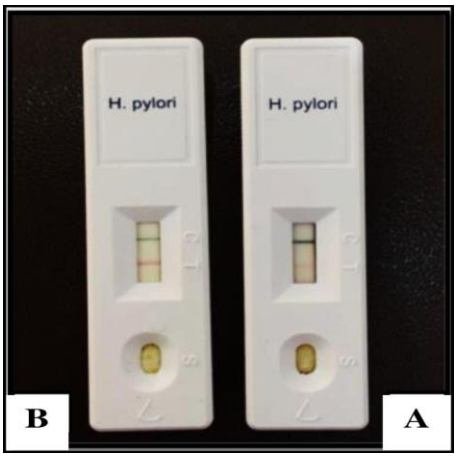
b-Urea Breath Test

- The patient ingests **urea labeled with carbon isotope** (^{13}C or ^{14}C).
- *H. pylori* urease breaks down urea into **ammonia and labeled CO_2**, which is detected in exhaled breath.
- Indicates **active infection**.



c-Stool Antigen Test

- Detects ***H. pylori*** **antigens** in stool samples.
- Indicates **active infection**.



10-Haemophilus

A. Specimens:

- Respiratory specimens: expectorated sputum, bronchoalveolar lavage.
- Pus, blood, and cerebrospinal fluid (CSF) depending on infection site.

B. Microscopic Detection (Gram Stain):

- Centrifuge body fluids (2000 rpm for 10 min) to concentrate bacteria.
- Prepare smears from the pellet.
- *Haemophilus* spp. appear as pale pink coccobacilli and may be difficult to see against protein-rich backgrounds.

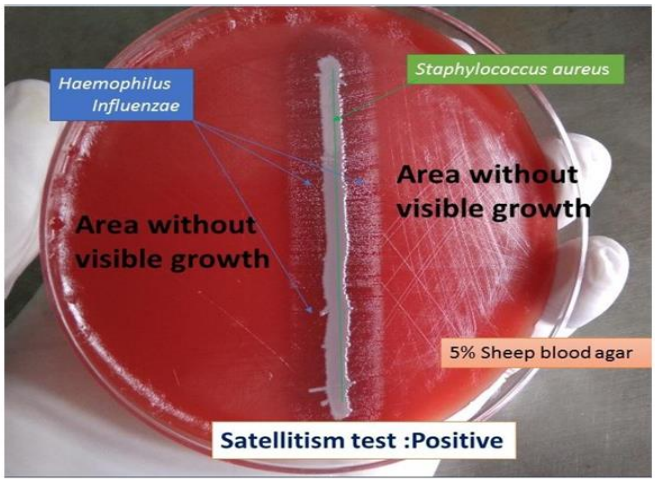
C. Colony Morphology:

Culture Media	Colony Morphology
Chocolate agar	Colonies are smooth, flat or convex, buff or slightly yellow. Encapsulated strains produce tan, mucoid colonies.
Sheep blood agar	Due to absence of V factor (NAD). Small colonies may appear near NAD-producing bacteria such as *Staphylococcus aureus* (**satellite phenomenon**).

❖ Satellite phenomenon:

The **satellite phenomenon** refers to the growth of fastidious bacteria, especially *Haemophilus* species, as **small colonies surrounding other bacteria** that provide essential growth factors.

On **sheep blood agar**, *Haemophilus* cannot grow because the medium lacks **V factor (NAD)**. However, when **NAD-producing bacteria** such as *Staphylococcus aureus* are present, they release NAD into the medium. As a result, *Haemophilus* grows only **around these bacteria**, forming tiny "satellite" colonies.



D. Incubation Conditions:

- Facultative anaerobes; grow aerobically or anaerobically.
- Growth enhanced by 5–10% CO_2 (candle jar, CO_2 pouch, or CO_2 incubator).

E. Molecular Methods:

- PCR assays for capsular types **a** and **f** from CSF, plasma, serum, or whole blood for rapid screening and outbreak evaluation.

Serological Test (Antigen Detection):

Detection of *Haemophilus influenzae* type b (Hib) is based on identifying the **capsular polysaccharide antigen** directly in clinical specimens such as **cerebrospinal fluid (CSF) and urine**. This is done using **commercial particle agglutination assays**, which rely on an **antigen–antibody reaction**. A visible agglutination indicates the presence of the Hib antigen.

11-*Bordetella* and *Brucella*

Identification of Bordetella pertussis

A. Specimen Collection

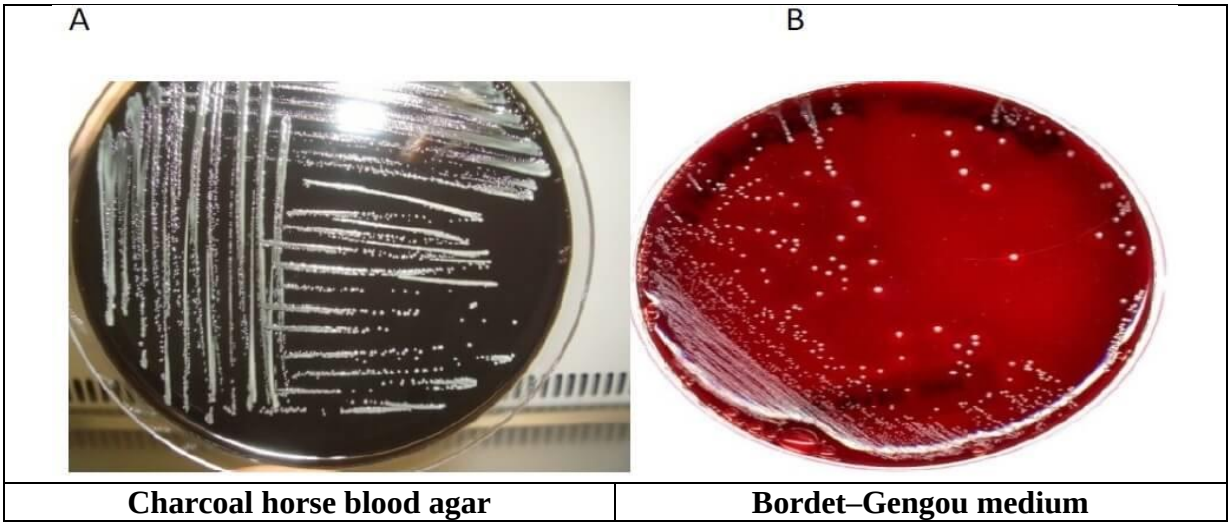
- **Nasopharyngeal swab** collected during the **paroxysmal stage** of the disease

B. Microscopic Examination

- **Gram stain:** Minute **Gram-negative coccobacilli**, may resemble *H. influenzae*
- **Toluidine blue stain:** Shows **bipolar metachromatic granules**
- **Capsule:** Present, especially in freshly isolated strains

C. Culture media and colony characters

Culture Medium	Incubation Conditions	Colony Characteristics / Notes
Bordet–Gengou medium (potato-blood-glycerol + Penicillin G)	35–37 °C, strict aerobe, moist environment, 3–7 days (use a sealed plastic bag)	Small, smooth, convex, slow-growing, hemolytic (virulent strains)
Charcoal horse blood agar *(medium of choice)*	30–37 °C, strict aerobe	Small, smooth, glistening, better growth than Bordet–Gengou
Stainer–Scholte broth *(commercial medium)*	35–37 °C	Turbid growth
Cyclodextrin solid medium *(commercial medium)*	35–37 °C	Small, smooth colonies



Charcoal horse blood agar	**Bordet–Gengou medium**

D. Biochemical reactions :

Test	Result
Catalase	Positive (+)
Oxidase	Positive (+)
Citrate	Negative (-)
Methyl Red (MR)	Negative (-)
Voges Proskauer (VR)	Negative (-)
Urease	Negative (-)
Motility	Non-motile
Nitrate Reduction	Negative (-)
Indole	Negative (-)

Note:

- Does not require X or V factors
- Produces acid (no gas) from glucose and lactose
- Metabolism mainly by oxidation of amino acids
- Carbohydrates not usually utilized

Identification *Brucella* spp.

A. Specimen Collection

- Blood
- Bone marrow
- Other clinical specimens from humans or animals

B. Microscopic Examination

- Small Gram-negative coccobacilli or short rods

C. Culture media and colony characters

Culture Medium	Incubation Conditions	Colony Characteristics
Blood culture media	37 °C, aerobic, 2–5 days	Small, smooth, convex, non-pigmented
Trypticase soy agar	37 °C, aerobic (*B. abortus* requires 5–10% CO_2)	Small, smooth, translucent
Enriched media	37 °C, aerobic	Slow-growing, smooth colonies

D. Biochemical reaction

Test	Result
Oxidase	Positive
Catalase	Positive
Urease	Positive (rapid)
H_2S production	Many strains positive
Nitrate reduction	Nitrate → Nitrite positive
Carbohydrate utilization	Utilized but **no significant acid or gas production**

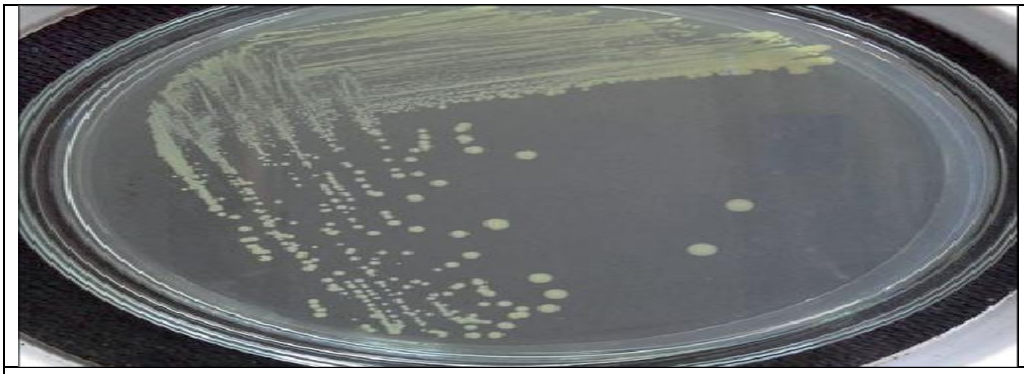
E. Serology test

-Rose Bengal Test (RBT)

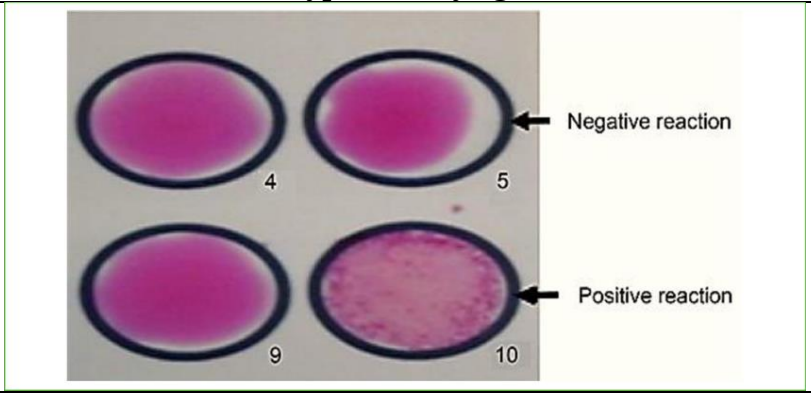
Spot test for brucella diagnosis(humans and animals). Detects specific antibodies (IgM and IgG).

Procedure

- Place a **drop (30 µl) of undiluted serum** on a slide.
- Add a **drop of Rose Bengal Brucella antigen** next to the serum.
- Mix both drops using a **disposable stirring stick**, spreading them over the full surface of the circle.
- **Observe for agglutination** (clumping indicates a positive reaction).



Trypticase soy agar



Rose Bengal Test (RBT)

12-*Chlamydia*

Identification of *Chlamydia trachomatis*

A. Specimen Collection

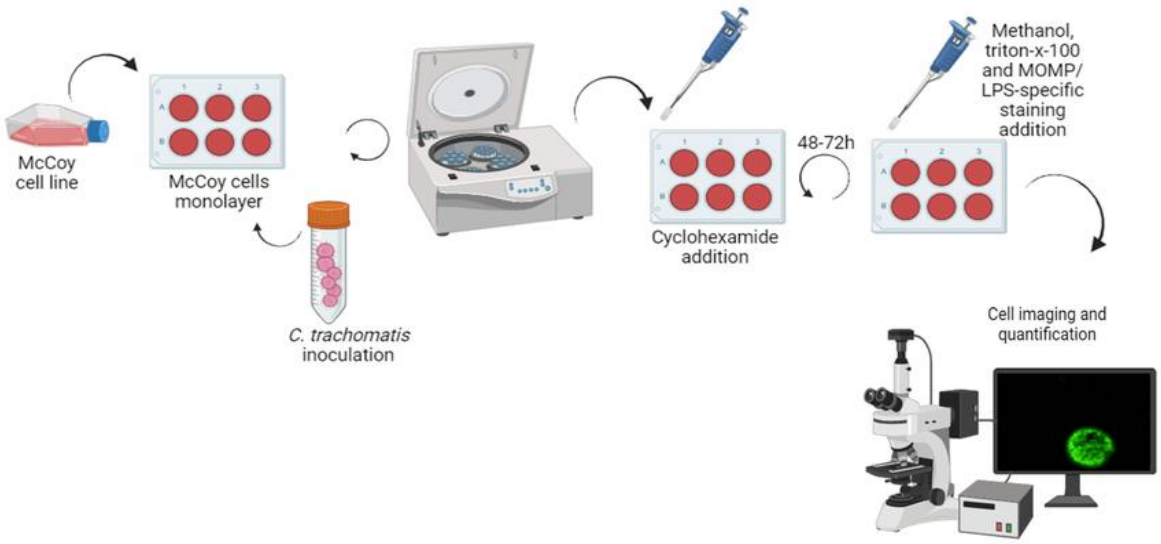
- Conjunctival swabs (newborns, trachoma cases)
- Genital specimens
- Clinical samples are collected from infected epithelial cells

B. 1. Indirect Method: Culture

- **Obligate intracellular organism**, therefore cultured only in **living cells**
- **Cell lines used:**
  - McCoy cells *(most commonly used)*
  - HeLa cells
  - Monkey kidney cells
- **Cycloheximide-treated McCoy cells** are preferred to inhibit host cell protein synthesis and enhance chlamydial growth

Procedure:

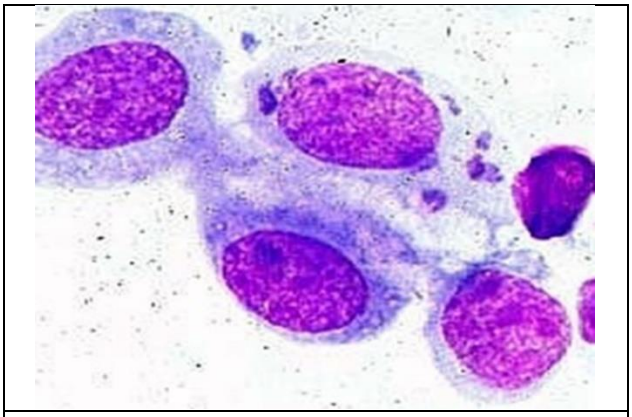
1. Clinical specimens are shaken with **5-mm glass beads** to release elementary bodies (EBs).
2. Specimens are centrifuged onto a **cell monolayer** grown on a coverslip in a vial (**shell vial technique**) to enhance adherence.
3. Incubation is carried out for **48–72 hours**.
4. Infected cell monolayers are stained using **fluorescein-labeled monoclonal antibodies**.
5. **Intracytoplasmic inclusions** are visualized under a fluorescence microscope.



B.2. Direct Detection Methods

1. Cytologic Examination

- Specimens: Cell scrapings from:
  - Conjunctiva of newborns
  - Patients with ocular trachoma
- **Staining method:** Giemsa stain
- **Finding:** Presence of **intracytoplasmic inclusion bodies**



Giemsa stain

2. Antigen Detection and Nucleic Acid Hybridization

- Commercially available diagnostic kits
- Used to overcome limitations of cell culture
- Detect chlamydial antigens or nucleic acid directly from clinical specimens

13-Spirochetes

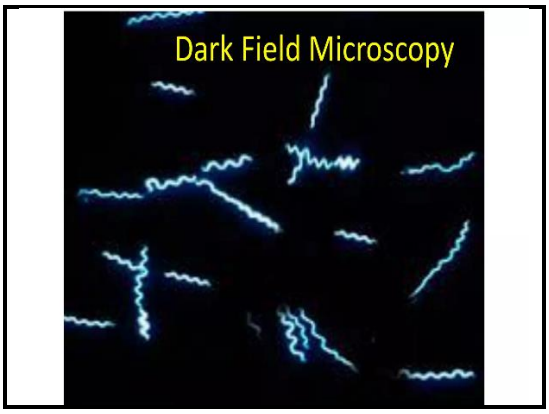
Laboratory Diagnosis of *Treponema pallidum*

A. Specimens

- **Tissue fluid or exudate** from early skin or mucosal lesions (primary chancre)
- **Blood** for serologic testing

- **Cerebrospinal fluid (CSF)** in suspected cases of neurosyphilis

B. Direct Detection Methods



1. Dark-Field Microscopy

- **Specimen:** Tissue fluid or exudate from the hard chancre
- **Procedure:**
    1. A drop of tissue fluid is placed on a clean glass slide.
    2. A coverslip is applied to obtain a thin preparation.
    3. The slide is examined immediately using a **dark-field microscope**.
- **Result:** Slender, actively motile spirochetes with characteristic corkscrew movement are observed.

2. Immunofluorescence

- **Specimen:** Tissue fluid or exudate
- **Procedure:**
    1. The specimen is spread on a glass slide.
    2. The slide is air-dried.
    3. It is examined using **fluorescent-labeled antibodies** specific for *T. pallidum*.
- **Result:** *Treponema pallidum* appears as bright, fluorescent spirochetes.

3. Nucleic Acid Amplification Tests (NAATs)

- Used to detect *T. pallidum* DNA directly from clinical specimens.
- These tests serve as supportive modern diagnostic methods.

C. Indirect Detection Methods (Serologic Tests)

- Serologic Tests for Syphilis

Table 48.2: Diagnostic tests for syphilis

A. Demonstration of treponemes in the exudate	1. Dark-ground microscopy 2. Direct fluorescent-antibody staining for *Treponema pallidum* (DFA- Tp) 3. Silver impregnation method (Levaditi's stain) 4. Enzyme immunoassay, Polymerase chain reaction (PCR).
B. Serological tests	**a. Nontreponemal tests** **Nonspecific *(reagin antibody)* tests using the cardiolipin antigen *(standard tests for syphilis or STS)*.** 1. Wassermann complement fixation test 2. Kahn flocculation test 3. Venereal Disease Research Laboratory (VDRL) test 4. Rapid Plasma Reagin (RPR) test 5. Toluidine red unheated serum test (TRUST) **b. Treponemal tests** **a. Group specific test using cultivable treponemal (Reiter strain) antigen** I. Reiter Protein CF (RPCF) test (1953) **b. Specific tests using pathogenic treponemes *(T. palidum)*** I. Test using live *T. pallidum* *Treponema pallidum* Immobilization (TPI) test **II. Tests using killed *T. pallidum*** a. Treponema pallidum agglutination (TPA) test b. Treponema pallidum immune adherence (TPIA) test c. Fluorescent Treponemal Antibody Absorption (FTA-ABS) test **III. Tests using *T. pallidum* extract** a. *Treponema pallidum* Hemagglutination Assay (TPHA) Microhemagglutination test for *Treponema pallidum* (MHA-TP) b. *Treponema pallidum* Enzyme Immunoassays (TP-EIA):

14-*Mycobacterium*

A. Specimen Collection

❖ Pulmonary Tuberculosis

- At least **three sputum specimens** should be collected.
- Preferably **early-morning samples** on **three consecutive days**.
- Collection should be done in a **well-ventilated or isolated area**.

❖ Induced Sputum

- For patients unable to expectorate sputum:
  - Inhalation of **warm hypertonic saline (5–15%) aerosol**
- Induced sputum must be labeled as **"induced"**.

❖ Alternative Specimens

- Bronchoscopy samples (if sputum cannot be obtained)
- Gastric aspirates (especially in children)
- For extrapulmonary TB:
  - Urine
  - Cerebrospinal fluid (CSF)
  - Pleural fluid
  - Pus
  - Biopsy specimens

❖ Specimen Transport

- Tissue specimens should be placed in: **Transport medium (e.g., Dubos medium)** or **Normal saline**

B. Microscopic examination

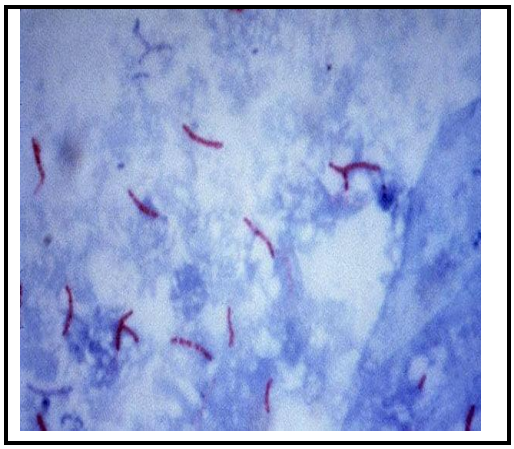
- *Mycobacterium tuberculosis* appears as **slender, straight or slightly curved rods**
- The bacilli are **non-motile, non-spore-forming, non-capsulated**, and **acid-fast**.
- Although structurally Gram-positive, they are **poorly stained by Gram stain**.

❖ Ziehl–Neelsen Staining
❖ Smear

1-Prepare a thin smear from the clinical specimen

(e.g., sputum).

2- Allow the smear to **air-dry**, then **heat-fix** gently

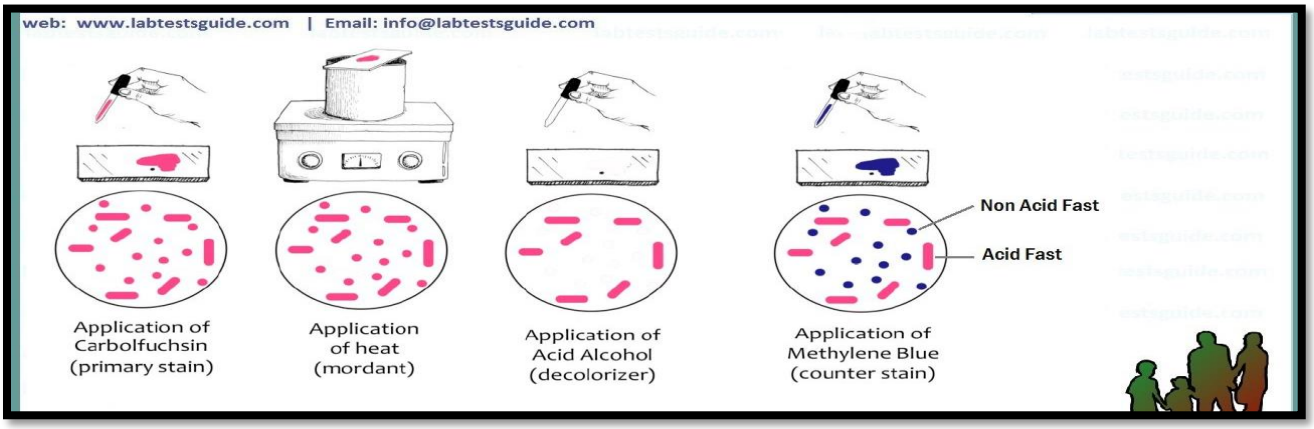


❖ Procedure: .

1. Stain with **carbol fuchsin** and heat for **3–5 min**.
2. Rinse with water.
3. Decolorize with **acid (20% H_2SO_4 or acid-alcohol)**.
4. Rinse and counterstain with **methylene blue (1–2 min)**.
5. Examine under **oil immersion (100×)**.

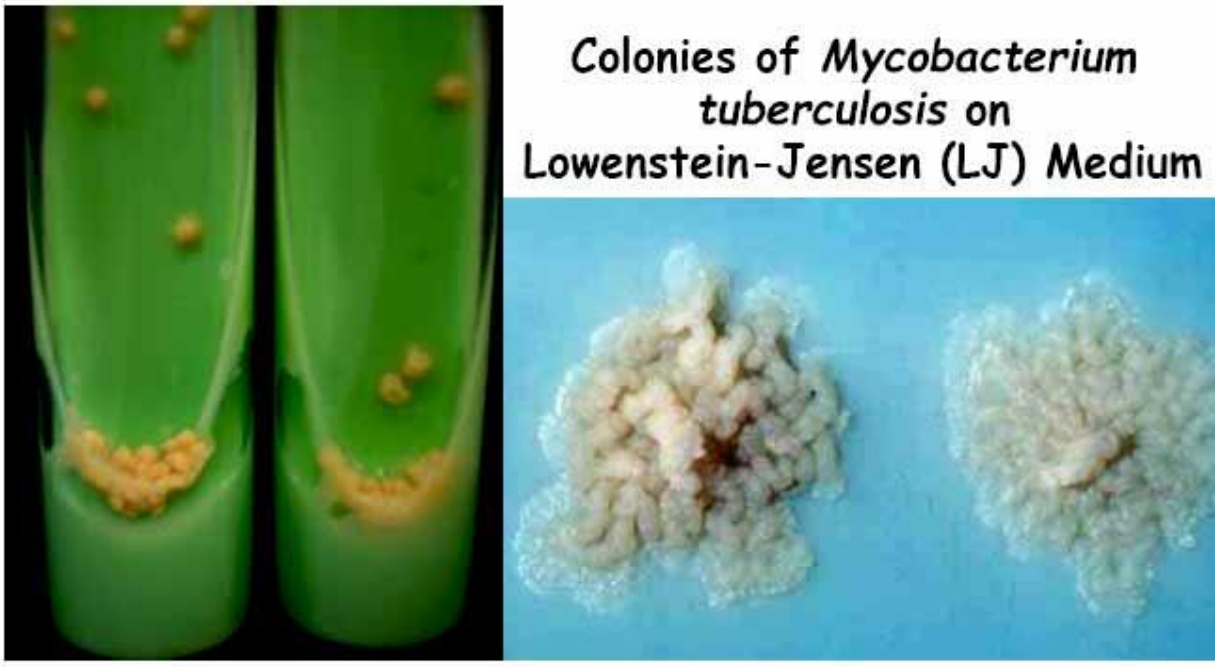
❖ Result:

- *M. tuberculosis* stains **bright red**
- Background tissue cells and other organisms stain **blue**



C. Cultural Characteristics

Feature	Mycobacterium tuberculosis	Mycobacterium bovis
Oxygen requirement	Obligate aerobe	Obligate aerobe
Optimal temperature	35–37 °C	35–37 °C
Optimal pH	6.4–7.0	~6.4–7.0
Growth rate	Slow (generation time 14–15 hours)	Slow
Common culture medium	Lowenstein–Jensen (LJ) medium	LJ medium
Growth on LJ glycerol	Good growth	Poor growth
Growth on LJ pyruvate	Not preferred	Better growth
Time of colony appearance	2–8 weeks	2–8 weeks
Colony texture	Dry, rough, tenacious	Smooth, moist
Colony shape	Raised, irregular	Flat
Colony surface	Wrinkled	Smooth
Colony color	Creamy white → yellowish/buff with time	White
Ease of emulsification	Difficult to emulsify	Easily disrupted



D. Biochemical reactions

Test	*M. tuberculosis*	*M. bovis*	*Atypical mycobacteria*
Production of niacin	+	–	–
Binding of neutral red	+	+	+/–
Hydrolysis of Tween 80	–	–	+
Production of enzymes:			
• Nitrate reduction	+	–	+/–
• Arylsulphatase	–	–	–/+
• Catalase at room temp	–	–	+
at 68°C	–	–	+
• Catalase-Peroxidase	Weak +	Weak +	Strong +
Nicotinamidase	+	–	–
• Pyrazinamidase	+	–	+/–
Susceptibility to:			
• Pyrazinamide	+	–	–
Uptake of iron	–	–	–/+

15-*Mycoplasma* and *Rickettsia*

Laboratory Diagnosis of *Mycoplasma pneumoniae*

A. Specimens

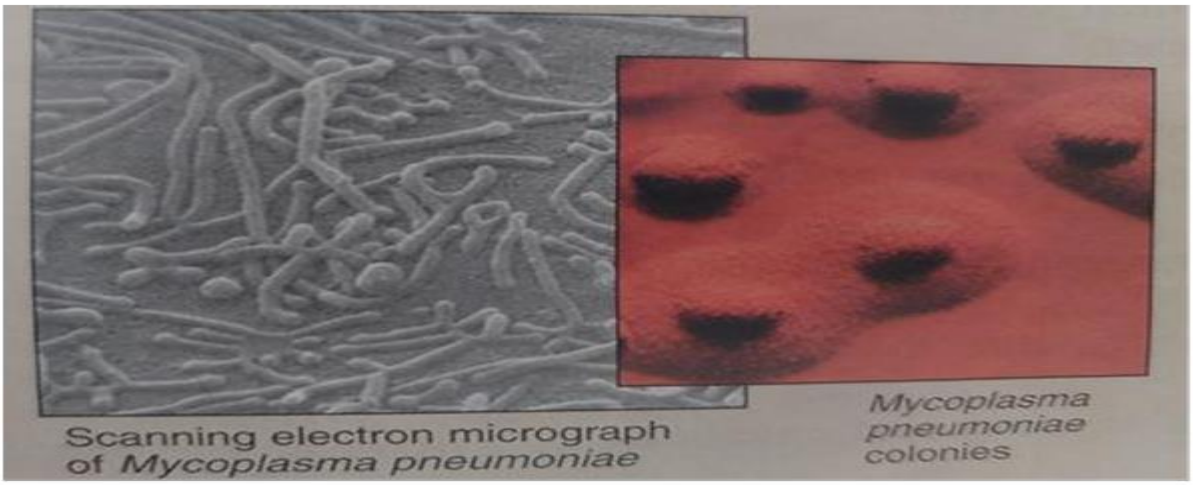
- Sputum
- Throat swabs

B. Direct Microscopic Examination

- Direct microscopic examination of clinical specimens is of **limited diagnostic value**.
- *Mycoplasma pneumoniae* cannot be visualized by **Gram stain** due to the absence of a cell wall.

C. Culture

- Specimens (sputum or throat swabs) are inoculated onto **special enriched media** containing sterols.
- The organism grows **slowly**.
- **Incubation period:** approximately **8–15 days**.
- Colonies may show a characteristic **fried egg appearance**.
- Culture is **rarely performed routinely** because it is time-consuming.



D. Serologic Tests

- Serological methods are commonly used to detect **specific antibodies** against *M. pneumoniae*.
- These tests are useful for **clinical diagnosis**, especially when culture is impractical.

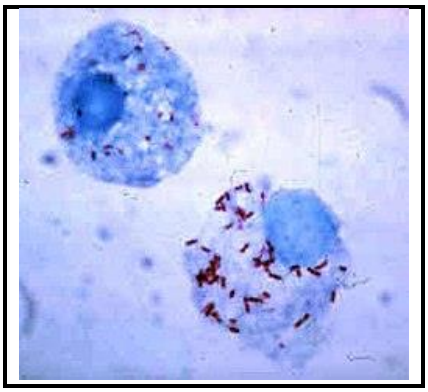
Laboratory Diagnosis of *Rickettsia*

A. Specimen Collection

- **Blood (serum):** For serological testing
- **Skin biopsy (from rash lesion):** Especially in cases of spotted fever

- **Whole blood / tissue samples:** For molecular testing (PCR)

B. Microscopic Examination



- Observed using **light microscopy** after staining the samples with:
  - **Giemsa stain**
- Appears as **short rods or coccobacilli inside host cells**

C. Culture

- **Not routinely cultured** in clinical laboratories
- **Reason:**
  - Rickettsia are **obligate intracellular organisms**
  - Require **living cells** (cell culture or embryonated eggs)

D. Serological Tests

1-Indirect Immunofluorescence Assay (IFA)

2- ELISA

E. Direct Detection Methods

- **Immunofluorescence staining**
  - For direct detection of antigens in skin biopsy or tissue samples
- **Histochemical staining**
  - Used in certain tissue samples