HLS
Practical

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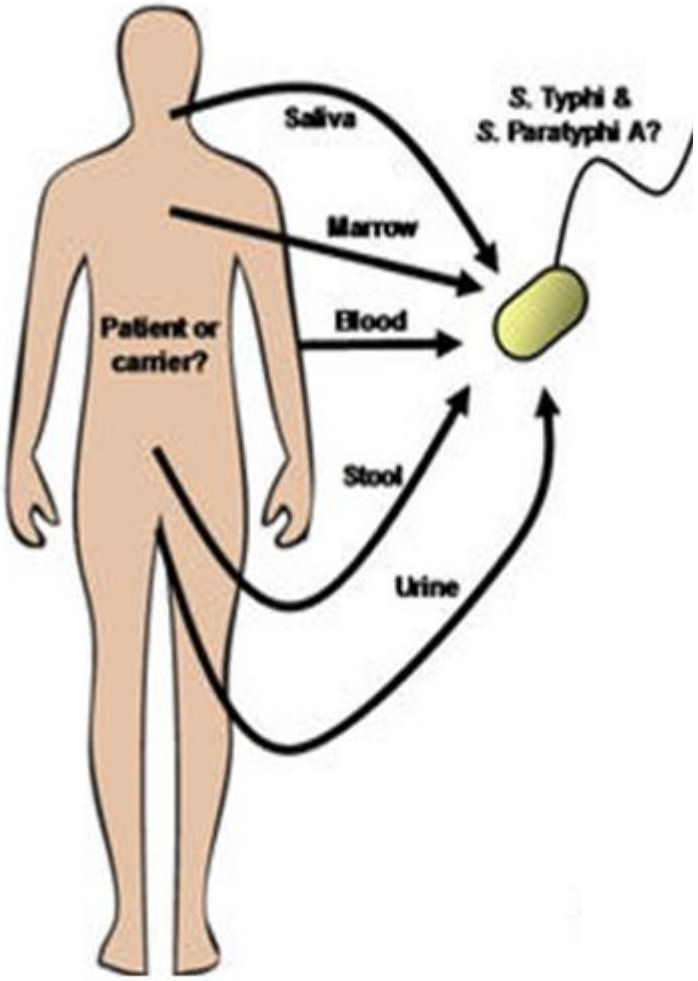
Diagnosis of Salmonella

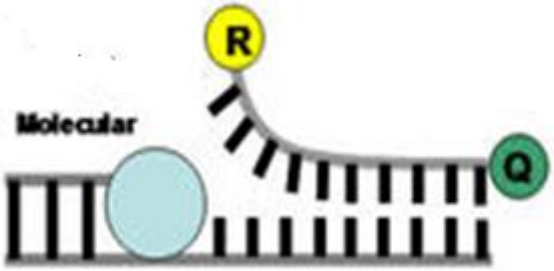
Which Sample?



Which Method?

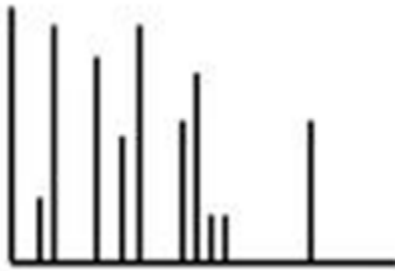
Microbiological

Serological



Molecular

Mass Spectrometry



Diagnosis of salmonellosis

Cultural properties

Grow easily on simple culture media and on selective and differential media that contain biliary salts and lactose.They grow On MacConkey's or Deoxycholate-citrate agar (DCA) medium, they produce pale yellow colonies being non lactose fermenters.

- **Salmonella growing on XLD agar; Xylose Lysine Deoxycholate agar is a selective growth medium used in the isolation of Salmonella (black dots) and Shigella species from clinical samples and from food**
- **Produce H2S, colonies have a "cat-eye" appearance.**

Media used for *Salmonella* isolation

1. **Enrichment cultures**
2. ***Salmonella* selective media**

Enrichment cultures

Enrichment cultures: The specimen (usually stool) is put into **selenite F** or **tetrathionate broth**, both of which inhibit replication of normal intestinal bacteria and permit multiplication of salmonellae. After incubation for 1–2 days, it is plated on differential and selective media.

Diagnosis of salmonellosis

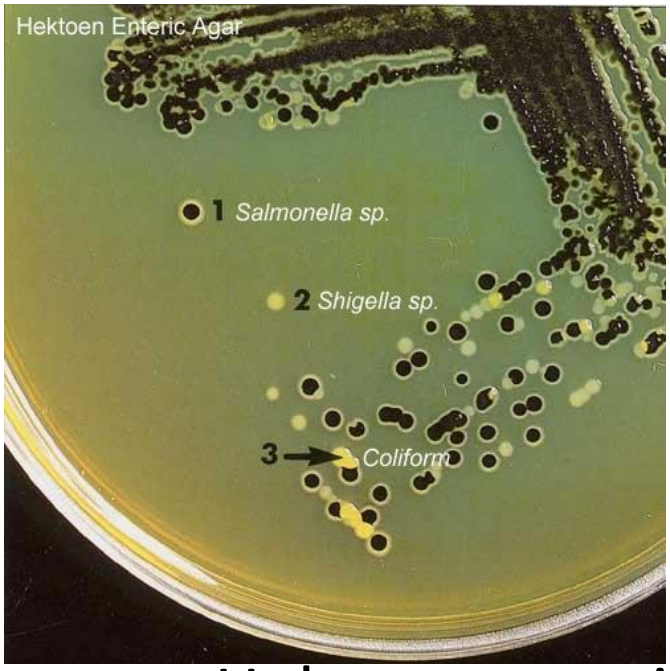
Salmonella selective media:

Favor growth of *salmonellae* and *shigellae* over other *Enterobacteriaceae* including

Salmonella-Shigella (SS) agar

Shigella: colorless colonies without black centers

Lactose fermenter flora: pink to red colonies



Hektoen enteric agar

Salmonella: colorless colonies with black centers

Principle of Hektoen enteric agar

Hektoen Enteric Agar (HE) is a selective and differential medium designed to isolate and differentiate members of the species Salmonella and Shigella from other Enterobacteriaceae.

Bile salts and the dyes bromothymol blue and acid fuchsin inihibit the growth of most Gram-positive organisms.	**Lactose, sucrose, and salicin** provide fermentable carbohydrates to encourage the growth and differentiation of enterics.	**Sodium thiosulfate** provides a source of sulfur.	**Ferric citrate**: H2S indicator

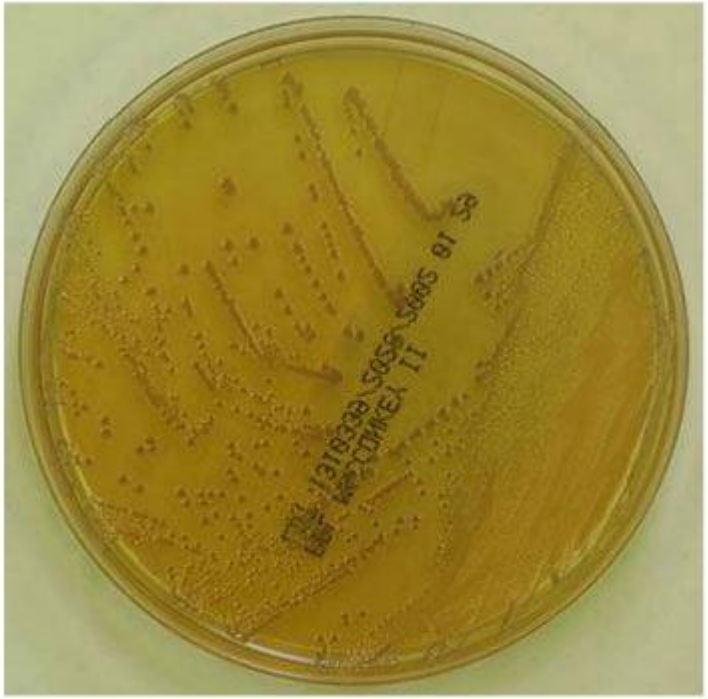
Enterics that ferment one or more of the carbohydrates will produce (**orange, yellow or salmon coloured colonies**). Non-fermenters will produce (**translucent colonies, light green or greenish blue**). Organisms that reduce sulfur to hydrogen sulfide will produce black colonies or blue-green colonies with a black center.

Diagnosis of salmonellosis

Suspected colonies from solid media are identified by biochemical reaction patterns

- **Motile**
- **Lactose negative; Lactose + means that the organism can use lactose as an energy source and produce acids, whilst lactose – means they cannot.**
  - **S. Typhi ferment glucose, mannitol and sorbitol to produce acid or acid and gas**
  - Some S. paratyphi ferments these with production of acid and gas
  - To differentiate between S paratyphi A and B;
    - A is H2S -& citrate –
    - B is H2S + & Citrate +
    - S paratyphi C, S. typhimurium and enteritides are similar to S. paratyphi B. To differentiate use serological testing (slide agglutination test
- **Indole test negative**
- **Methyl red test: positive**
- **Voges-Proskauer test: negative;**
- **Citrate :positive (growth on Simmon's citrate agar)**
- **Urease :negative**

Lactose test



lactose negative organism
growing on
MacConkey agar

Escherichia coli growing on MacConkey agar.

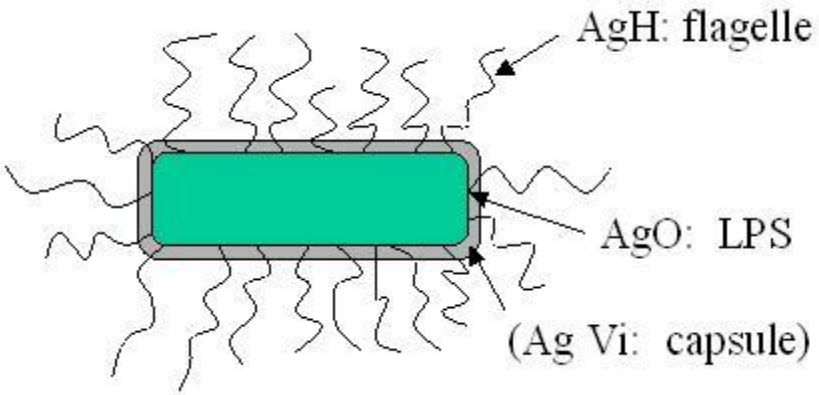
Diagnosis of salmonellosis

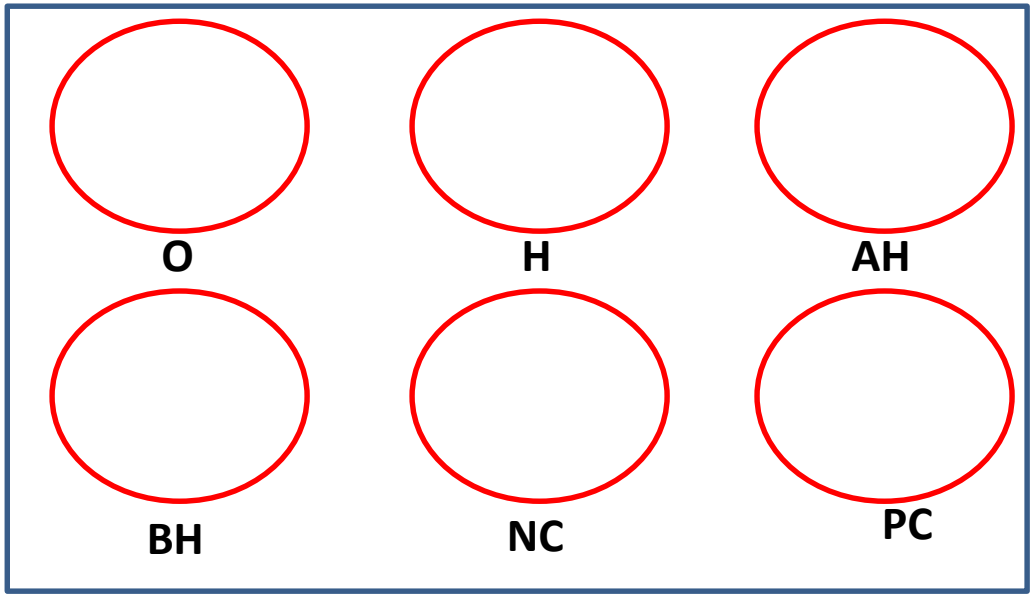
Serologic Methods (Widal test)

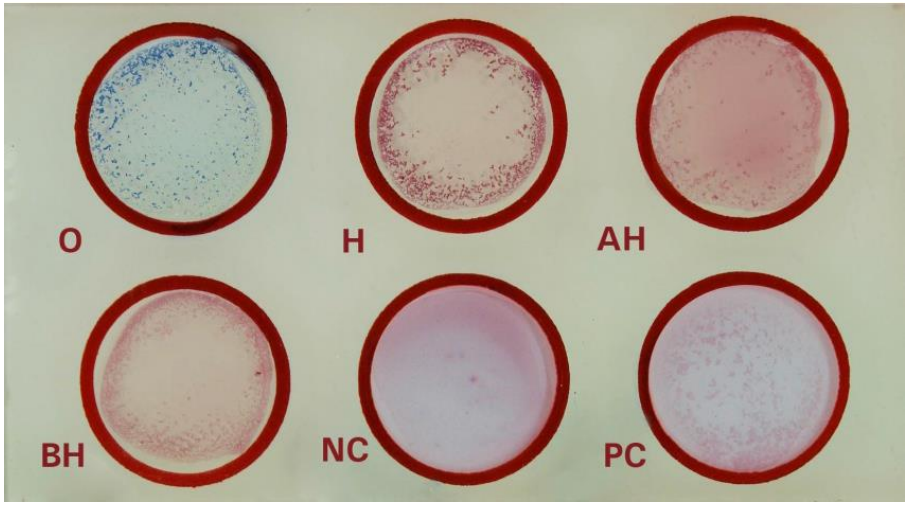
- Principle: Patients' suffering from enteric fever would possess antibodies in their sera against *S. t*yphi O antigen, *S. t*yphi H antigen and *S. p*aratyphi AH antigen and *S. p*aratyphi BH antigen which can be detected by slide widal test. 2 circles as controls.
- Procedure: One drop each of undiluted patients' serum samples are placed on the circled card and one drop of each of the four Salmonella antigens are added separately and gently rotated for one minute. Appearance of agglutination gives qualitative results

Diagnosis of salmonellosis

Serologic Methods (Widal test)







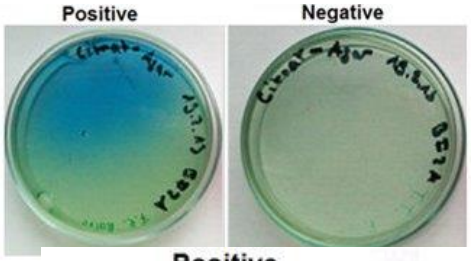
Citrate Utilization Test

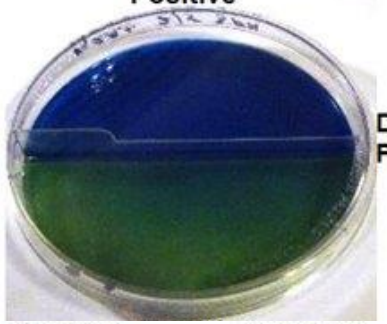
- **Use**: to determine bacterial ability to use citrate as the sole source of carbon.
- **Culture medium**: **Simmons citrate agar**; contains source of citrate & pH indicator bromthymol blue (**neutral; green** & **alkaline; blue**).
- **Principle**: citrate use → ammonia production → alkaline pH.
  - **Results**:
  - 1- **Positive**: The usual colour change is from **green (neutral)** to **blue (alkaline).**
  - 2- **Negative**: No growth, colour remains green.
- **Important citrate-positive bacteria**:

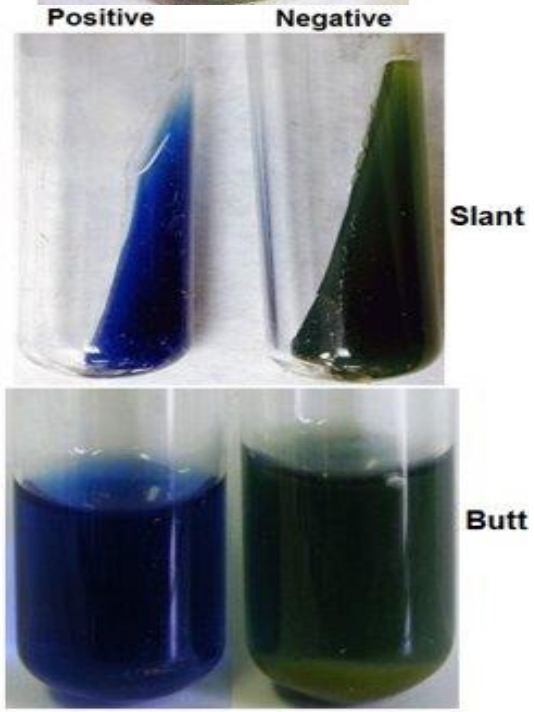
1- Klebsiella sp.

2- Citrobacter sp.

3- Proteus sp.







Urea Hydrolysis

- **Use**: to determine bacterial ability to hydrolyze urea (by urease enzyme) into CO_2 & ammonia which alkalinizes the medium.
- **Culture medium**: **Christensen's urea agar** or **Stuart's urea broth**: both contain urea, & phenol red indicator.
- **Method**:
- 1- Streak agar surface with portion of well-isolated colony or inoculate urea broth with 1-2 drops from overnight enrichment broth.
- 2- Leave cap on loosely & incubate at 35°C.
- **Results**:
- 1- **Positive**: enzyme present, ammonia produced, high pH (**bright pink colour**).
- 2- **Negative**: enzyme absent, NO colour change (**yellow orange**).
- **Important urease-positive bacteria**:
- Proteus sp.
- Helicobacter sp.

Indole Test

- **Use**: to determine bacterial ability to degrade amino acid tryptophan (by tryptophanase enzyme) into indole (+ Kovac's reagent (yellow) indicator) → red colour.
- **Method**:

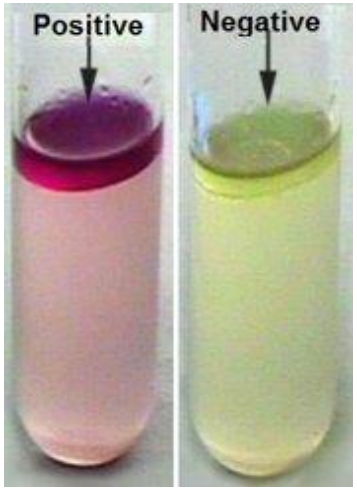
1- Inoculate tryptophane broth with 1-2 drops from overnight bacterial enrichment broth.

2- Incubate at 35°C.

3- Add 0.5 mL (5-10 drops) of Kovac's reagent.

- **Results**:
- **Positive**: enzyme present, indole produced, red ring on top of broth e.g. **E.coli.**
- **Negative**: enzyme absent, indole NOT produced, NO colour change or clear yellow ring e.g. **Klebsiella sp., Enterobacter sp., Salmonella sp.**

Kovac's Reagent



Methyl Red (MR) & Voges-Proskauer (VP) Tests

- **Use**:

1- **MR tests** for **acids production** from glucose fermentation.

2- **VP tests** for **acetoin production** from glucose fermentation.

- **Culture media**: MRVP Glucose Broth, & **Reagents**:

1- Methyl Red indicator for acids produced using **mixed acid fermentation pathway** using pyruvate as a substrate.

2- VP indicators (5% Alpha-naphthol & potassium hydroxide) for acetoin production using **2,3-butanediol fermentation pathway**.

- **Method**:

1- Inoculate tube aseptically with inoculating loop.

2- Incubate at 35°C for 48 hours of incubation.

3- Separate bacterial broth into 2 separate tubes.

4- Add few drops of MR to one tube.

5- Add both VP reagents to the other tube, shake vigorously then allow to sit for 5-10 minutes.

Methyl Red (MR) & Voges-Proskauer (VP) Tests Results

MR

- **Positive**: acids, pH <4.2, red
- **Negative**: NO acids produced, pH >6.2, yellow.

Negative Positive

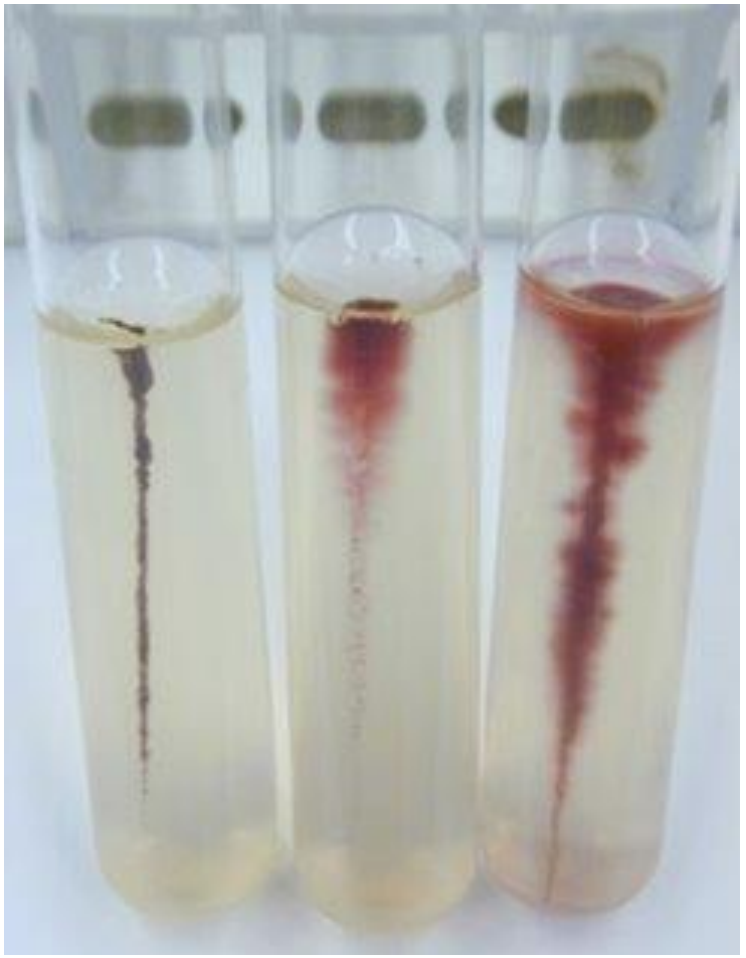
VP

- **Positive**: acetoin present, red.
- **Negative**: acetoin absent, NO colour change.

Positive Negative

Motility test

- Motility in semisolid agar: Positive (motile); fuzzy growth feathering away from stab line creating cloudy appearance & Negative (nonmotile); growth strictly along stab line.



Yersinia pestis

Diagnosis

Acceptable Specimen Types .

- Bronchial wash/tracheal aspirate (≥ 1 ml) .
- Whole blood: 5-10 ml blood in EDTA, and/or Inoculated blood culture bottle .
- Aspirate or biopsy of liver, spleen, bone marrow, lung, or bubo

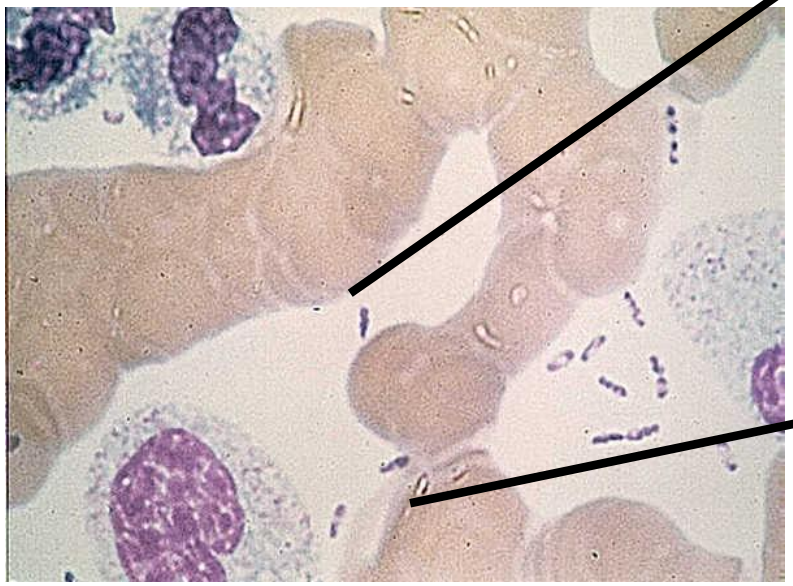
Diagnosis

- Blood and bubo aspirates and sputum should be Giemsa stained. Smears typically show the bacillus to have a **bipolar or "safety pin" appearance.**
- Send smears to a reference lab for fluorescent antibody microscopy.
- Most Gram-negative bacteria produce colonies within 24 h*; Y. pestis* do not. Because Cultures grow slower (1.25 hours/generation time) than other bacteria and thus require longer incubation times for optimal growth

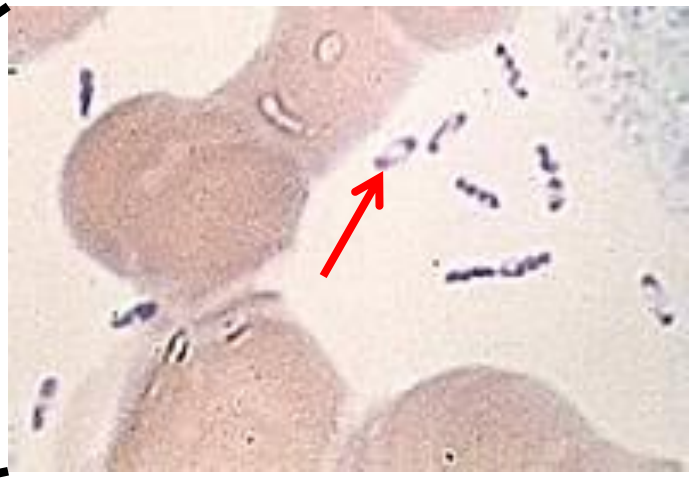
Diagnosis

Staining pattern

Gram-negative rods (0.5 - 0.8 x 1- 3 µm) Bipolar staining (resembling closed safety pin) may be evident with Gram stain but more apparent with Giemsa stain



Giemsa staining



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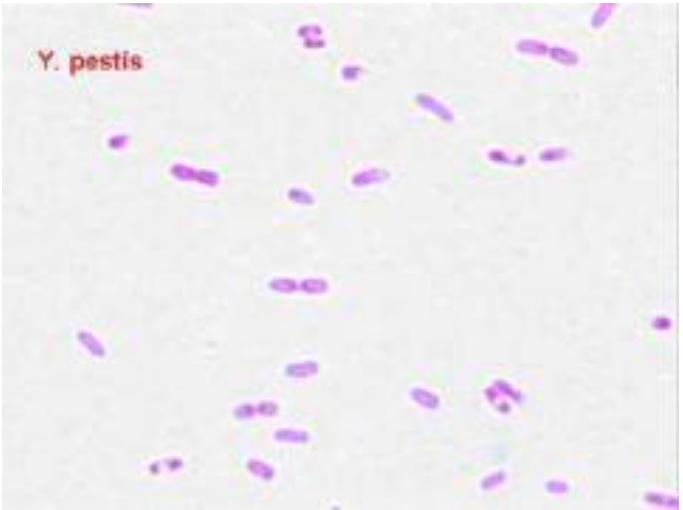


Gram staining

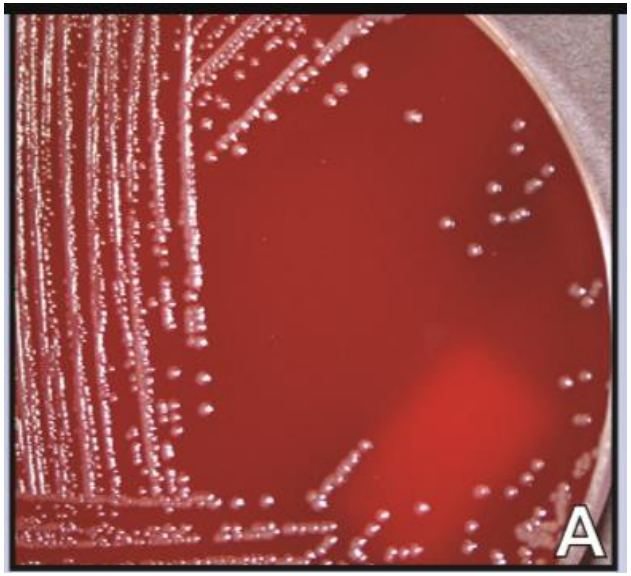
Diagnosis

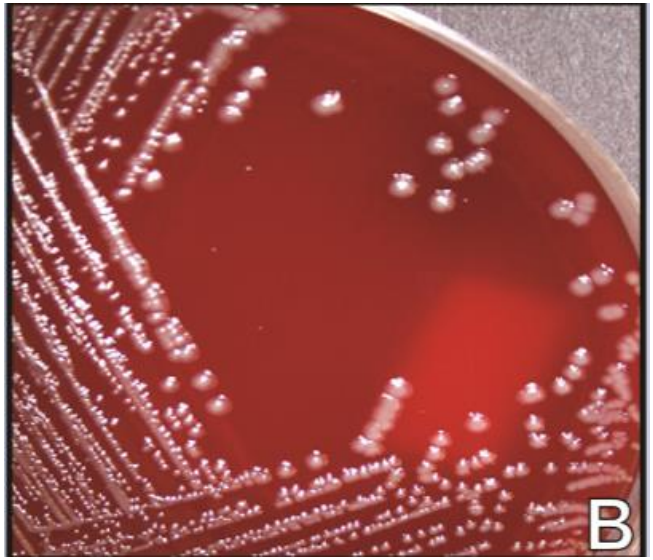
Colony Morphology

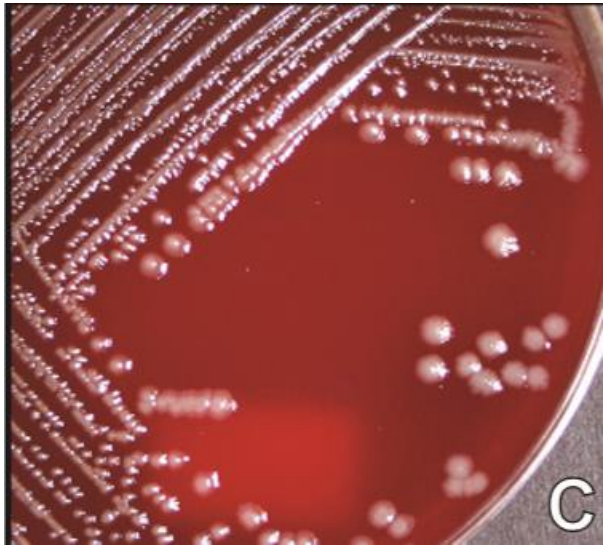
- Grey-white translucent colonies on Blood Agar (BA) and Chocolate Agar (CA) at ambient and 35/37^{o}C (growth faster at 28^{o}C).
- “Fried egg” appearance on BA in older cultures

Diagnosis

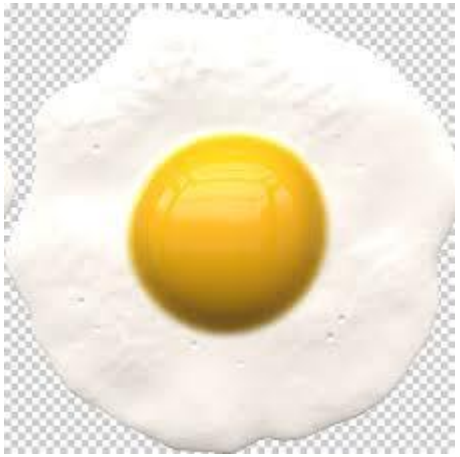
Colony Morphology

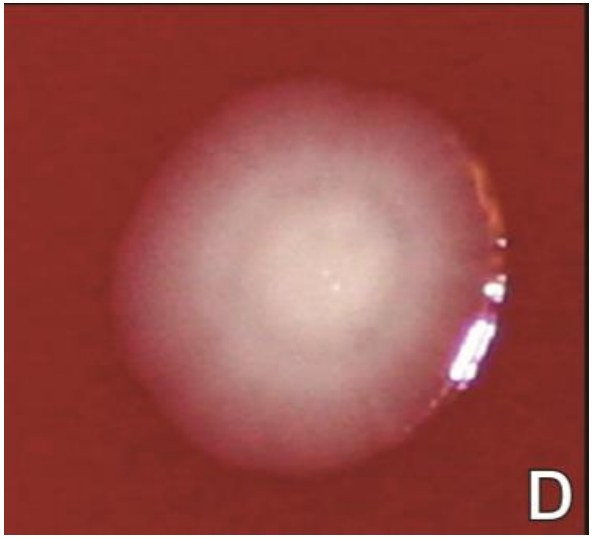






Yersinia pestis growth on BA at (A) 48 h, (B) 72 h, (C) 96 h, (D) 96 h "Fried egg"

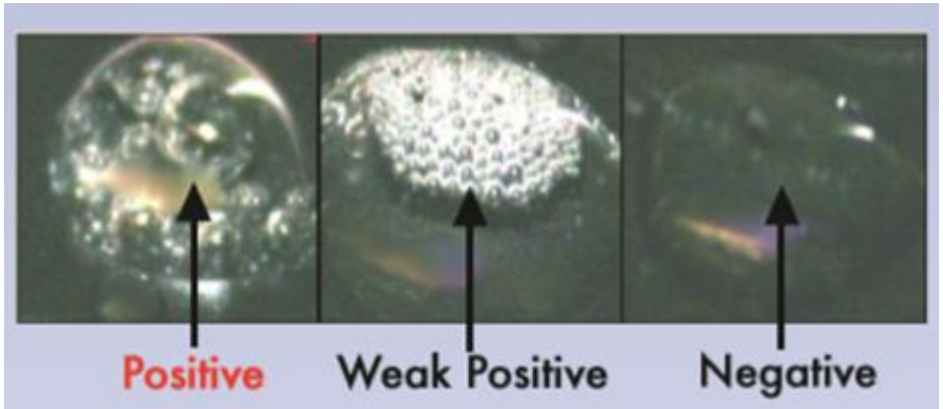




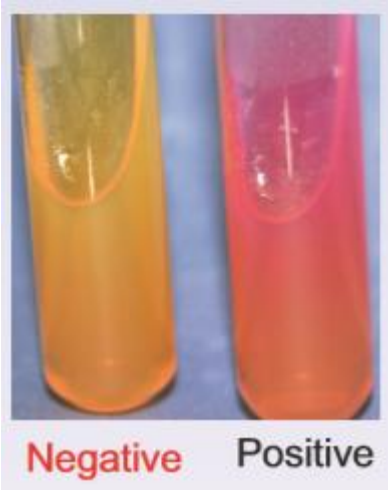
Diagnosis

Additional Lab Identification

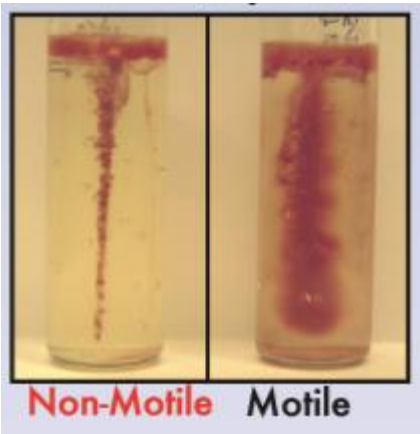
Catalase: positive



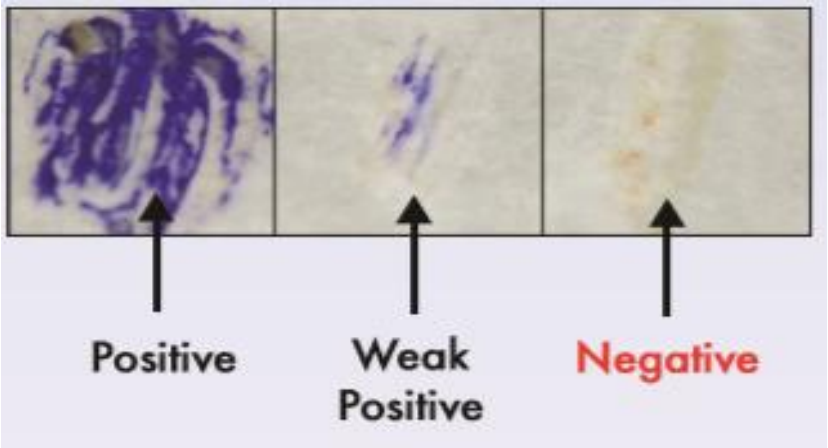
Urease: negative



Motility: nonmotile



Oxidase: negative Indole: negative



Oxidase Test

- **Use**:
- To determine **aerobic** bacteria's ability to produce cytochrome c oxidase enzyme (electron transport chain)

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- **Principle**:
- Oxidation of a substrate to **indophenol** , a dark purple colored end product .
- Results:
- 1- Positive: enzyme present & substrate oxidized to end-product indophenol (dark purple colour).
- 2- Negative: enzyme absent & substrate remains reduced (No colour).

Catalase Test

- **Use**: to detect bacterial catalase enzyme which catalyzes breakdown of ***hydrogen peroxide (H_2O_2)*** into ***water (H_2O)*** & ***↑O_2 oxygen***.

Results:

1- **Positive**: immediate or rapid copious bubbles

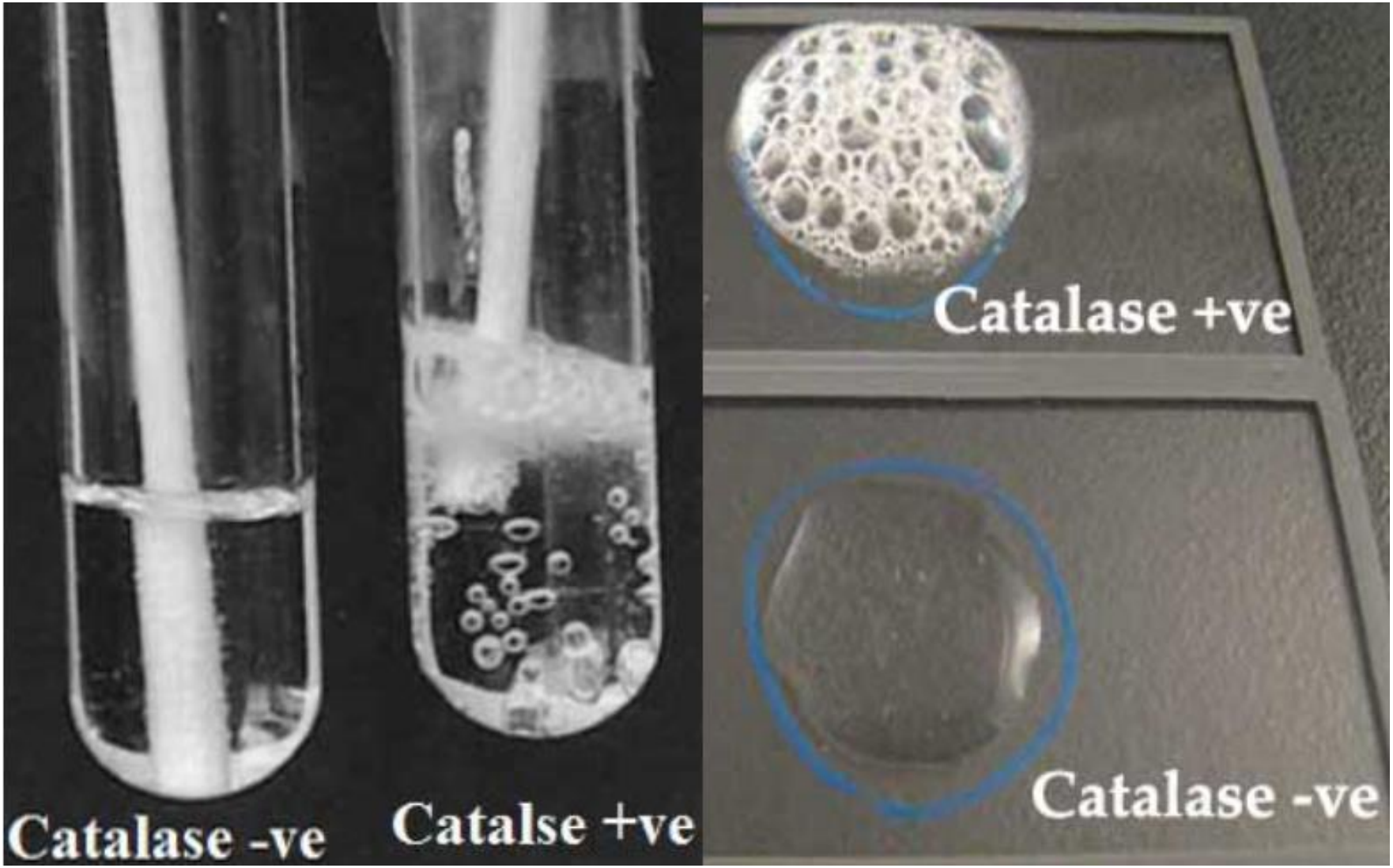
2- **Negative**: NO or slow few bubbles (***Strep spp***.).

Warning:

1- Do NOT do test on blood agar as RBCs contain catalase enzyme → False-positive result.

2- Enterococci produce peroxidase which slowly catalyzes breakdown of H2O2 → False positive (weakly positive).

Catalase Test



Diagnosis

Grey-white translucent, non-hemolytic colonies on BA or CA (24 h), Yellow and opaque (48 h).

↓

Gram-negative rods bipolar staining (closed safety pin)

↓

*Catalase: positive *Motility: nonmotile
* Urease: negative *Oxidase: negative * Indole: negative

No
Continue laboratory identification procedure

Yes
Immediatelly notify the physician to treat and to take the the proper isolation precautions

Brucellosis

Brucellosis

Specimen collection, transport, and processing

- A definitive diagnosis of brucellosis requires isolation of the organisms in cultures of blood, bone marrow, CSF, pleural and synovial fluids, urine, abscesses, or other tissues.
- If processing will be delayed, the specimen may be held in the refrigerator.

Brucellosis

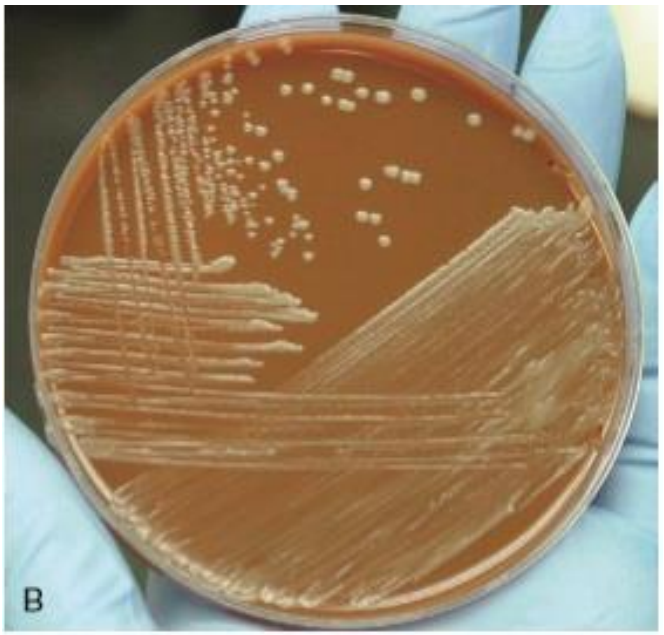
Direct detection methods

- Conventional and real-time polymerase chain reaction (PCR) assays are reliable and specific means of directly detecting Brucella organisms in clinical specimens.

Brucellosis

Cultivation

- Brucella can grow on blood and chocolate agars
- More enriched agars including Brucella agar or infusion base agar are used to isolate *Brucella*
- All subculture plates should be held for a minimum of 7 days.
- On culture, colonies appear small, convex, smooth, translucent, nonhemolytic, and slightly yellow and opalescent after at least 48 hours of incubation
- Brucella spp. are catalase and urease positive, and most strains are oxidase positive



Brucellosis

Serologic test

- Is widely used (e.g., serum agglutination test [SAT] or microplate agglutination [MAT]) because isolating brucellae is difficult
- A titer of 1 : 160 or greater in the SAT is considered diagnostic if this result fits the clinical and epidemiologic findings.

Diagnosis Q fever

- Serology (rise in titer)
  - IFA, CF, ELISA, microagglutination
  - The indirect IFA is the most dependable and widely used method.
- DNA detection methods
  - PCR
- Isolation of organism
  - Risk to laboratory personnel
  - Rarely done