HLS
Practical

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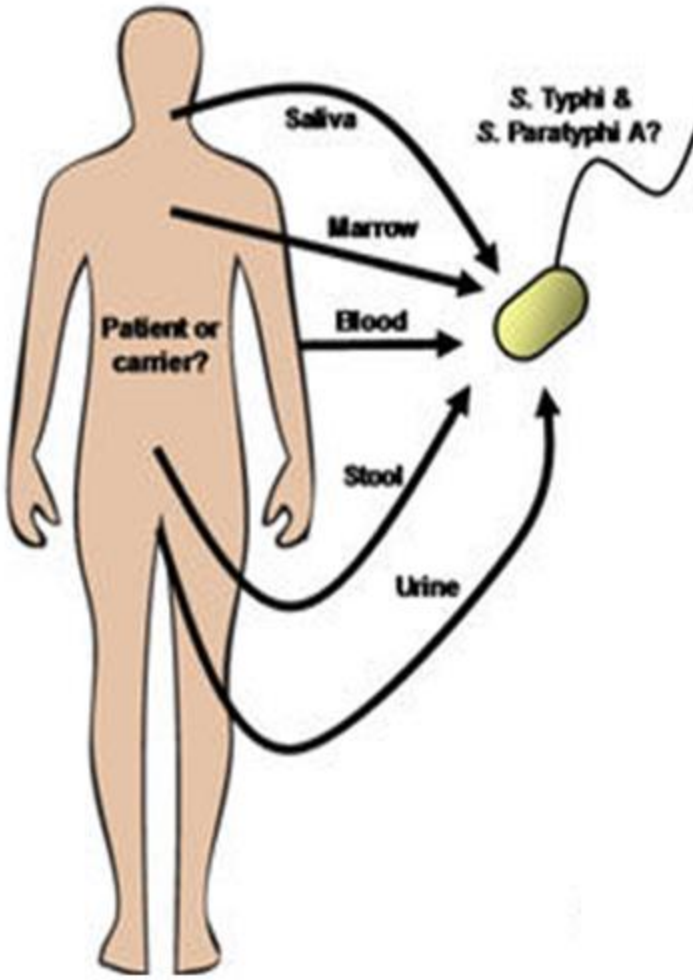
Diagnosis of Salmonella

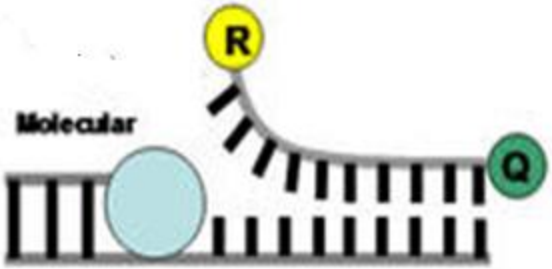
Which Sample?



Which Method?

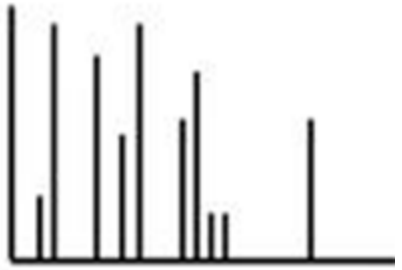
Microbiological

Serological



Molecular

lass Spectrometry



Diagnosis of salmonellosis

Cultural properties

- Grow easily on simple culture media and on selective and differential media that contain biliary salts and lactose.

Media used for *Salmonella* isolation

1. Enrichment cultures
2. *Salmonella* selective media

- **Enrichment cultures**

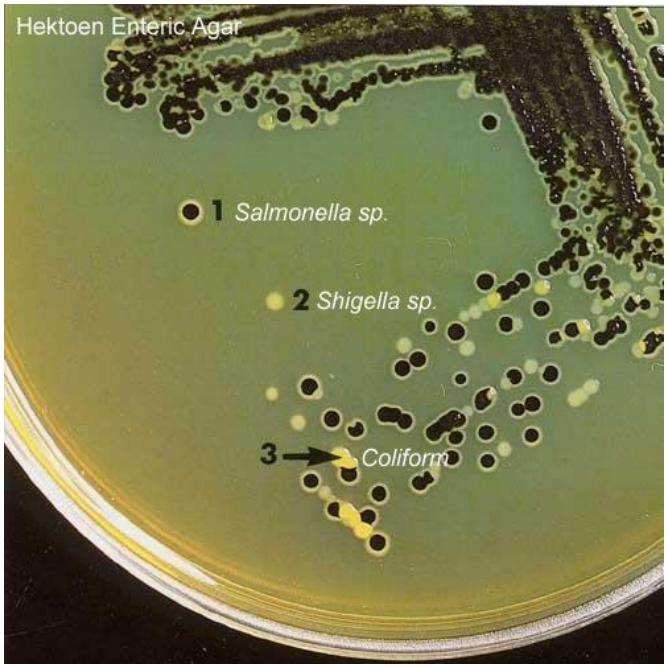
Enrichment cultures: The specimen (usually stool) also is put into **selenite F** or **tetrathionate** broth, both of which inhibit replication of normal intestinal bacteria and permit multiplication of salmonellae. After incubation for 1–2 days, this is plated on differential and selective media.

Diagnosis of salmonellosis

Salmonella selective media:

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

1. Salmonella-Shigella (SS) agar
2. Hektoen enteric agar



Shigella: colorless colonies without black centers

Salmonella: colorless colonies with black centers

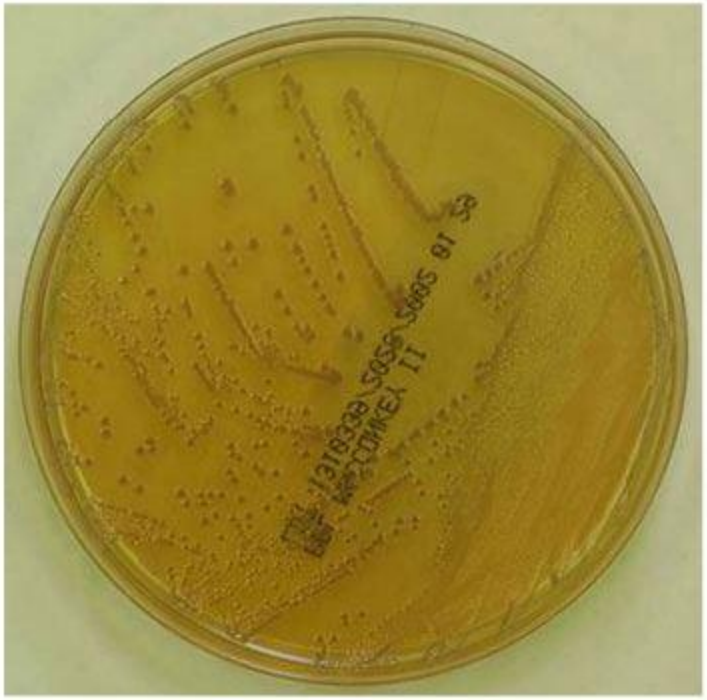
Lactose fermenter flora: pink to red colonies

Diagnosis of salmonellosis

Suspected colonies from solid media are identified by biochemical reaction patterns

- **Motile**
- **Lactose negative**
- **acid from glucose, mannitol, maltose, and sorbitol.**
- **Indole test negative**
- **Methyl red test positive**
- **Voges-Proskauer test negative**
- **Urease negative**

Lactose test



lactose negative organism growing on MacConkey agar

Escherichia coli growing on MacConkey agar.

Diagnosis of salmonellosis

S. typhi

Glucose	Lactose	Maltose	Mannite	Sucrose	Indole	MR	VP	Citrate	urease	H2S
A	_	A	A	_	_	+	_	_	_	+

S. paratyphi A

Glucose	Lactose	Maltose	Mannite	Sucrose	Indole	MR	VP	Citrate	urease	H2S
A/G	_	A/G	A/G	_	_	+	_	_	_	_

S. paratyphi B

Glucose	Lactose	Maltose	Mannite	Sucrose	Indole	MR	VP	Citrate	urease	H2S
A/G	_	A/G	A/G	_	_	+	_	+	_	+

A/G produce Acid and Gas
A produce Acid only

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

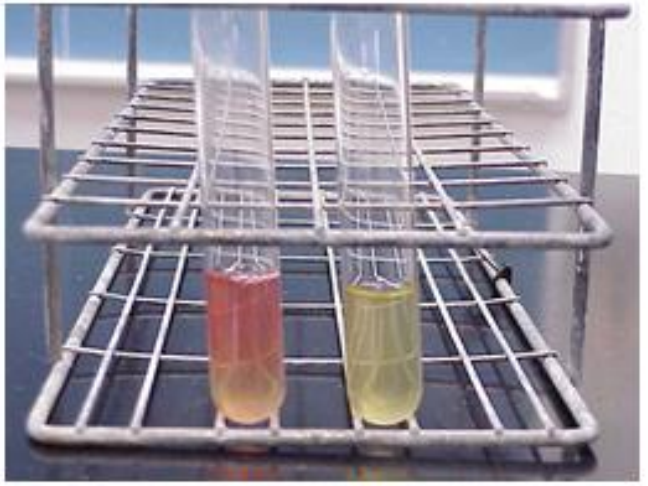
MR

- **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

Citrate Utilization Test

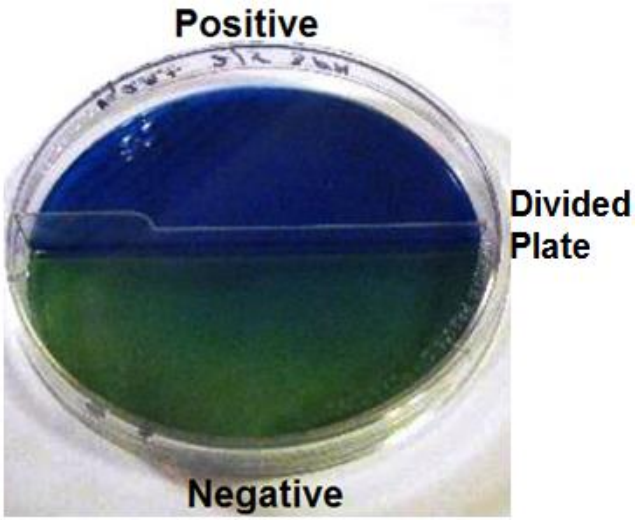
- **Use**: to determine bacterial ability to use citrate as the sole source of carbon.
- **Culture medium**: **Simmons citrate agar**; contains source of citrate & pH indicator bromthymol blue (**neutral; green** & **alkaline; blue**).
- **Principle**: citrate use → ammonia production → alkaline pH.

Citrate Utilization Test

- **Results**:

1- **Positive**: The usual colour change is from **green (neutral)** to **blue (alkaline).**

2- **Negative**: No growth, colour remains green.

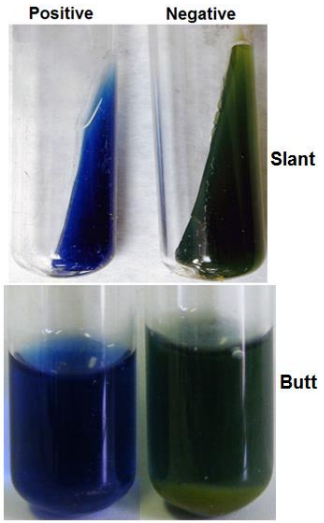


- **Important citrate-positive bacteria**:

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.

Urea Hydrolysis

- **Results**:

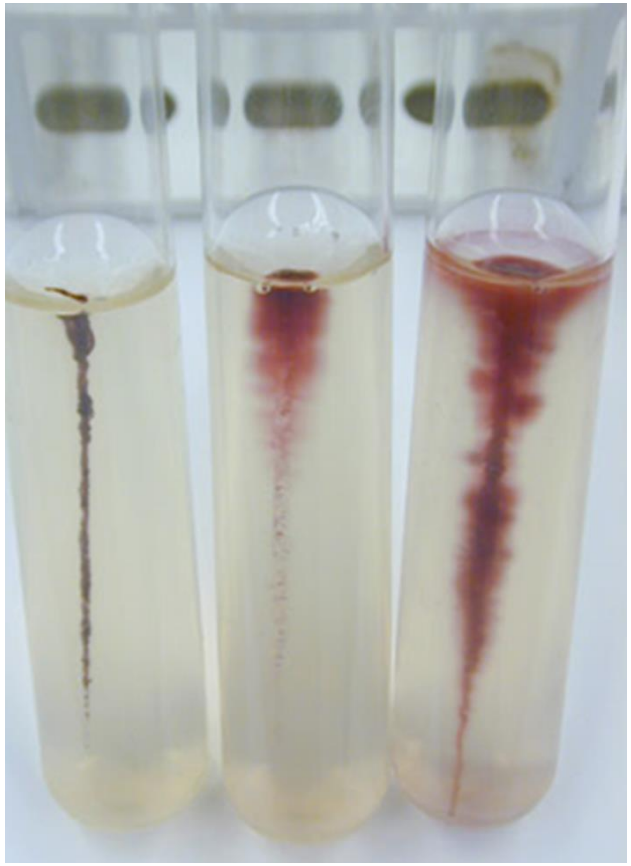
1- **Positive**: enzyme present, ammonia produced, high pH (**bright pink colour**).

2- **Negative**: enzyme absent, NO colour change (**yellow orange**).

- **Important urease-positive bacteria**:
- Proteus sp.
- Helicobacter sp.

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.

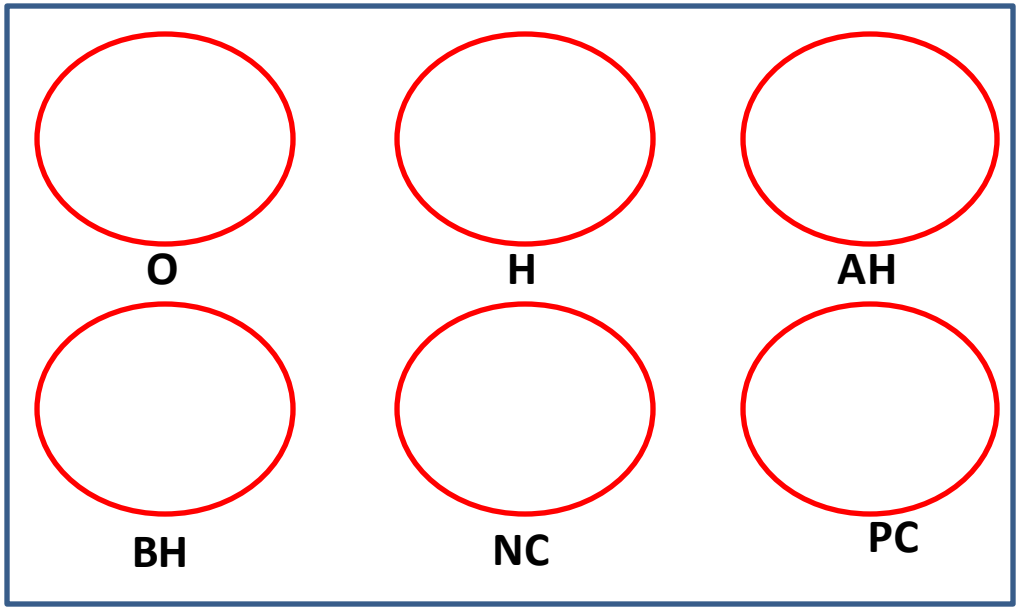


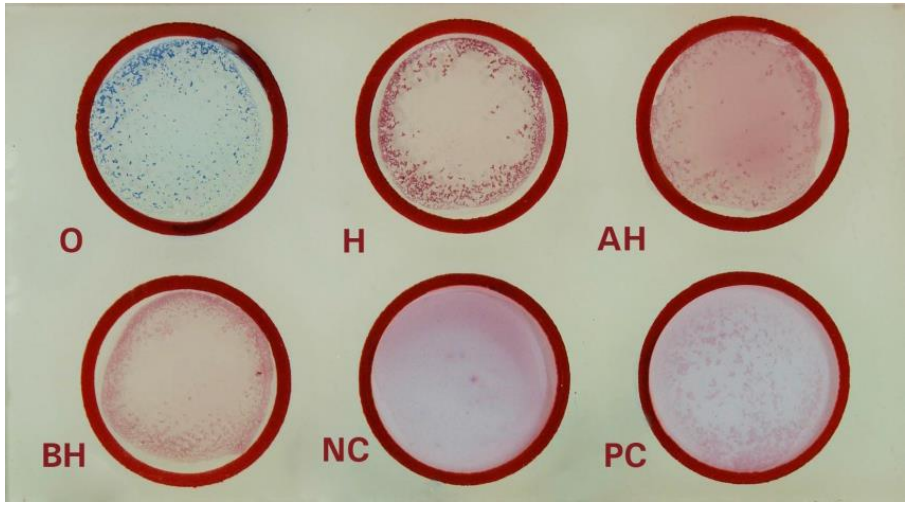
Diagnosis of salmonellosis

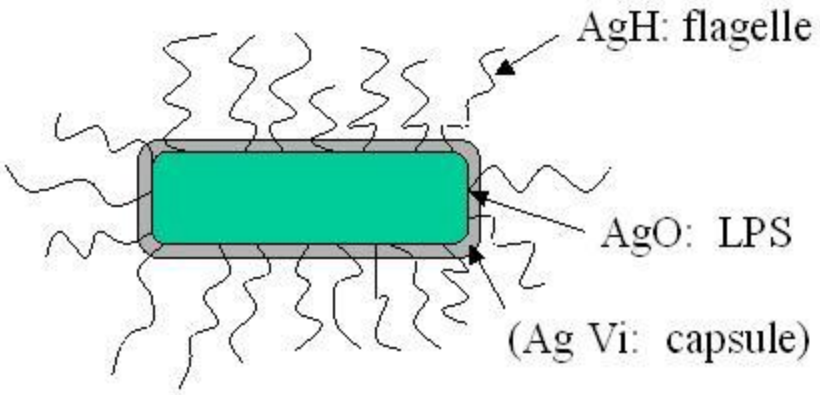
Serologic Methods (Widal test)

Slide agglutination tests with specific sera.

Serologic techniques are used to identify unknown cultures with known sera **and may also be used to determine antibody titers in patients with unknown illness**







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 A H antigen and *S. p*aratyphi BH antigen which can be detected by slide widal test.
- Procedure: One drop each of undiluted patients' serum samples for the four antigens 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

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

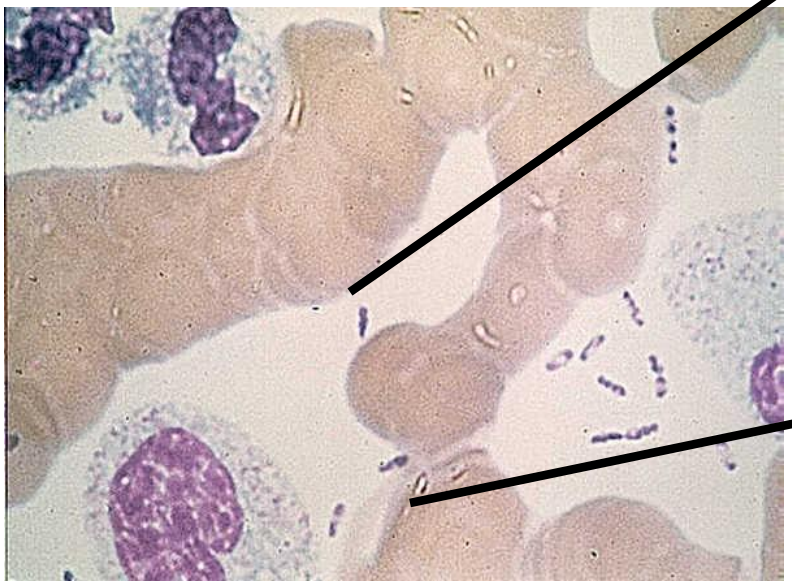
Diagnosis

- 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

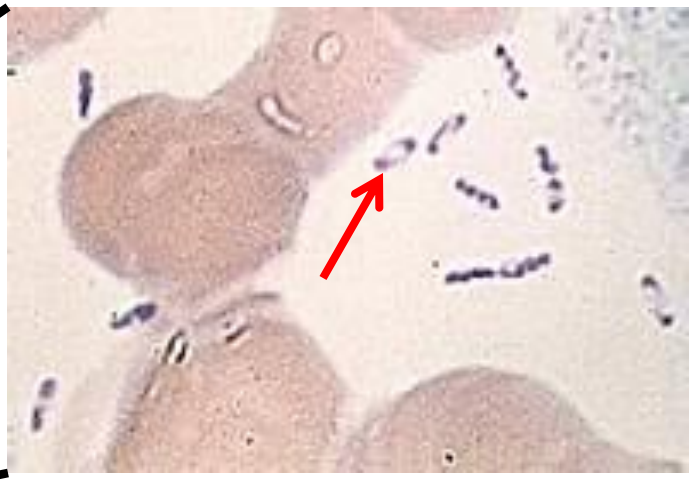
Diagnosis

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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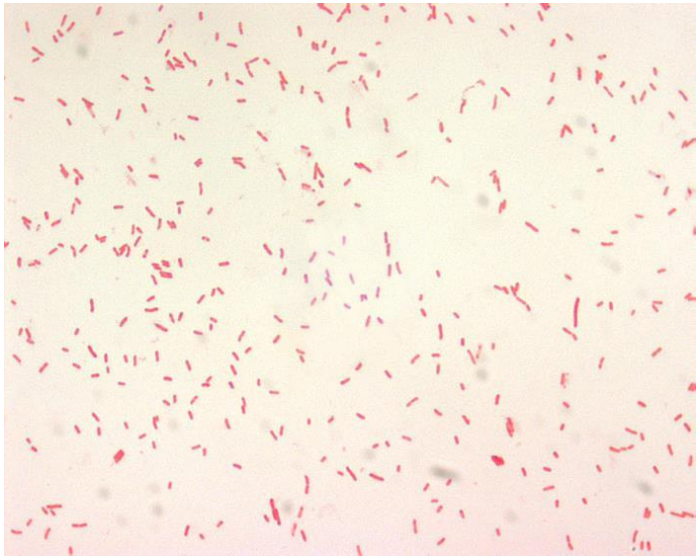


Gram staining

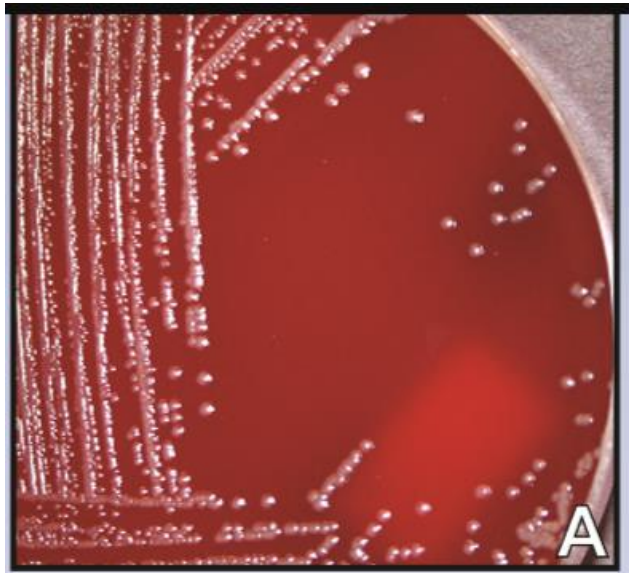
Diagnosis

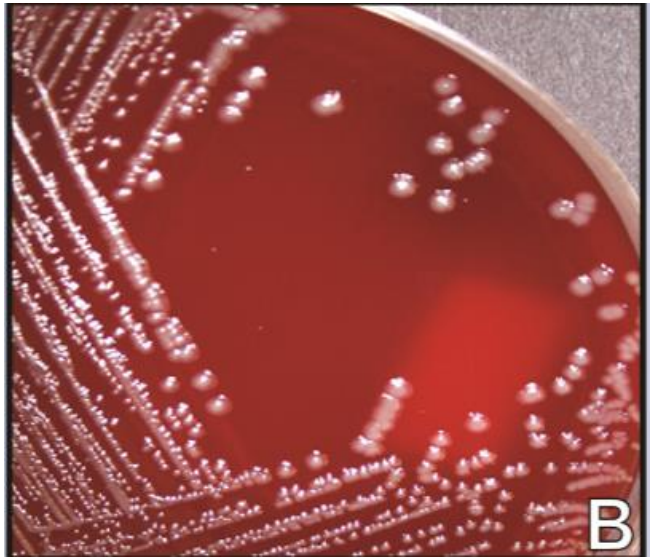
Colony Morphology

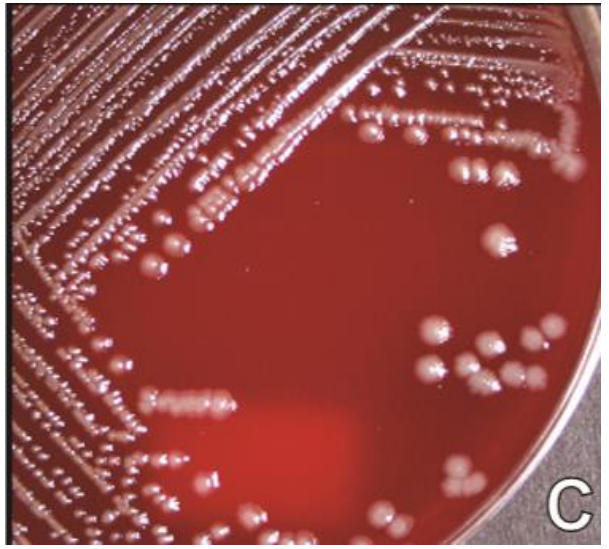
- Grey-white translucent colonies on Blood Agar (BA) and Chocolate Agar (CA) at ambient and 35/37^{o}C (growth faster at 28^{o}C).
- “Fried egg” appearance on BA in older cultures

Diagnosis

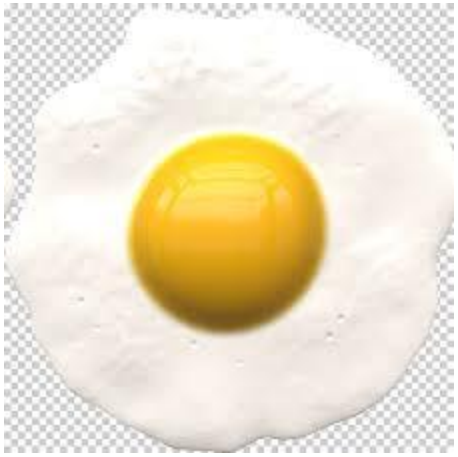
Colony Morphology

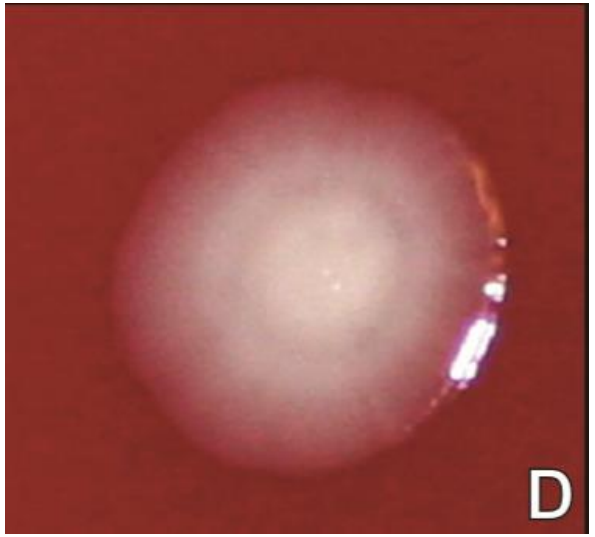






Yersinia pestis growth on BA at (A) 48 h, (B) 72 h, (C) 96 h, (D) 96 h "Fried egg"

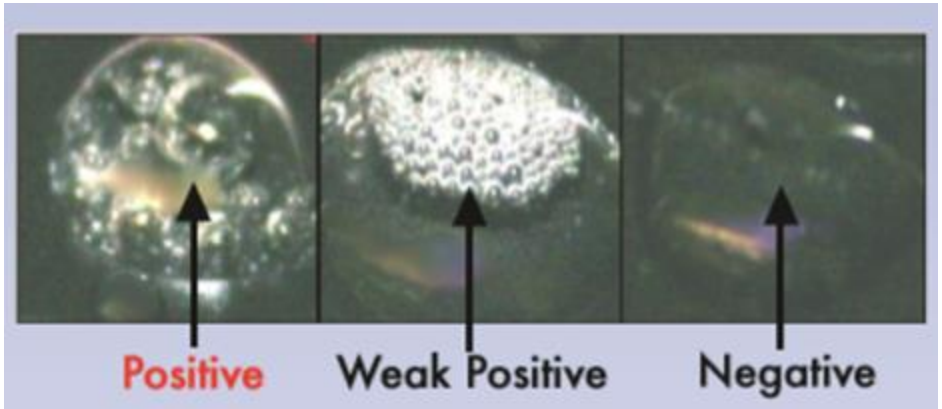




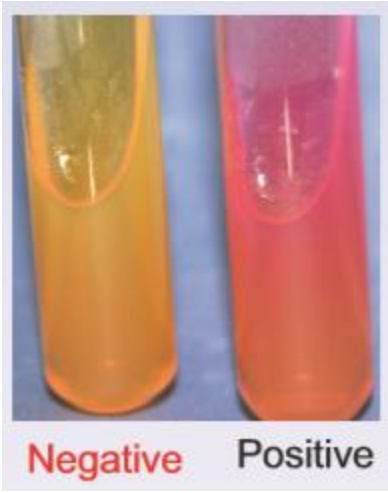
Diagnosis

Additional Lab Identification

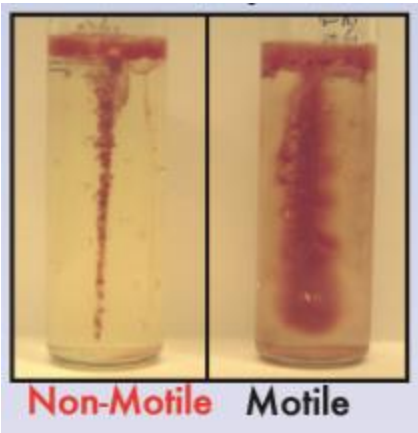
Catalase: positive



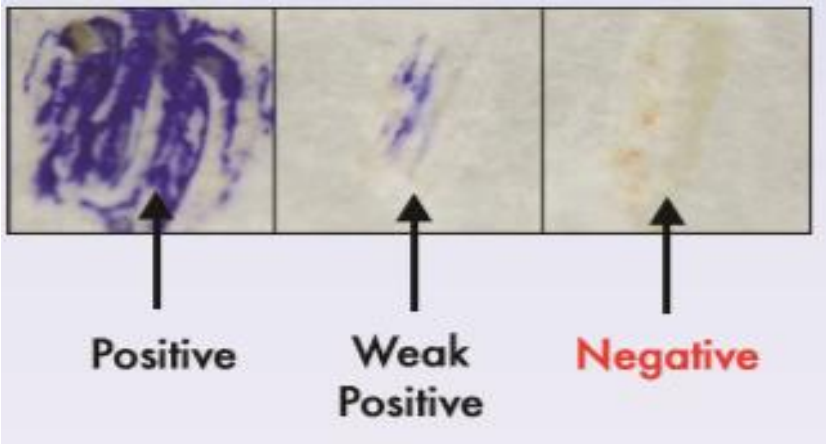
Urease: negative



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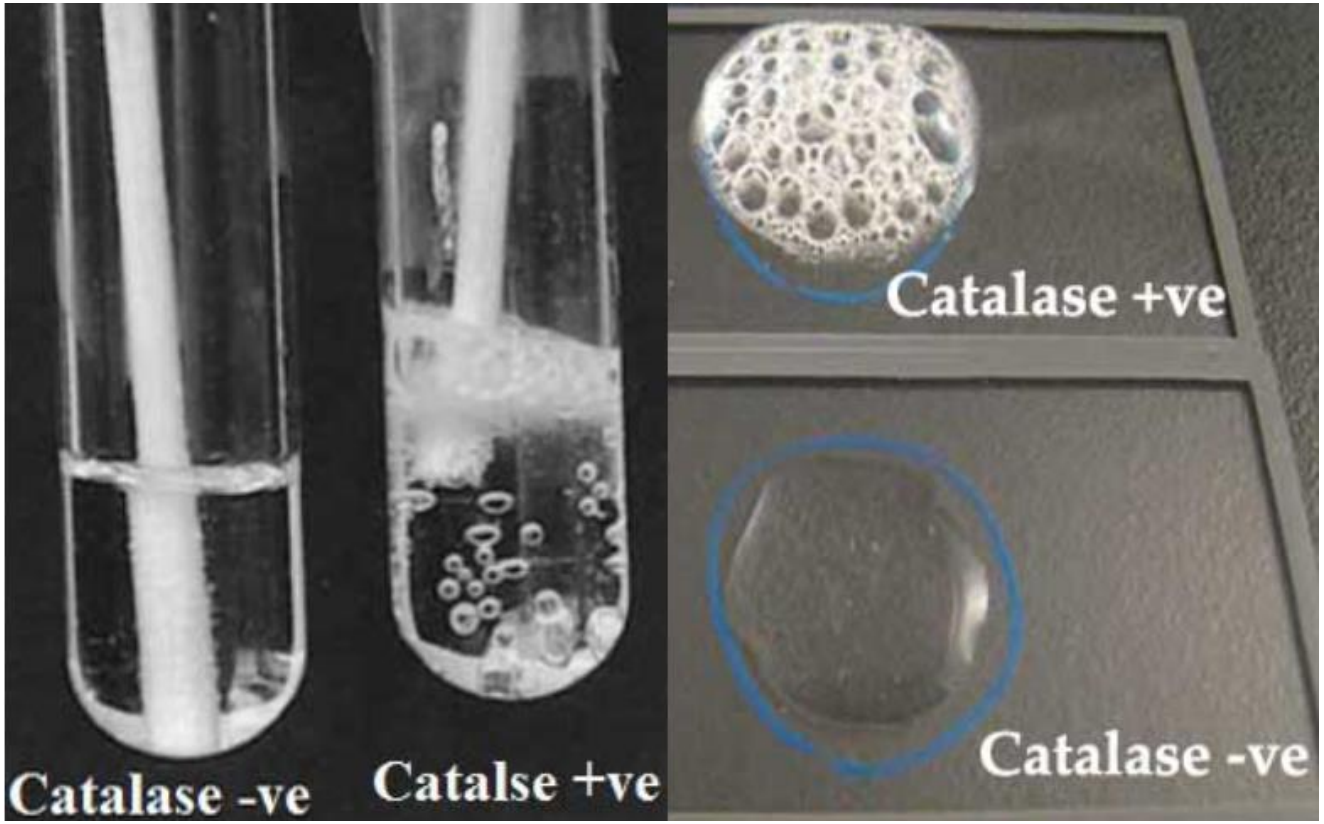
- **Principle**:
- Oxidation of a substrate to **indophenol** , a dark purple colored end product .
- Results:
- 1- Positive: enzyme present & substrate oxidized to end-product indophenol (dark purple colour).
- 2- Negative: enzyme absent & substrate remains reduced (No colour).

Catalase Test

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



Catalase Test

- **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 H_2O_2 → False positive (weakly positive).

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

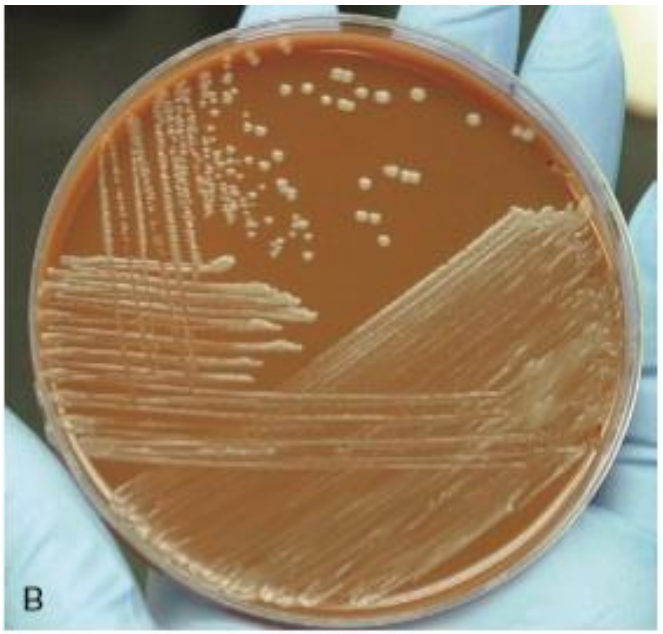
Specimen collection, transport, and processing

- 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

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.

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.

Diagnosis Q fever

- Serology (rise in titer)
  - IFA, CF, ELISA, microagglutination
- DNA detection methods
  - PCR
- Isolation of organism
  - Risk to laboratory personnel
  - Rarely done