



دانشگاه اصفهان

به نام خدا

دانشکده علوم و فناوری های زیستی، گروه سلولی و مولکولی و میکروبیولوژی،  
آزمایشگاه میکروبیولوژی

## آزمایشگاه باکتری شناسی ۲

بررسی خصوصیات ماکروسکوپی و میکروسکوپی و انجام آزمون های  
بیوشیمیایی جهت شناسایی باکتری های سالمونلا و شیگلا

تهیه کننده: سهیلا عباسی

# Salmonella

- Causes Infections in Humans and vertebrates,
- Enteric Fever ( Typhoid fever )
- Gastroenteritis
- Septicemias,
- **Carrier state a concern**

# Bacteriology –Typhoid fever

- The Genus Salmonella belong to Enterobacteriaceae
- Facultative anaerobe
- Gram negative bacilli
- Distinguished from other bacteria by Biochemical and antigen structure



# Salmonella

- A Very complex group
- Contains more > 2,000 spp
- Typed on the basis of Serotyping, and species typing
- Divided into two groups
  - 1 Enteric fever group
  - 2 **Food poisoning group – Septicemias.**

# Key points

- There are more than 2000 different antigenic types of Salmonella; those pathogenic to man are serotypes of *S. enterica*.
- Most serotypes of *S. enterica* cause food-borne gastroenteritis and have animal reservoirs.
- *S. enterica* serotypes Typhi and Paratyphi cause typhoid fever.

# Enteric Fever

## Typhoid Fever

- **Caused by Salmonella typhi, and other Groups called as Paratyphoid A, B, C**
- Salmonella typhi - Causes Typhoid
- Salmonella Paratyphi A,B,C Causes Paratyphoid fevers.
- Food Poison group
- Spread from Animals – Humans
- Causes Gastroenteritis – Septicemias, Localized Infection



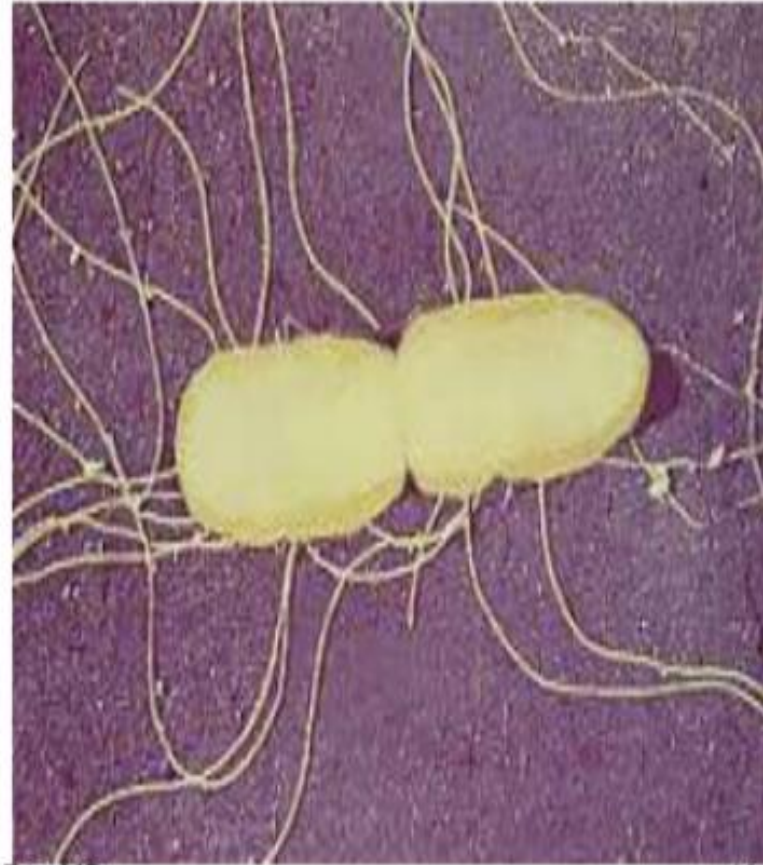
# Typhoid Mary



- A famous example is “Typhoid” Mary Mallon, who was a food handler responsible for infecting at least 78 people, killing 5. These highly infectious carriers pose a great risk to public health.

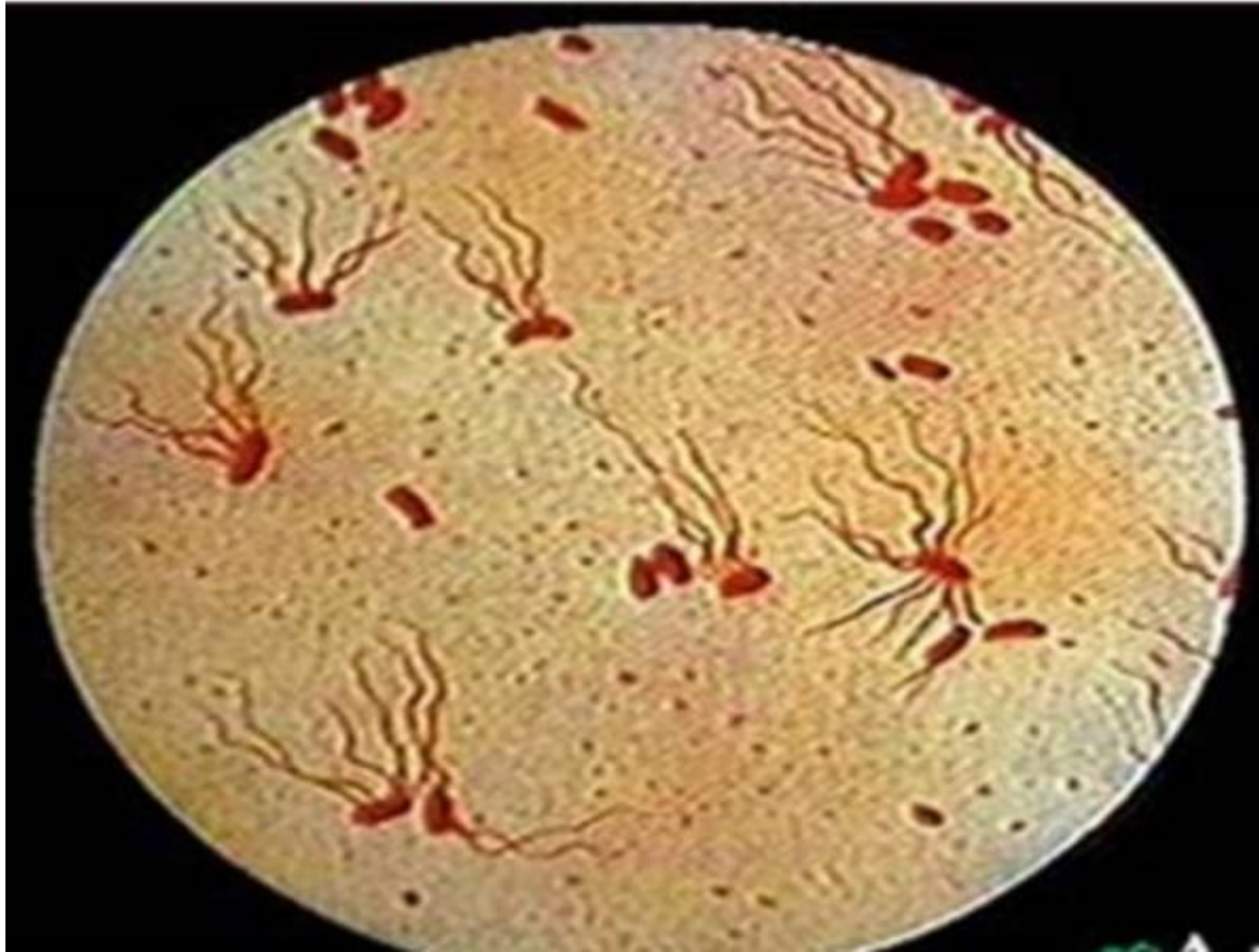
# Morphology of Salmonella

- Gram negative bacilli
- 1-3 / 0.5 microns,
- Motile by peritrichous flagella





## **S.typhi with Flagella**



## Different types of Salmonella

I - *enterica*

II - *salamae*

IIIa - *arizonae*

IIIb - *diarizonae*

IV - *houtenae*

V - *bongori*

VI - *indica*

# Cultural Characters

- Aerobic / Facultatively anaerobic
- Grows on simple media – Nutrient agar,
- Temp 15 – 41°C / 37°C
- Colonies appear as large 2 -3 mm, circular, low convex,
- **On MacConkey medium appear Colorless ( NLF )**

Selective Medium - Wilson Blair Bismuth sulphide medium. Produce Jet black colonies  
H<sub>2</sub>S produced by Salmonella typhi

# Specimen collection

Blood

Serum

Urine

Feces

BoneMarrow

Bile

Pus

CSF

Sputum

Gall bladder

Liver

Spleen

Mesentric lymph nodes

## Ideal specimen

First week	Blood (culture)
Second week	Serum (Antibodies)
Third week	Stool
Fourth week	Urine



- Shed throughout the course of the disease and also in convalescence
- Valuable in patients on antibiotics ( drug does not eliminate the bacilli from the gut)
- Fecal samples plated directly on

MacConkey

DCA / XLD

Wilson Blair Media

- Enrichment also done in **selenite or tetrathionate broth** , incubated for 6 to 8 hours and subcultured.

# سالمونلا و شیگلا روی SS آگار



*Salmonella* on SS Agar



*Shigella* on SS Agar



اشريشياکلی



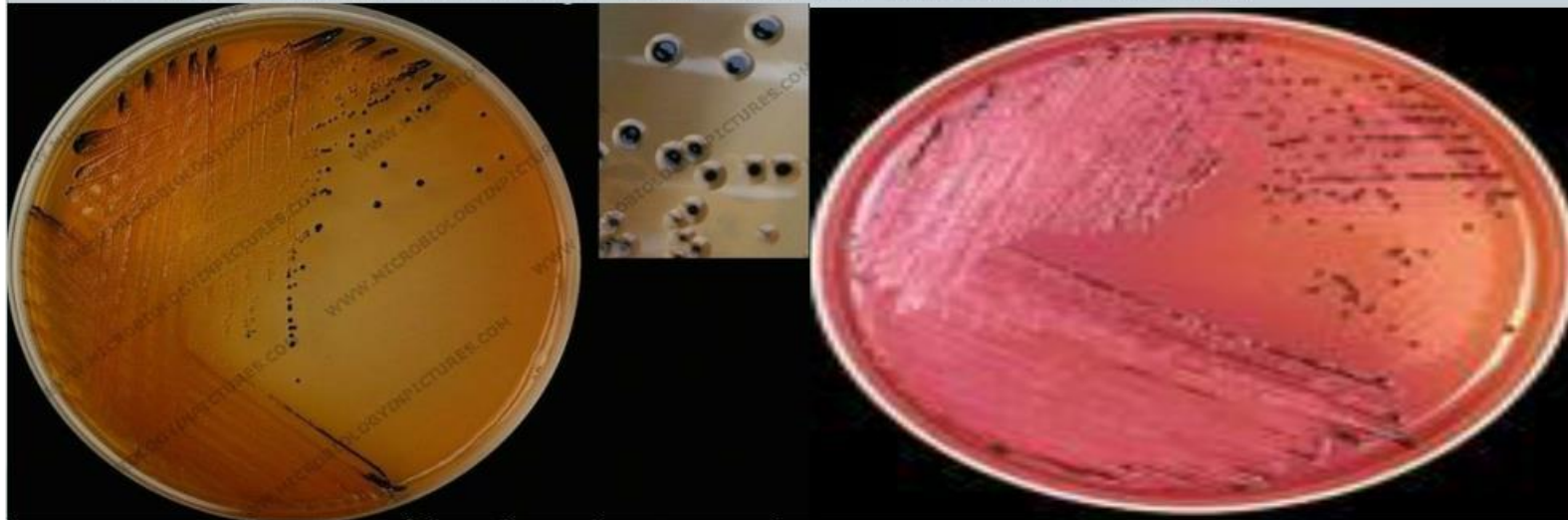
سالمونلا



شيگلا

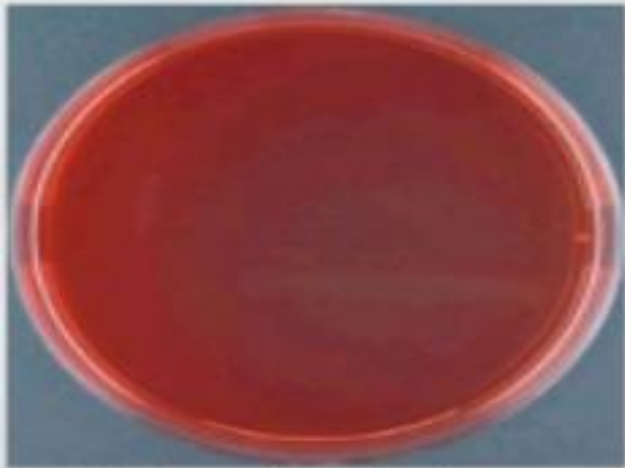
## Laboratory Diagnosis

- Enrichment and selective media for *Salmonella* in faeces is Selenite broth
- Differential media is XLD and SSA
- XLD-pink colonies with black centers
- SSA- black colonies with silver metallic sheen.

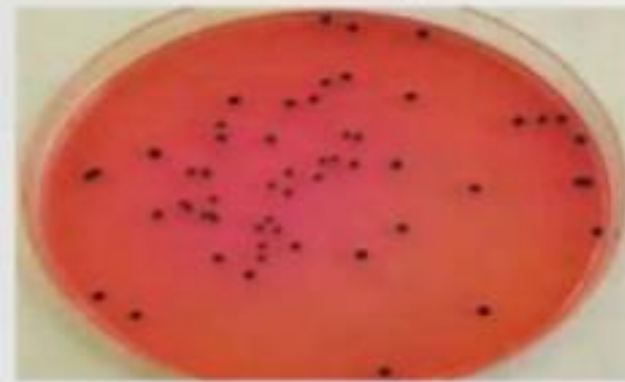




# Salmonella on XLD



Uninoculated plate



Salmonella on XLD is red, with or without black centers

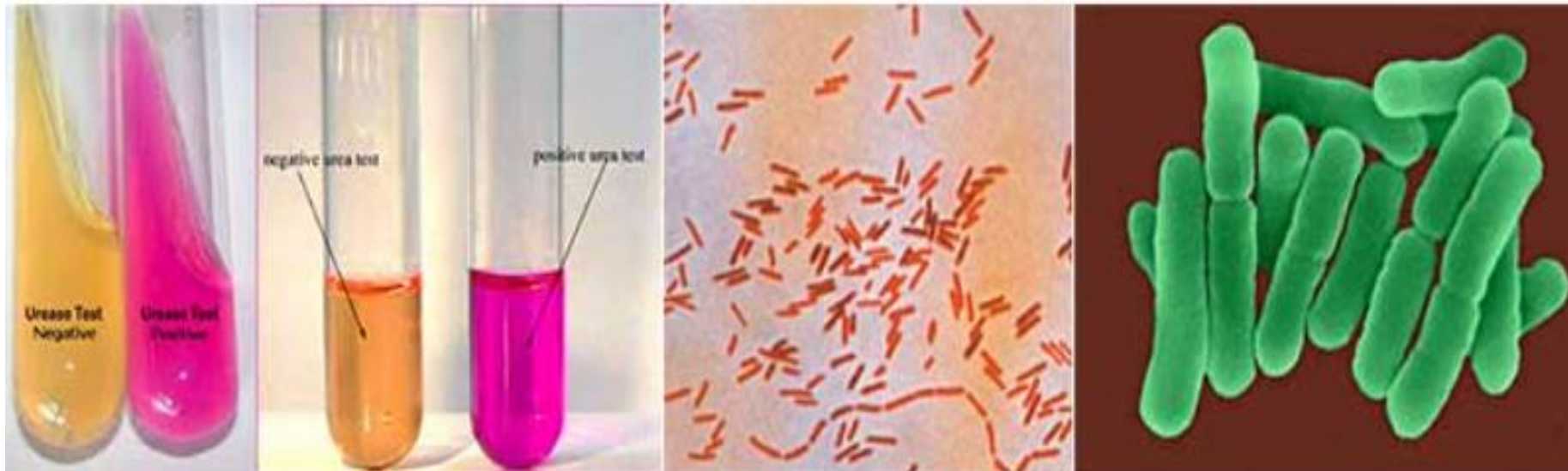


Pale non lactose non sucrose fermenting  
colonies – DCLS

Red, black centred colonies – XLD

- Rule out proteus by urease test
- Check for purity by subculturing in nutrient agar
- Do biochemical reactions and sugars
- Do serotyping by slide agglutination test

# Biochemical Test and Identification of *Salmonella Typhi*



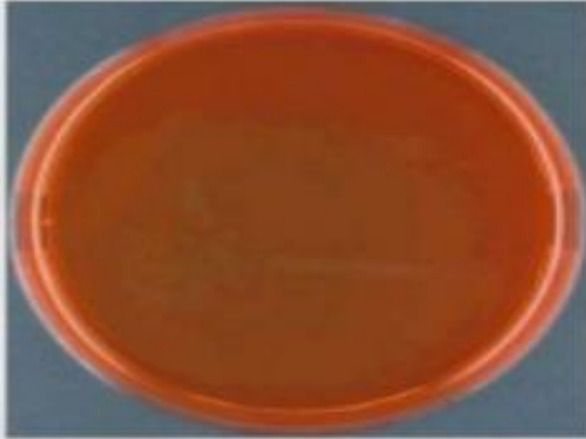
# Interpretation

Provisional report – given on third or fourth day and inform the clinician

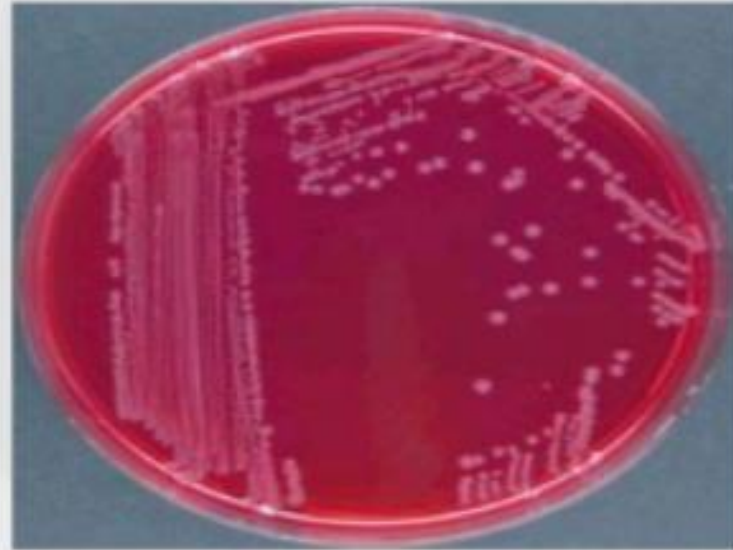
Secondary confirmation test panel:

1. Citrate agar slope
2. Lysine decarboxylase medium with control
3. Salicin peptone water
4. ONPG
5. Mac Conkey secondary purity plate
6. Nutrient agar slope
7. Sensitivity agar plate

# Salmonella on BGA



an uninoculated plate



Salmonella on BGA . The colonies are small, opaque, pink or white. Can also be transparent and colorless.



## **Enrichment Medium**

### **Liquid Medium**

- Selenite F medium
- Tetrathionate broth
- Above medium are used for isolation of Salmonella from contaminated specimens
- Particularly stool specimens..



# Identifying Enteric Organisms

- Isolates which are Non lactose fermenting
- Motile, Indole positive
- Urease negative
- Ferment Glucose, Mannitol, Maltose
- Do not ferment Lactose, Sucrose
- Typhoid bacilli are anaerogenic
- Some of the Paratyphoid form acid and gas
- Further identification done by slide agglutination tests

## Resistance of Salmonella

- 55° c – 1 hour
- 60° c – 15 MT
- Boiling ,Chlorination,  
Pasteurization Destroy the  
Bacilli.

# Antigenic structure of Salmonella

- Two sets of antigens
- Detection by serotyping
- **1 Somatic or O Antigens** contain long chain polysaccharides ( LPS ) comprises of heat stable polysaccharide commonly.
- **2 Flagellar or H Antigens** are strongly immunogenic and induces antibody formation rapidly and in high titers following infection or immunization. The flagellar antigen is of a dual nature, occurring in one of the two phases.

# Salmonella

## Antigenic Structure

- H – Flagellar antigens
- O – Somatic antigen,
- Vi – Surface antigen in some species only
- H antigens also called flagellar antigens, heat labile protein,
- Boiling destroys antigenicity
- When mixed with Antiserum produces agglutination and fluffy clumps are produced
- H antigens are strongly immunogenic Induces antibodies rapidly,

## Antigens – Salmonella ( cont )

- O Antigens
  - Forms integral part of Cell wall,
  - Like Endotoxin
  - O Antigens unaffected by boiling.
  - When mixed with antiserum produce chalky clumps are formed, take more time reaction, at high temp 50° – 55° c
  - O antigens are less immunogenic. than H antigens



## Antigen (Vi) – Salmonella ( contd )

- Vi antigens
- Many strains in S.typhi covers the O antigens- prevents agglutination.
- Resembles like K antigens
- Destroyed after boiling at 60° c / 1 hour.
- Vi a polysaccharide
- Acts as virulence factor, protects the bacilli against Phagocytosis and activity of Complement
- Poorly immunogenic
- Low titer of antibodies are produced, Not diagnostic

## Classification of Salmonella

- Classified on the basis of Kauffmann-White Scheme
- Structure of O and H antigens are taken into consideration,
- More than 2000 species characterized.

# Kauffmann – White scheme

• Serotype	O antigens	H antigens	
		Phase <b>1</b>	<b>2</b>
1. Typhi	9, <b>12</b> , (Vi)	d	1,2
2 Paratyphi A	1,2, <b>12</b>	a	-
3 Paratyphi B	1,4,5, <b>12</b>	b	1,2
4 Typhimurium	1,4,5, <b>12</b>	l	1,7
5 Enteritidis	1,9, <b>12</b>	g m	1,2

<b>Capsule</b>	Negative (-ve)
<b>Catalase</b>	Positive (+ve)
<b>Citrate</b>	Negative (-ve)
<b>Flagella</b>	Positive (+ve)
Gas	Negative (-ve)
Gelatin Hydrolysis	Negative (-ve)
<b>Gram Staining</b>	Negative (-ve)
Growth in KCN	Negative (-ve)
H <sub>2</sub> S	Positive (+ve)
<b>Indole</b>	Negative (-ve)
Motility	Motile
<b>MR (Methyl Red)</b>	Positive (+ve)
MUG Test	Negative (-ve)

<b>MR (Methyl Red)</b>	Positive (+ve)
MUG Test	Negative (-ve)
Nitrate Reduction	Positive (+ve)
<b>Oxidase</b>	Negative (-ve)
Pigment	Negative (-ve)
<b>Shape</b>	Rod
<b>Spore</b>	Negative (-ve)
TSIA (Triple Sugar Iron Agar)	Alkali/Acid
<b>Urease</b>	Negative (-ve)
<b>VP (Voges Proskauer)</b>	Negative (-ve)
Fermentation of	
Adonitol	Negative (-ve)
Arabinose	Negative (-ve)
Arabitol	Negative (-ve)

Arabitol	Negative (-ve)
Cellobiose	Negative (-ve)
DNase	Negative (-ve)
Dulcitol	Negative (-ve)
Erythritol	Negative (-ve)
Esculin Hydrolysis	Negative (-ve)
Glucose	Positive (+ve)
Glycerol	Negative (-ve)
Inositol	Negative (-ve)
Lactose	Negative (-ve)
Malonate	Negative (-ve)
Maltose	Positive (+ve)
Mannitol	Positive (+ve)

Arabitol	Negative (-ve)
Cellobiose	Negative (-ve)
DNase	Negative (-ve)
Dulcitol	Negative (-ve)
Erythritol	Negative (-ve)
Esculin Hydrolysis	Negative (-ve)
Glucose	Positive (+ve)
Glycerol	Negative (-ve)
Inositol	Negative (-ve)
Lactose	Negative (-ve)
Malonate	Negative (-ve)
Maltose	Positive (+ve)
Mannitol	Positive (+ve)



Xylose	Positive (+ve)
Enzymatic Reactions	
Acetate Utilization	Negative (-ve)
Arginine Dehydrolase	Negative (-ve)
Esculin Hydrolysis	Negative (-ve)
Lipase	Negative (-ve)
Lysine	Positive (+ve)
ONPG ( $\beta$ -galactosidase)	Negative (-ve)
Ornithine Decarboxylase	Negative (-ve)
Peroxidase	Negative (-ve)
Tyrosine Hydrolysis	Negative (-ve)

# Shigella

# MORPHOLOGY AND STAINING:

- Short rods
- - Non-encapsulated
- - Non-motile
- - Non-spore former
- - Gram-negative



# Factors Contributing Spread

- Spread is always from **a human resource** and generally involves one of the *five f's*:
  - **food,**
  - **fingers,**
  - **feces,**
  - **flies or**
  - **fomites.**
- This is in contrast to salmonellae, which are often spread to humans from infected animals.<sup>13</sup>

# Morphology & Physiology

- Small Gram-negative, facultatively anaerobic, **coliform** bacillus
  - Non-motile (no H antigen)
  - Possess capsule (K antigen) and O antigen
  - **K antigen** not useful in serologic typing, but can interfere with O antigen determination
  - **O antigens**: A, B, C, D correspond respectively to the four species
  - Non-lactose fermenting
  - Bile salts resistant: trait useful for selective media
- ferment glucose
- reduce nitrates** ( $\text{NO}_3$  to  $\text{NO}_2$  or  $\text{N}_2$ )
- are **oxidase** negative



# Taxonomy

Family *Enterobacteriaceae*

2. ***Shigella dysenteriae***: most serious form of bacillary dysentery
  3. ***Shigella flexneri***: shigellosis in underdeveloped countries
- ***Shigella sonnei***: shigellosis in developed countries
  - ***Shigella boydii***

## Culture Media for Identification

- Commonly used primary isolation media include MacConkey, Hektoen Enteric Agar, and Salmonella-Shigella (SS) Agar. These media contain bile salts to inhibit the growth of other Gram-negative bacteria and pH indicators to differentiate lactose fermenters (Coliforms) from non-lactose fermenters such as Shigella

# Diagnosis



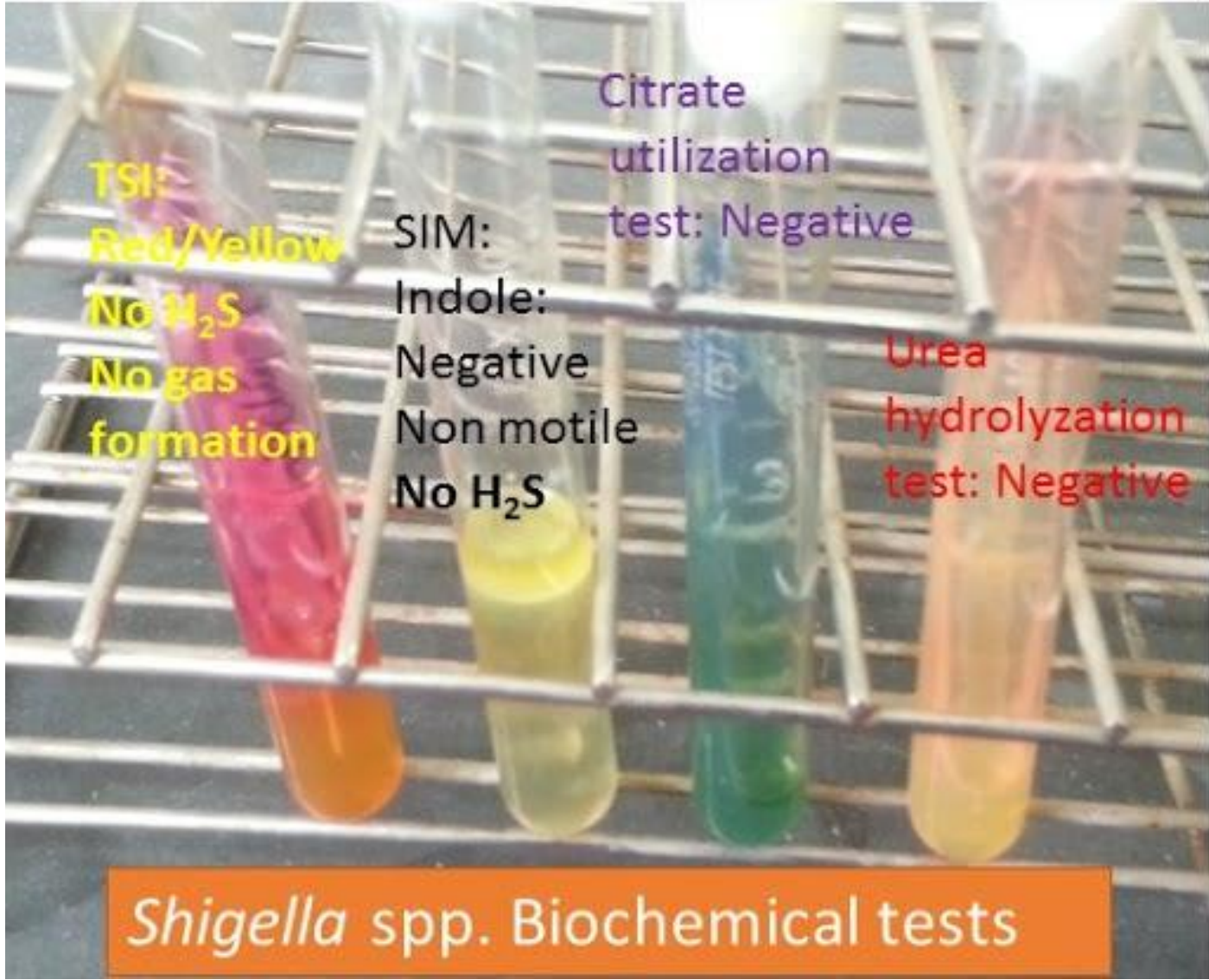
## Figure:

Several media have been designed to selectively grow enteric bacteria and allow differentiation of *Salmonella* and *Shigella* from *E. coli*. The primary plating media shown here are eosin methylene blue (EMB) agar, MacConkey agar, ENDO agar, Hektoen enteric (HE) agar and Salmonella-Shigella (SS) agar.

<http://www.textbookofbacteriology.net/Shigella.html>









# Shigella species



Non Fermentation

*S dysenteriae*

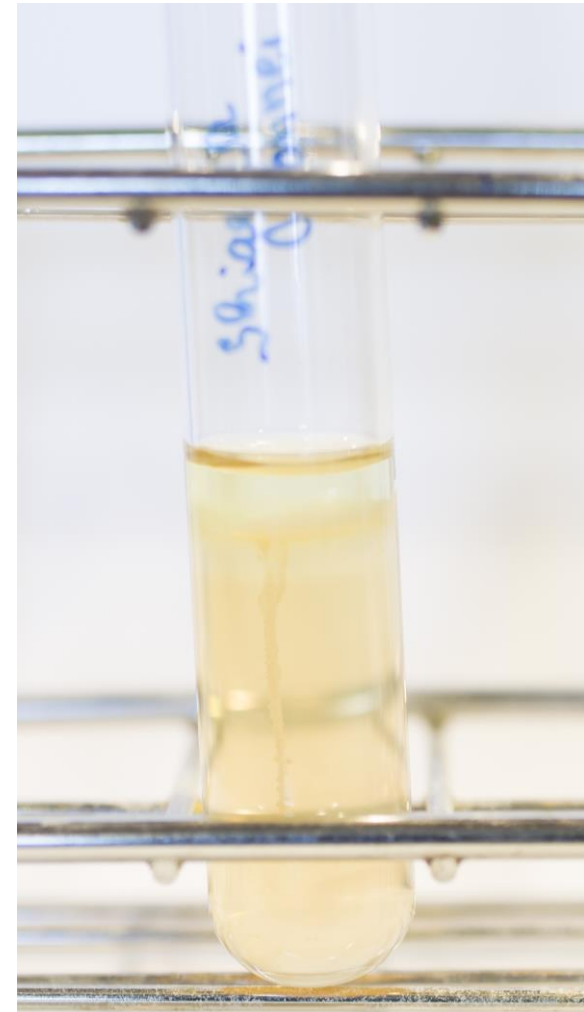


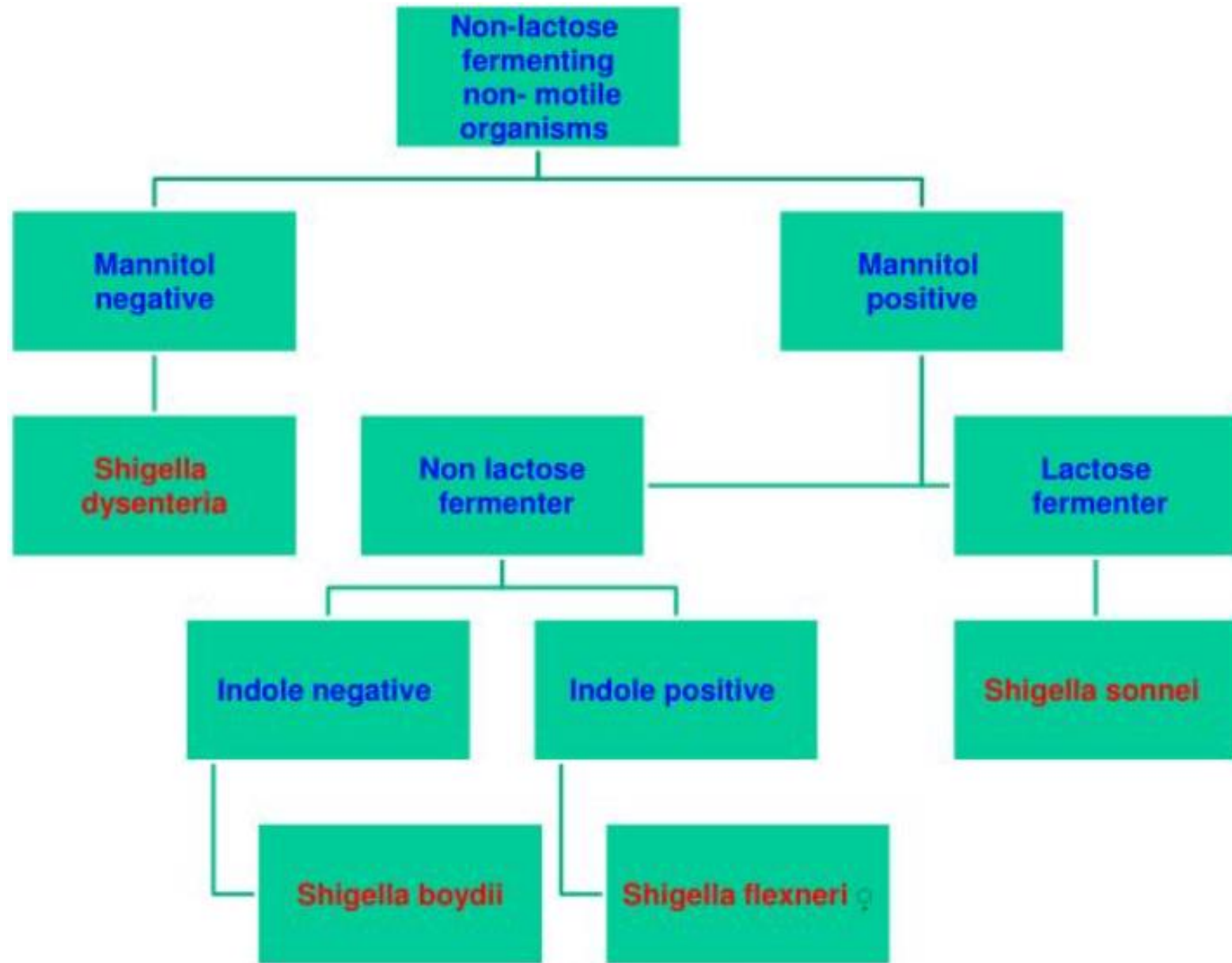
Fermentation

*S flexneri*

*S boydii*

*S sonnei*





**Table 7.9b.** Third-stage table for the enterobacteria (part 1)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Motility	+	+	+	+	+	+	+	+	+	+	+	d	+	+	+
Yellow pigment	-	-	-	-	-	-	-	-	-	-	-	-	-	+	d
Red pigment	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MacConkey growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Simmons' citrate	d	-	+	+	+	-	+	+	+	-	+	+	+	+	d
Christensen's citrate	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	d	d	d	-	-	d	+	-	-
Gelatin hydrolysis	-	-	-	-	-	-	-	-	-	-	-	d	-	+	d
Growth in KCN medium	+	+	+	+	-	-	+	+	-	-	+	+	-	+	d
H <sub>2</sub> S (PbAc paper)	-	-	-	-	-	-	d	+	+	+	-	-	d	-	-
H <sub>2</sub> S from TSI	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
Glucanate	-	-	+	d	+	-	-	-	-	-	+	+	+	+	-
Malonate	+	+	+	+	+	-	-	+	-	+	+	+	+	d	d
ONPG	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Phenylalanine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine dihydrolase	-	+	+	+	+	+	+	d	+	-	-	+	-	+	-
Lysine decarboxylase	-	-	-	-	-	-	-	-	-	+	+	-	d	-	-
Ornithine decarboxylase	+	+	-	-	-	+	+	d	d	+	+	+	+	+	-
Selenite reduction	-	d	+	+	+	-	+	+	+	d	+	+	+	+	-
Casein hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DNase production	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Carbohydrates [in Peptone Water medium], gas from glucose	-	-	+	d	+	-	+	+	+	+	+	+	+	+	-
acid from:															
adonitol	-	-	-	-	-	-	-	-	+	-	+	d	-	-	-
arabinose	+	-	-	-	-	-	+	+	+	d	+	+	+	+	+
cellobiose	+	+	+	+	+	+	+	+	+	-	+	+	+	+	d
dulcitol	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-
glycerol	d	d	d	d	+	-	d	+	+	d	+	d	+	d	d
inositol	-	-	-	-	-	-	-	-	-	-	+	-	-	d	-
lactose	+	-	d	d	+	-	+	+	+	-	+	d	d	+	d
maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d
mannitol	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
raffinose	+	-	-	d	-	+	-	d	-	-	+	d	+	+	d
rhamnose	+	-	-	-	-	+	+	+	+	-	+	+	+	+	+
salicin	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
sorbitol	-	-	-	d	+	+	+	+	+	-	+	+	-	-	d
sucrose	-	+	-	+	+	+	-	d	d	-	+	+	+	+	+
trehalose	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
xylose	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+
starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MR test (37 °C) <sup>a</sup>	+	+	d	+	+	+	+	+	+	+	d	d	+	-	d
MR test (RT) <sup>b</sup>	+	+	-	-	-	-	+	+	+	+	-	-	-	-	d
VP test (37 °C) <sup>a</sup>	-	-	+	-	-	-	-	-	-	-	d	d	-	d	-
VP test (RT) <sup>b</sup>	-	-	+	d	-	-	-	-	-	-	d	d	d	d	d
Indole	-	-	-	-	-	-	+	d	+	+	-	-	-	-	-

1 *Buttiauxella agrestis*

2 *Cedecea davisae*

3 *Cedecea lapagei*

4 *Cedecea species 3*

5 *Cedecea neteri*; *Cedecea species 4*

6 *Cedecea species 5*

7 *Citrobacter amalonaticus*; '*Levinea amalonatica*'

8 *Citrobacter freundii* '*Escherichia freundii*';

'*Salmonella coli*'; '*S. ballerup*'; '*S. hormaechei*';

Bethesda-Ballerup group

9 *Citrobacter koseri*; *C. diversus*; *Levinea malonatica*

10 *Edwardsiella tarda*; *E. anguillimortifera*; Asakusa group; Bartholomew group

11 *Enterobacter aerogenes*; *Klebsiella mobilis*; (NOT '*Aerobacter aerogenes*'

Beijerinck)

12 *Enterobacter cloacae*; '*Cloaca cloacae*'; '*Aerobacter cloacae*'

13 *Enterobacter gergoviae*

14 *Enterobacter sakazakii*

15 *Erwinia herbicola*; '*Bacterium typhiflavum*'; *Enterobacter agglomerans*;

*Pantoea agglomerans*;

Table 7.9c Third-stage table for the enterobacteria (part 2)

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Motility	+	d	+	+	+	d	-	-	-	-	-	+	+	+	d
Yellow pigment	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Red pigment	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MacConkey growth	+	+	+	+	+	+	+	+	+	d	+	+	+	+	+
Simmons' citrate	-	-	d	-	-	d	+	+	d	+	-	+	-	d	-
Christensen's citrate	-	d	+	d	-	+	+	+	+	+	d	+	d	+	d
Urease	-	-	-	-	-	+	+	d	+	-	-	+	+	+	+
Gelatin hydrolysis	d	-	-	-	-	d	-	-	-	-	-	-	-	+	+
Growth in KCN medium	+	-	-	+	-	+	+	d	d	d	+	+	+	+	+
H <sub>2</sub> S (PbAc paper)	-	-	d	-	-	d	-	-	-	-	-	d	+	+	+
H <sub>2</sub> S from TSI	-	-	-	-	-	-	-	-	-	-	-	-	-	+	d
Gluconate	-	-	-	-	-	+	+	+	-	-	-	-	-	d	-
Malonate	+	-	-	-	+	d	+	+	-	+	+	-	-	-	-
ONPG	+	d	+	+	+	+	+	+	+	+	-	+	-	-	-
Phenylalanine	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Arginine dihydrolase	-	d	d	-	+	-	-	-	d	-	-	-	-	-	-
Lysine decarboxylase	-	d	+	d	+	+	+	d	+	-	d	-	-	-	-
Ornithine decarboxylase	-	d	+	+	-	+	-	-	-	-	-	+	+	+	-
Selenite reduction	+	d	+	+	+	+	+	+	-	d	-	+	d	+	d
Casein hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	d	+
DNase production	-	-	-	-	-	-	-	-	-	-	-	-	-	d	+
Carbohydrates [in Peptone Water medium], gas from glucose	+	d	+	+	+	+	+	+	d	+	-	+	+	+	+
acid from:															
adonitol	+	-	+	-	-	-	+	+	+	+	+	-	-	-	-
arabinose	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
cellobiose	+	-	+	+	+	d	+	+	+	+	+	+	-	-	-
dulcitol	+	d	-	+	-	-	d	d	-	+	-	-	-	-	-
glycerol	-	+	-	-	-	+	+	+	d	+	d	d	d	+	+
inositol	-	-	-	-	-	-	+	+	d	+	+	-	-	-	-
lactose	+	d	d	d	d	-	+	+	d	+	-	+	-	-	-
maltose	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
mannitol	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
raffinose	d	d	-	-	+	-	+	+	+	+	+	+	-	-	-
rhamnose	+	+	+	+	+	+	+	+	d	+	+	+	-	-	-
salicin	+	d	+	d	+	d	+	+	+	+	+	+	-	-	-
sorbitol	-	+	-	-	-	d	+	+	+	+	+	d	-	-	-
sucrose	d	d	-	d	-	-	+	+	d	+	+	+	-	-	+
trehalose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	d
xylose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
starch	-	-	-	-	-	-	d	d	d	+	d	-	-	-	-
MR test (37 °C) <sup>a</sup>	+	+	+	+	+	d	d	d	+	+	+	+	+	d	d
MR test (RT) <sup>b</sup>	+	+	d	+	+	-	d	d	+	+	+	+	+	d	d
VP test (37 °C) <sup>a</sup>	-	-	-	-	-	d	d	d	-	-	-	-	-	-	-
VP test (RT) <sup>b</sup>	-	-	-	-	-	+	d	d	-	-	-	-	-	d	-
Indole	+	+	d	+	-	-	+	-	-	-	-	d	+	-	-

<p>16 <i>Escherichia adecarboxylata</i></p> <p>17 <i>Escherichia coli</i></p> <p>18 <i>Escherichia fergusonii</i></p> <p>19 <i>Escherichia hermannii</i></p> <p>20 <i>Escherichia vulneris</i></p> <p>21 <i>Hafnia alvei</i>; '<i>Enterobacter alvei</i>'</p> <p>22 <i>Klebsiella oxytoca</i></p> <p>23 <i>Klebsiella pneumoniae</i> subsp. <i>aerogenes</i>; '<i>K. aerogenes</i>'; <i>K. pneumoniae (sensu lato)</i>; (NOT '<i>Aerobacter aerogenes</i>' Beijerinck)</p>	<p>24 <i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>; <i>K. ozaenae</i></p> <p>25 <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>; <i>K. pneumoniae</i> (<i>sensu stricto</i>); Friedländer's pneumobacillus</p> <p>26 <i>Klebsiella pneumoniae</i> subsp. <i>rhinoscleromatis</i>; <i>K. rhinoscleromatis</i></p> <p>27 <i>Kluyvera</i> spp.</p> <p>28 <i>Morganella morganii</i>; '<i>Proteus morganii</i>'; Morgan's no. 1 bacillus</p> <p>29 <i>Proteus mirabilis</i></p> <p>30 <i>Proteus penneri</i>; <i>P. vulgaris</i> biogroup I</p>
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Table 7.9c Third-stage table for the enterobacteria (part 2)

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Motility	+	d	+	+	+	d	-	-	-	-	-	+	+	+	d
Yellow pigment	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Red pigment	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MacConkey growth	+	+	+	+	+	+	+	+	+	+	d	+	+	+	+
Simmons' citrate	-	-	d	-	-	d	+	+	d	+	-	+	-	d	-
Christensen's citrate	-	d	+	d	-	+	+	+	+	+	d	+	d	+	d
Urease	-	-	-	-	-	-	+	+	d	+	-	-	+	+	+
Gelatin hydrolysis	d	-	-	-	-	-	d	-	-	-	-	-	-	+	+
Growth in KCN medium	+	-	-	+	-	+	+	d	d	d	d	+	+	+	+
H <sub>2</sub> S (PbAc paper)	-	-	d	-	-	d	-	-	-	-	-	d	+	+	+
H <sub>2</sub> S from TSI	-	-	-	-	-	-	-	-	-	-	-	-	-	+	d
Gluconate	-	-	-	-	-	+	+	+	-	-	-	-	-	d	-
Malonate	+	-	-	-	+	d	+	+	-	+	+	+	-	-	-
ONPG	+	d	+	+	+	+	+	+	+	+	-	+	-	-	-
Phenylalanine	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Arginine dihydrolase	-	d	d	-	+	-	-	-	d	-	-	-	-	-	-
Lysine decarboxylase	-	d	+	d	+	+	+	+	d	+	-	d	-	-	-
Ornithine decarboxylase	-	d	+	+	+	+	-	-	-	-	-	+	+	+	-
Selenite reduction	+	d	+	+	+	+	+	+	-	d	-	+	d	+	d
Casein hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	d	+
DNase production	-	-	-	-	-	-	-	-	-	-	-	-	-	d	+
Carbohydrates [in Peptone Water medium], gas from glucose	+	d	+	+	+	+	+	+	d	+	-	+	+	+	+
acid from:															
adonitol	+	-	+	-	-	-	+	+	+	+	+	-	-	-	-
arabinose	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
cellobiose	+	-	+	+	+	d	+	+	+	+	+	+	-	-	-
dulcitol	+	d	-	+	-	-	d	d	-	+	-	-	-	-	-
glycerol	-	+	-	-	-	+	+	+	d	+	d	d	d	+	+
inositol	-	-	-	-	-	+	+	d	+	+	-	-	-	-	-
lactose	+	d	d	d	d	-	+	+	d	+	-	+	-	-	-
maltose	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
mannitol	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
raffinose	d	d	-	-	+	-	+	+	+	+	+	+	-	-	-
rhamnose	+	+	+	+	+	+	+	d	+	+	+	-	-	-	-
salicin	+	d	+	d	+	d	+	+	+	+	+	+	-	-	-
sorbitol	-	+	-	-	-	d	+	+	+	+	+	d	-	-	-
sucrose	d	d	-	d	-	-	+	+	d	+	+	+	-	-	+
trehalose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	d
xylose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
starch	-	-	-	-	-	-	d	d	d	+	d	-	-	-	-
MR test (37 °C) <sup>a</sup>	+	+	+	+	+	d	d	d	+	+	+	+	+	d	d
MR test (RT) <sup>b</sup>	+	+	d	+	+	-	d	d	+	+	+	+	+	d	d
VP test (37 °C) <sup>a</sup>	-	-	-	-	-	d	d	d	-	-	-	-	-	-	-
VP test (RT) <sup>b</sup>	-	-	-	-	-	+	d	d	-	-	-	-	-	d	-
Indole	+	+	d	+	-	-	+	-	-	-	-	-	d	+	-

- |  |   |
|--|---|
| <b>16</b> <i>Escherichia adecarboxylata</i>  | <b>24</b> <i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i> ; <i>K. ozaenae</i>  |
| <b>17</b> <i>Escherichia coli</i>  | <b>25</b> <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> ; <i>K. pneumoniae</i> ( <i>sensu stricto</i> ); Friedländer's pneumobacillus |
| <b>18</b> <i>Escherichia fergusonii</i>  | <b>26</b> <i>Klebsiella pneumoniae</i> subsp. <i>rhinoscleromatis</i> ;<br><i>K. rhinoscleromatis</i>   |
| <b>19</b> <i>Escherichia hermannii</i>   | <b>27</b> <i>Kluyvera</i> spp.  |
| <b>20</b> <i>Escherichia vulneris</i>  | <b>28</b> <i>Morganella morganii</i> ; ' <i>Proteus morganii</i> '; Morgan's no. 1 bacillus   |
| <b>21</b> <i>Hafnia alvei</i> ; ' <i>Enterobacter alvei</i> '  | <b>29</b> <i>Proteus mirabilis</i>  |
| <b>22</b> <i>Klebsiella oxytoca</i>  | <b>30</b> <i>Proteus penneri</i> ; <i>P. vulgaris</i> biogroup 1  |
| <b>23</b> <i>Klebsiella pneumoniae</i> subsp. <i>aerogenes</i> ; ' <i>K. aerogenes</i> ';<br><i>K. pneumoniae</i> ( <i>sensu lato</i> ); (NOT ' <i>Aerobacter aerogenes</i> '<br>Beijerinck) |   |

RT, room temperature (18–22 °C); <sup>a</sup> incubation for two days; <sup>b</sup> incubation for five days

Other symbols used in the table are explained in Tables 5.1 and 5.2 on n.47.



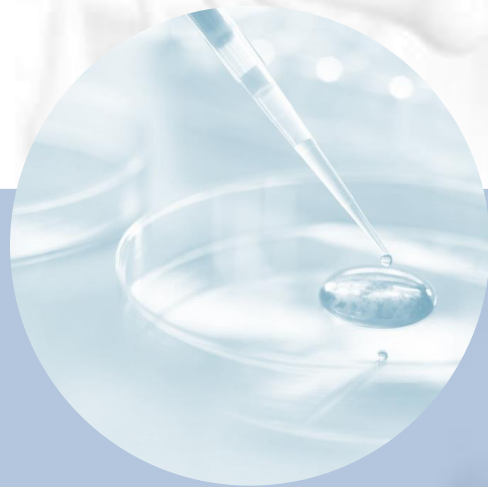
Table 7.9d. Third-stage table for the enterobacteria (part 3)

	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Motility	d	d	d	+	d	+	-	+	-	+	+	+	+	+
Yellow pigment	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Red pigment	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MacConkey growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Simmons' citrate	d	-	d	+	+	d	-	-	-	+	+	+	+	-
Christensen's citrate	+	d	+	+	+	+	+	+	+	+	+	+	+	+
Urease	+	+	-	+	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	+	d	-	-	-	-	-	-	-	-	d	d	d	-
Growth in KCN medium	+	+	+	+	+	-	-	-	-	-	-	-	+	-
H <sub>2</sub> S (PbAc paper)	+	+	d	+	d	d	+	d	d	+	+	+	+	+
H <sub>2</sub> S from TSI	d	d	-	-	-	d	d	-	d	+	+	+	+	d
Gluconate	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Malonate	-	-	-	-	-	-	-	-	-	-	+	+	-	-
ONPG	-	-	-	-	-	-	-	-	-	-	-	d	+	-
Phenylalanine	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Arginine dihydrolase	-	-	-	-	-	+	-	+	d	+	+	+	+	+
Lysine decarboxylase	-	-	-	-	-	+	+	-	+	+	+	+	+	+
Ornithine decarboxylase	-	-	-	-	-	+	-	+	+	+	+	+	+	-
Selenite reduction	+	d	d	+	d	d	d	+	+	+	+	+	+	+
Casein hydrolysis	d	-	-	-	-	-	-	-	-	-	-	-	-	-
DNase production	+	+	d	d	d	d	d	-	d	d	d	d	-	-
Carbohydrates [in Peptone Water medium], gas from glucose	+	+	d	-	-	+	-	+	d	+	+	+	+	-
acid from:														
adonitol	-	-	d	+	-	-	-	-	-	-	-	-	-	-
arabinose	-	-	-	-	-	-	+	+	+	+	+	+	+	-
cellobiose	-	-	-	-	-	-	-	-	-	d	d	-	d	-
dulcitol	-	-	-	-	-	d	+	+	-	+	+	-	-	d
glycerol	+	+	d	d	+	d	d	d	-	d	d	-	d	d
inositol	-	-	-	+	+	-	-	-	-	d	d	-	-	-
lactose	-	-	-	-	-	-	-	-	-	-	-	d	-	-
maltose	+	+	-	-	-	+	d	+	-	+	+	+	+	+
mannitol	-	-	-	+	-	+	+	+	+	+	+	+	+	+
raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
rhamnose	-	-	-	d	-	+	d	+	d	+	+	+	+	-
salicin	+	-	-	d	-	-	-	-	-	-	-	-	d	-
sorbitol	-	-	-	-	-	+	d	+	-	+	+	+	+	+
sucrose	+	+	d	d	d	-	-	-	-	-	-	-	-	-
trehalose	d	+	-	-	+	-	+	+	+	+	+	+	+	+
xylose	+	+	-	-	-	+	d	-	d	+	+	+	+	+
starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MR test (37 °C) <sup>a</sup>	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MR test (RT) <sup>b</sup>	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VP test (37 °C) <sup>a</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP test (RT) <sup>b</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole	+	+	+	+	+	-	-	-	-	-	-	-	-	-

31 *Proteus vulgaris* biogroup 232 *Proteus vulgaris* biogroup 333 *Providencia alcalifaciens*; *Proteus inconstans*; '*Proteus inconstans* A'; '*Proteus providenciae*'; '*Providencia providenciae*'34 *Providencia rettgeri*; '*Proteus rettgeri*'; '*Retterella rettgeri*'35 *Providencia stuartii*; '*Proteus stuartii*'; '*Proteus inconstans* B'; '*Proteus providenciae* B'36 *Salmonella choleraesuis*38 '*Salmonella paratyphi* A'39 '*Salmonella pullorum*'40 *Salmonella* subgenus I; '*S. kauffmannii*'; '*S. enterica*'; '*S. enteritidis* serotype (bioser) xyz41 *Salmonella* subgenus II; '*S. salamae*'; '*S. dar-es-salaam*'42 *Salmonella* subgenus III; '*Arizona arizonae*'; '*A. hinshawii*'; '*Salmonella arizonae*'43 *Salmonella* subgenus IV; '*S. houtenae*'

Table 7.9e. Third-stage table for the enterobacteria (part 4)

	45	46	47	48 <sup>c</sup>	49	50	51	52 <sup>d</sup>	53	54 <sup>e</sup>	55	56	57	58	59	
Motility	+	+	+	+	+	+	-	-	-	+	+	+	+	-	d	
Yellow pigment	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Red pigment	-	-	d	-	-	d	-	-	-	-	-	-	-	-	-	
MacConkey growth	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	
Simmons' citrate	+	+	+	+	+	d	-	-	-	-	d	d	-	-	-	
Christensen's citrate	+	+	+	+	+	+	-	-	+	d	+	+	+	-	-	
Urease	-	d	-	-	-	-	-	-	-	+	+	+	+	-	+	
Gelatin hydrolysis	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	
Growth in KCN medium	+	+	d	+	+	d	-	-	-	d	d	+	+	-	-	
H <sub>2</sub> S (PbAc paper)	-	d	-	d	-	d	-	-	-	-	-	-	-	-	-	
H <sub>2</sub> S from TSI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Gluconate	d	+	+	+	+	d	-	-	-	-	-	-	-	-	-	
Malonate	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	
ONPG	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	
Phenylalanine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Arginine dihydrolase	-	-	-	-	-	-	d	d	-	-	-	-	-	-	-	
Lysine decarboxylase	+	+	d	+	+	-	-	-	-	-	-	-	-	-	-	
Ornithine decarboxylase	+	+	-	+	-	-	+	-	-	+	+	+	+	-	-	
Selenite reduction	d	+	+	d	+	d	+	d	-	d	d	d	d	-	d	
Casein hydrolysis	d	+	d	d	+	d	-	-	-	-	-	-	-	-	-	
DNase production	d	+	d	d	-	d	-	-	-	-	-	-	-	-	-	
Carbohydrates [in Peptone Water medium], gas from glucose	d	d	-	-	-	d	-	-	-	-	-	-	-	-	-	
acid from:																
adonitol	-	d	+	d	+	-	-	-	-	-	-	-	-	-	-	
arabinose	+	-	+	+	+	+	+	d	-	+	+	+	+	+	+	
cellobiose	d	-	+	+	+	d	-	-	-	+	+	+	+	-	-	
dulcitol	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	
glycerol	+	+	+	+	+	d	d	d	-	+	+	+	+	d	+	
inositol	+	+	+	+	+	d	-	-	-	d	d	d	-	-	-	
lactose	-	-	+	d	+	d	-	-	-	-	d	-	-	-	-	
maltose	+	+	+	+	+	+	+	d	-	d	+	+	+	d	+	
mannitol	+	+	+	+	+	+	+	d	-	+	+	+	+	+	+	
raffinose	+	-	+	+	-	d	d	d	-	-	+	-	-	-	-	
rhamnose	-	-	-	+	+	+	+	-	-	-	+	+	-	-	+	
salicin	+	+	+	+	+	+	-	-	+	d	+	+	-	+	d	
sorbitol	+	+	-	+	+	d	-	d	-	+	+	+	+	-	-	
sucrose	+	+	+	+	-	+	-	-	+	+	+	+	-	-	-	
trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
xylose	+	-	+	+	+	+	-	-	-	d	+	+	+	+	+	
starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
MR test (37 °C) <sup>a</sup>	+	-	d	+	+	d	+	+	-	+	+	+	+	d	+	
MR test (RT) <sup>b</sup>	-	-	-	-	-	d	+	+	+	d	+	+	+	+	+	
VP test (37 °C) <sup>a</sup>	-	+	d	-	-	d	-	-	-	-	-	-	-	-	-	
VP test (RT) <sup>b</sup>	d	+	d	-	-	d	-	-	-	d	d	d	-	-	-	
Indole	-	-	-	-	-	-	-	d	-	d	+	+	d	-	-	
<b>45</b> <i>Serratia liquefaciens</i> ; ' <i>Enterobacter liquefaciens</i> '; ' <i>Aerobacter liquefaciens</i> '								<b>52<sup>d</sup></b> <i>Shigella</i> spp. (excluding <i>S. sonnei</i> ); <i>S. boydii</i> (serotypes 1-15; Boyd's dysentery bacilli); <i>S. dysenteriae</i> (serotype 1: ' <i>S. shigae</i> '; Shiga's bacillus); [serotypes 2-10; Large-Sach's group; 2 = ' <i>S. schmitzii</i> '; ' <i>S. ambigua</i> '; Schmitz's bacillus]; <i>S. flexneri</i> (serotypes 1-5; Flexner's dysentery bacilli); [serotype 6; Boyd 88]; Manchester bacillus; see Table 7.9f)								
<b>46</b> <i>Serratia marcescens</i> ; ' <i>Erythrobacillus prodigiosus</i> '; ' <i>Chromobacterium prodigiosum</i> '								<b>53</b> <i>Tatumella ptyseus</i>								
<b>47</b> <i>Serratia marinorubra</i> ; <i>S. rubidaea</i> , <i>Serratia</i> biotype II (Bascomb <i>et al.</i> , 1971); <i>Serratia</i> Phenon B (Grimont <i>et al.</i> , 1977)								<b>54<sup>e</sup></b> <i>Yersinia enterocolitica</i> ; ' <i>Pasteurella X</i> '								
<b>48<sup>c</sup></b> <i>Serratia odorifera</i> biovar I								<b>55</b> <i>Yersinia fredericksonii</i>								
<b>49</b> <i>Serratia odorifera</i> biovar II								<b>56</b> <i>Yersinia intermedia</i>								
<b>50</b> <i>Serratia plymuthica</i>								<b>57</b> <i>Yersinia kristensenii</i>								
<b>51</b> <i>Shigella sonnei</i>								<b>58</b> <i>Yersinia pestis</i> ; ' <i>Pasteurella pestis</i> '; the plague bacillus								
								<b>59</b> <i>Yersinia pseudotuberculosis</i> ; ' <i>Pasteurella pseudotuberculosis</i> '								



با تشکر از توجه شما

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