ENTERIC FEVER

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Learning objectives

At the end of the session, the students will be able to understand:

- Classification and Nomenclature
- Antigenic Structure
- Typhoidal *Salmonella*
- Non-Typhoidal *Salmonella*

Mary Mallon (1869-1938) and the history of typhoid fever

Filio Marineli, Gregory Tsoucalas, Marianna Karamanou, George Androutsos
Medical School, University of Athens, Athens, Greece

Mary Mallon as "Typhoid Mary"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3959940/

INTRODUCTION

- Enteric fever is a potentially fatal multisystem illness caused by *Salmonella* Typhi (typhoid fever) and *S*. Paratyphi A, B and C (paratyphoid fever).

CLASSIFICATION AND NOMENCLATURE

- *Salmonella* – GNB (family Enterobacteriaceae)
- Discovery - Salmon and Smith (1885).
- *S.* Typhi, was first observed by Eberth (1880) and Gaffky (1884) - formerly called **Eberth-Gaffky bacillus** or **Eberthella Typhi**.

Clinical Classification

- Oldest, widely used

1. Typhoidal *Salmonella*:

- Includes serotypes *S. Typhi* and *S. Paratyphi*
- Restricted to human hosts → enteric fever (typhoid/paratyphoid fever)

2. Non-typhoidal *Salmonellae or NTS*:

- Colonize intestine of animals
- Also infect humans → gastroenteritis & septicemia

Molecular Classification

- Genus *Salmonella* consists of 2 species—

(1) *Salmonella enterica*, & (2) *S. bongori*.

- Within the species *S. enterica*, there are six subspecies - *enterica, salamae*, *arizonae, diarizonae, houtenae* and *indica*.
- Each subspecies is further differentiated into serotypes (based on O and H antigens as described in the Kauffmann–White scheme)

ANTIGENIC STRUCTURE

Three important antigens on their cell wall

- 1. Somatic antigen (O)
- 2. Flagellar antigen (H)
- 3. Surface envelope antigen (Vi)—found in some species

Antigenic Classification (Kauffmann–White Scheme)

Serogroup		Serotype name	O Ag	V_i Ag	H Ag	
New	Old				Phase 1	Phase 2
2	A	*S*.Paratyphi A	1,2,12	-	a	[1,5]
4	B	*S*.Paratyphi B	1,4,[5],12	-	b	1,2
		S.Typhimurium	1,4, [5],12	-	i	1,2
7	C1	*S*.Paratyphi C	6,7	+	c	1,5
		S.Choleraesuis	6,7	-	c	1,5
9	D1	*S*.Typhi	9,12	+	d	-
		S.Enteritidis	1,9,12	-	g,m	[1,7]

Nomenclature

- Taxonomically, the correct nomenclature of the members of salmonellae is very much complicated,
- E.g. *Salmonella* species *enterica* subspecies *enterica* serotype Typhi.

Difference between somatic (O) and flagellar (H) antigen

Somatic (O) antigen	Flagellar (H) antigen
It is a part of cell wall lipopolysaccharide (LPS)	Made up of protein flagellin , It confers motility to the bacteria
In Widal test, O antigen of *S.* Typhi is used	In Widal test, H antigens of *S.* Typhi, *S.* Paratyphi A and B are used
Less immunogenic	More immunogenic
O antibody appears early, disappears early: indicates recent infection	H antibody appears late, disappears late- Indicates convalescent stage
When O antigen reacts with O antibody forms compact, granular, chalky clumps ➢ Agglutination takes place slowly ➢ Optimum temperature for agglutination is 55°C	When H antigen reacts with H antibody- forms large, loose, fluffy clumps. ➢ Agglutination takes place rapidly ➢ Optimum temperature for agglutination is 37^0C
Serogrouping of salmonellae is based on the O antigen	Serogroups are differentiated into serotypes based on H antigen

Vi Antigen

- Surface polysaccharide envelope or capsular antigen covering the O antigen
- Named with belief that Vi antigen is related to virulence
- Expressed in only few serotypes - *S.* Typhi, *S.*Paratyphi C, *S. dublin* and some strains of *Citrobacter freundii* (Ballerup-Bethesda group)

- Poorly immunogenic & antibody titers are low

  Not helpful in diagnosis of cases
- Complete absence of Vi antibody poor prognosis
- Disappears early in convalescence. If persists

  carrier state
- Phage typing of *S.* Typhi - using Vi specific

  bacteriophages
- Vi antigens - used for vaccination

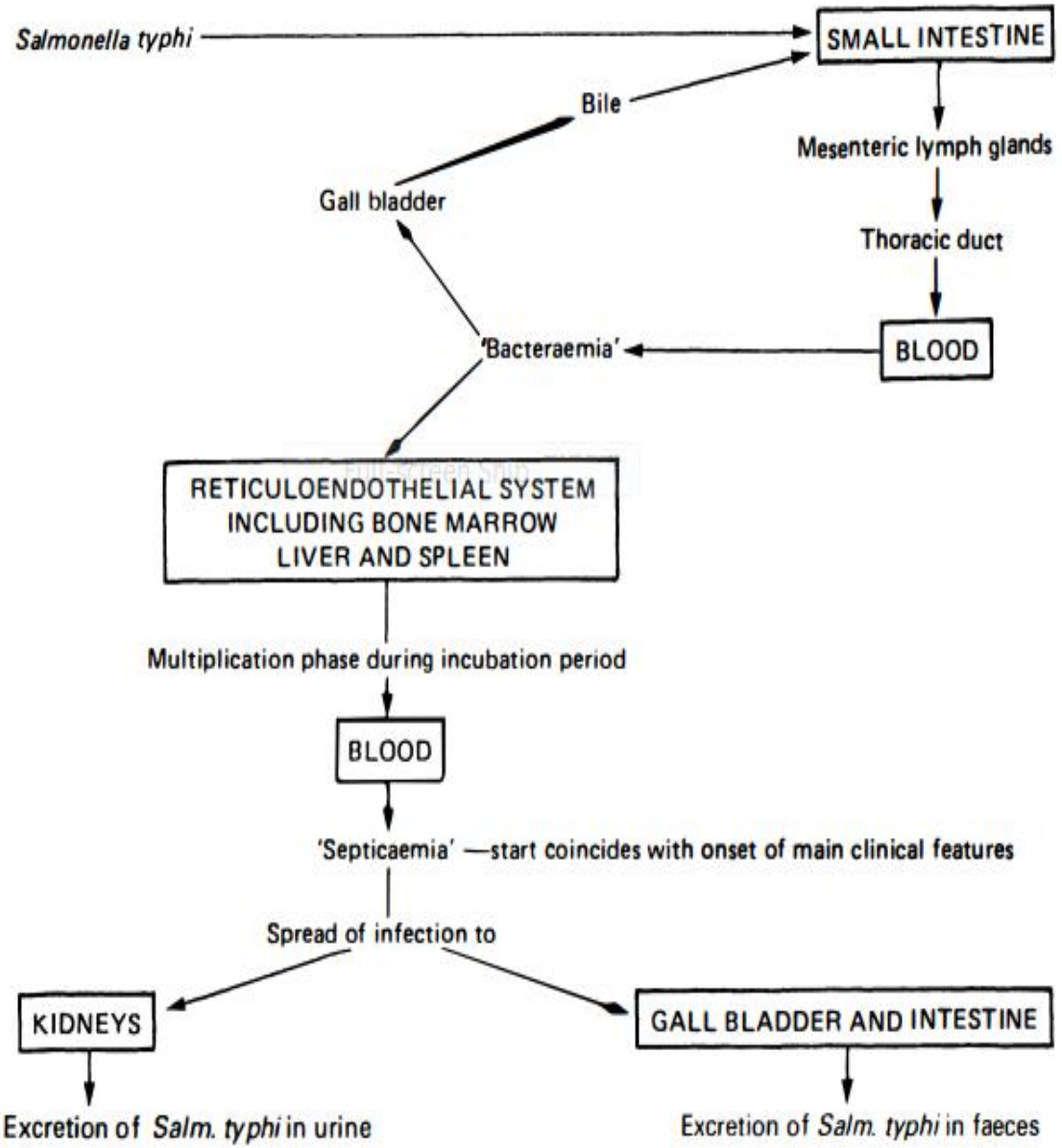
TYPHOIDAL SALMONELLA

- *S.* Typhi and
- *S.* Paratyphi A, B and C which cause enteric fever

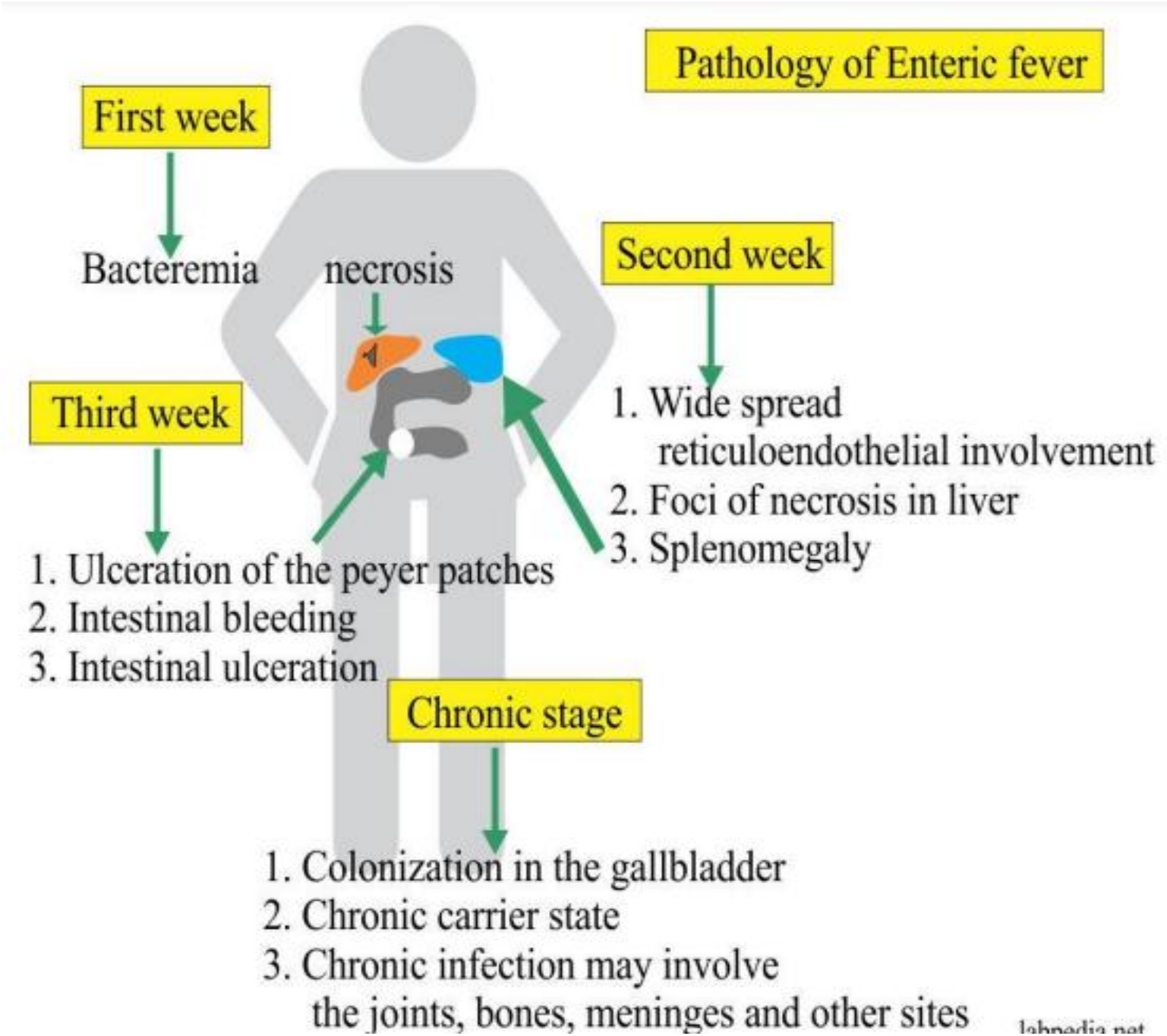
Pathogenesis

- **Transmission** - oral route (contaminated food & water).
- **Infective dose-** Minimum 10^3–10^6 bacilli

PATHOGENESIS OF TYPHOID FEVER



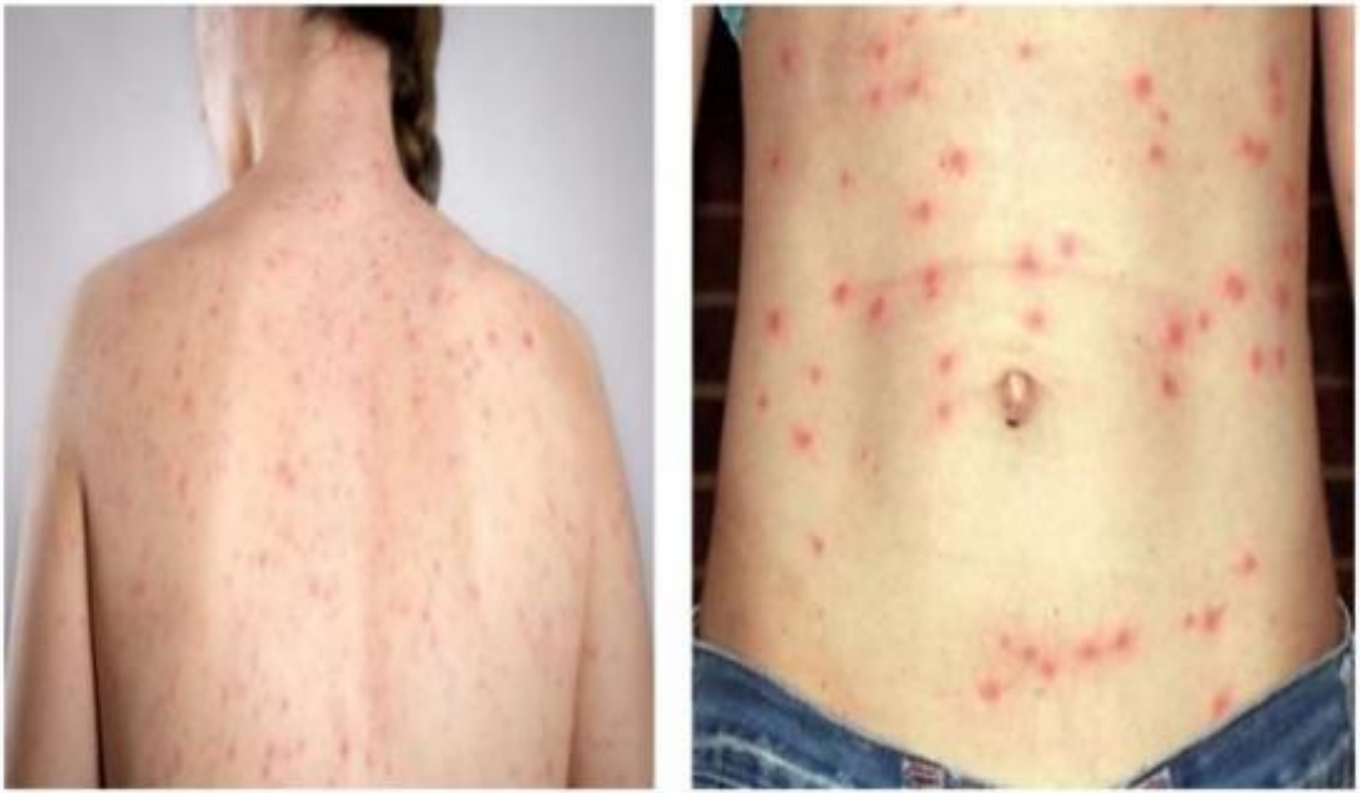
- **Primary bacteremia:** From inside macrophages spread via the lymphatics to enter the blood stream (transient primary bacteremia)
- **Spread:** Disseminate throughout reticuloendothelial tissues (liver, spleen, lymph nodes and bone marrow further multiplication
- **Secondary bacteremia occurs from the seeded organs -** clinical disease


Pathology of Enteric fever
First week
Bacteremia
necrosis
Second week
1. Wide spread
reticuloendothelial involvement
2. Foci of necrosis in liver
3. Splenomegaly
Third week
1. Ulceration of the peyer patches
2. Intestinal bleeding
3. Intestinal ulceration
Chronic stage
1. Colonization in the gallbladder
2. Chronic carrier state
3. Chronic infection may involve
the joints, bones, meninges and other sites

Clinical Manifestations of Enteric Fever

- Incubation period is about 10 - 14 days.
- **Fever (step ladder pattern of remittent fever)**
- **Other symptoms -** Headache, chills, cough, sweating, myalgia and arthralgia
- **Rashes** (called **rose spots)**
- **Early intestinal manifestations** - abdominal pain, nausea, vomiting and anorexia

ROSE SPOTS



2-4 mm diameter raised,disceret,irregular blanching pink maculae,s.

- **Important signs** - hepatosplenomegaly, epistaxis and relative bradycardia
- **Complications -** Gastrointestinal bleeding and intestinal perforation can occur mostly in the third and fourth weeks of illness
- **Neurologic manifestations** occur rarely

Epidemiology

- **Typhi vs Paratyphi:** *S*. Typhi infection is more common than *S*. Paratyphi A (ratio is 4:1).
- **Carriage:** Untreated patients become carriers and excrete *S*. Typhi in feces or urine.
- **Carriers are of two types:**

➢ Fecal carriers

➢ Urinary carriers

Duration of shedding:

➢ Temporary carriers - 3 months

➢ Chronic carriers - more than 1 year

Laboratory diagnosis

- Type of specimen to be collected depends on the **duration of illness.**

- **First week of illness:** Blood culture, bone marrow or duodenal aspirate culture
- **Second/third week of illness:** Serum specimen for serology (e.g. Widal test)
- **Third/Fourth week of illness:** Urine and stool culture.

Culture isolation

- Blood culture – positive in 90% of cases in 1st week of fever

➢ Conventional: BHI broth/agar

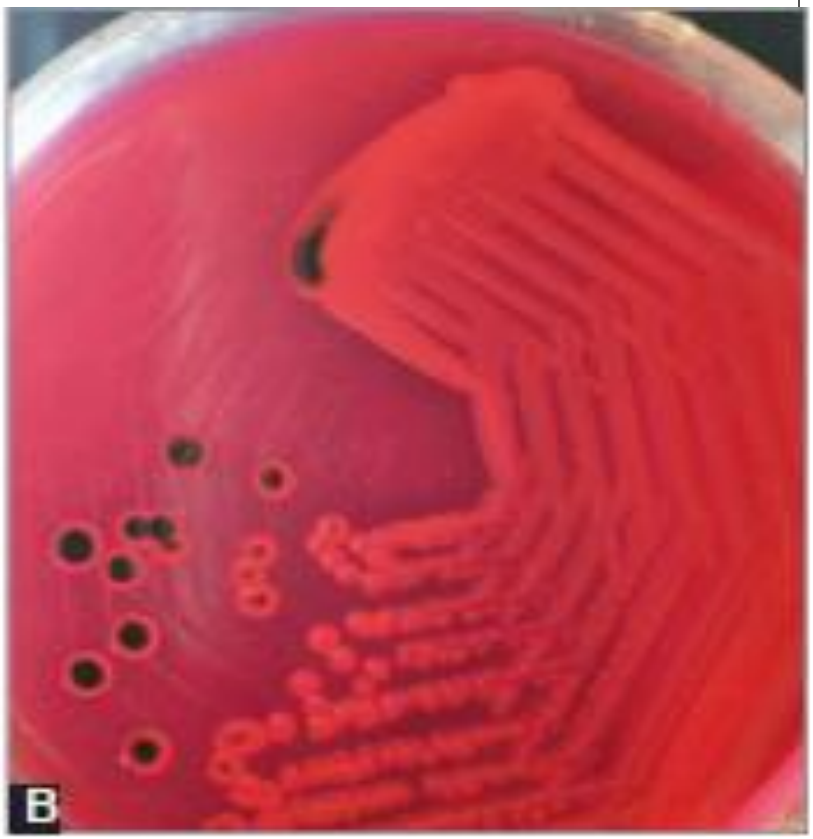
➢ Automated blood culture systems—BACTEC or BacT/ALERT

- Stool culture (in 3–4 weeks of illness):
  - Enrichment broth such as Selenite F broth, tetrathionate broth and gram-negative broth
  - Low selective medium: MacConkey agar (translucent NLF colonies)
  - Highly selective media: DCA, XLD agar, and Wilson Blair's Bismuth sulphite medium.
- Urine culture (in 3–4 weeks of illness)—on MacConkey agar.

Colonies of *S.* Typhi:

A. DCA (Deoxycholate citrate agar) showing pale colonies with black center; and

B. XLD agar (Xylose lysine deoxycholate) showing red colonies with black center



Other specimens-

- **Bone marrow culture –** done in first week of illness (55–90% sensitive) when blood culture is negative, especially when patient is on antibiotics
- **Duodenal aspirate culture** is recommended during first week of illness if both blood and bone marrow cultures turn negative

- Combination of blood, bone marrow, and intestinal secretions culture is the best method in the first week (sensitivity >90%)
- Rose spots, pus from suppurative lesions, CSF, sputum and autopsy specimens such as gallbladder, liver and spleen.

- **Culture smear and motility:** Motile, GNB
- **Biochemical identification**
  - Catalase positive and oxidase negative
  - ICUT: Indole(–), Citrate(+/–), Urease(–) ,TSI:K/A, gas(+) except in *S.* Typhi,
  - H2 S (*S.* Typhi- small speck, *S.* Paratyphi A-absent, *S.* Paratyphi B-abundant).
- **Slide agglutination test:** To confirm the serotype
- **AST**

- **Serum antibody detection (Widal test):** 2–3 weeks of illness

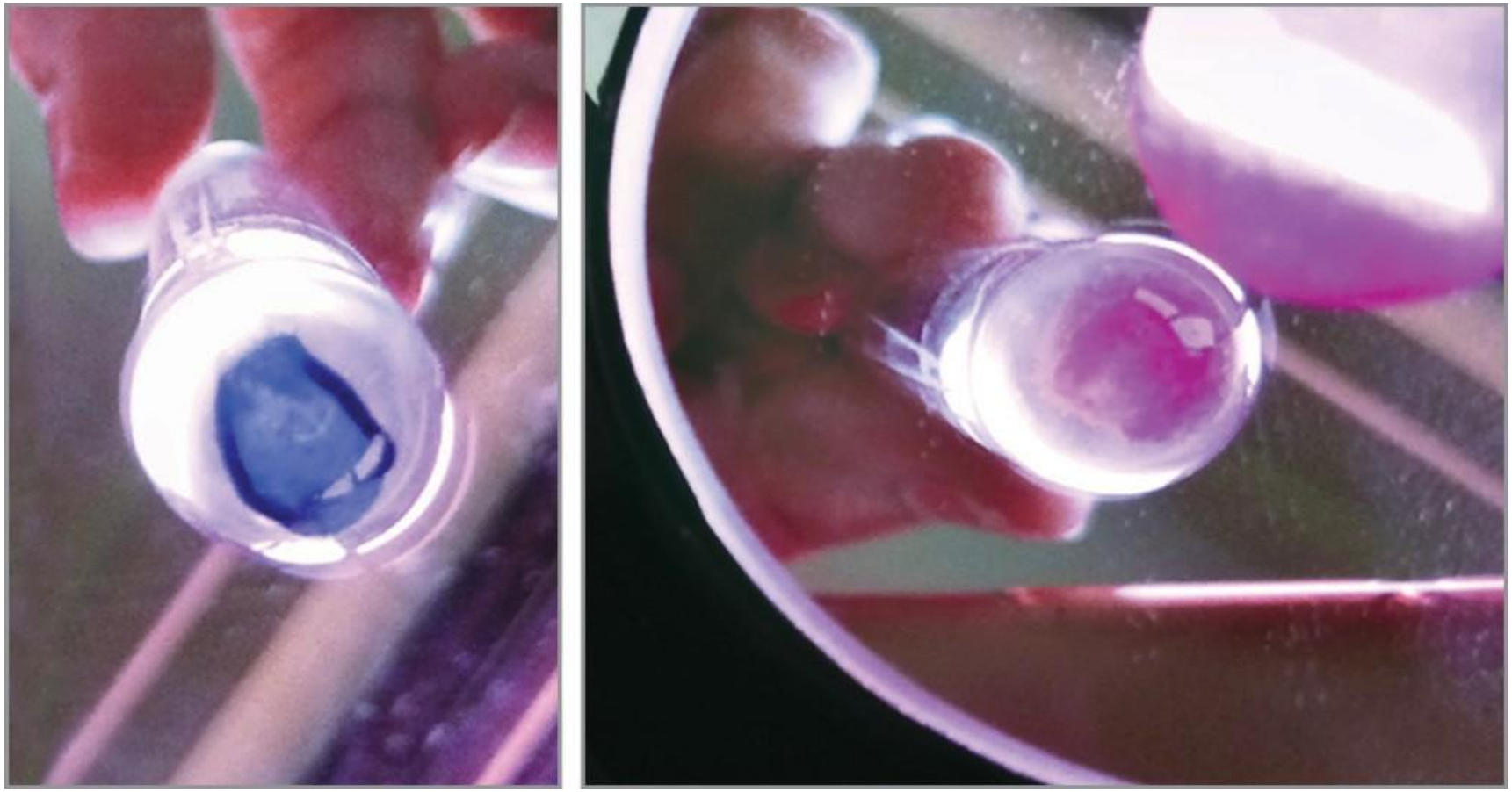
Antibodies are detected against TO, TH, AH, BH antigens

- In *S.* Typhi infection: ↑TO and TH antibodies
- In *S.* Paratyphi A infection: ↑TO and AH antibodies
- In *S.* Paratyphi B infection: ↑TO and BH antibodies.

Result and interpretation

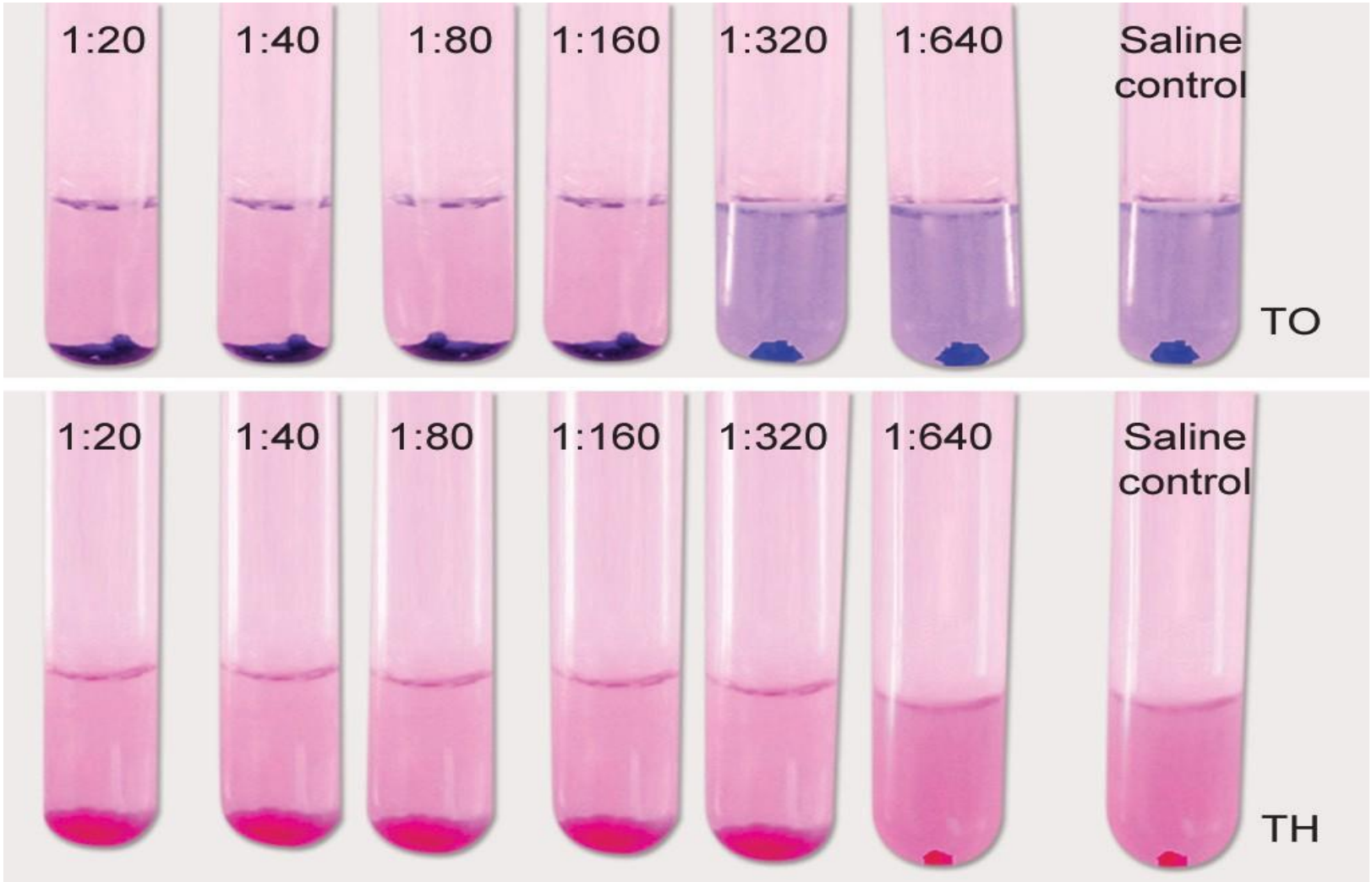
- O antibodies: Produce granular chalky clumps when react with O Ag
- H antibodies: Produce cottony woolly clumps when react with H Ag.

Widal test



O and H agglutination in Widal test (reading taken in a mirror)

Widal test



Widal test showing titre of TO 1:160 and TH 1:320.

Interpretation of Widal test

Widal test result	Suggestive of
Rise of TO and TH antibody	Enteric fever due to *S*.Typhi
Rise of TO and AH antibody	Enteric fever due to *S*.Paratyphi A
Rise of TO and BH antibody	Enteric fever due to *S*.Paratyphi B
Rise of only TO antibody	Recent infection -Due to any serotype -*S*.Typhi or *S*.Paratyphi A or B
Rise of only TH antibody	? Convalescent stage/ Anamnestic response
Rise of all three TH, AH, BH antibodies-	Post TAB vaccination

- **False-positive**: Widal test may occur due to:
  - Anamnestic response: It refers to a transient rise of titer due to unrelated infections (malaria, dengue) in persons who have had prior enteric fever
  - If bacterial antigen suspensions are not free from fimbriae
  - Persons with inapparent infection or
  - Persons with prior immunization (with TAB vaccine).

- **False-negative**: Widal test may occur in:
  - Early - stage (1st week of illness)
  - Late - stage (after fourth week)
  - Carriers
  - Patients on antibiotics
  - Due to prozone phenomena (antibody excess) - this can be obviated by serial dilution of sera.

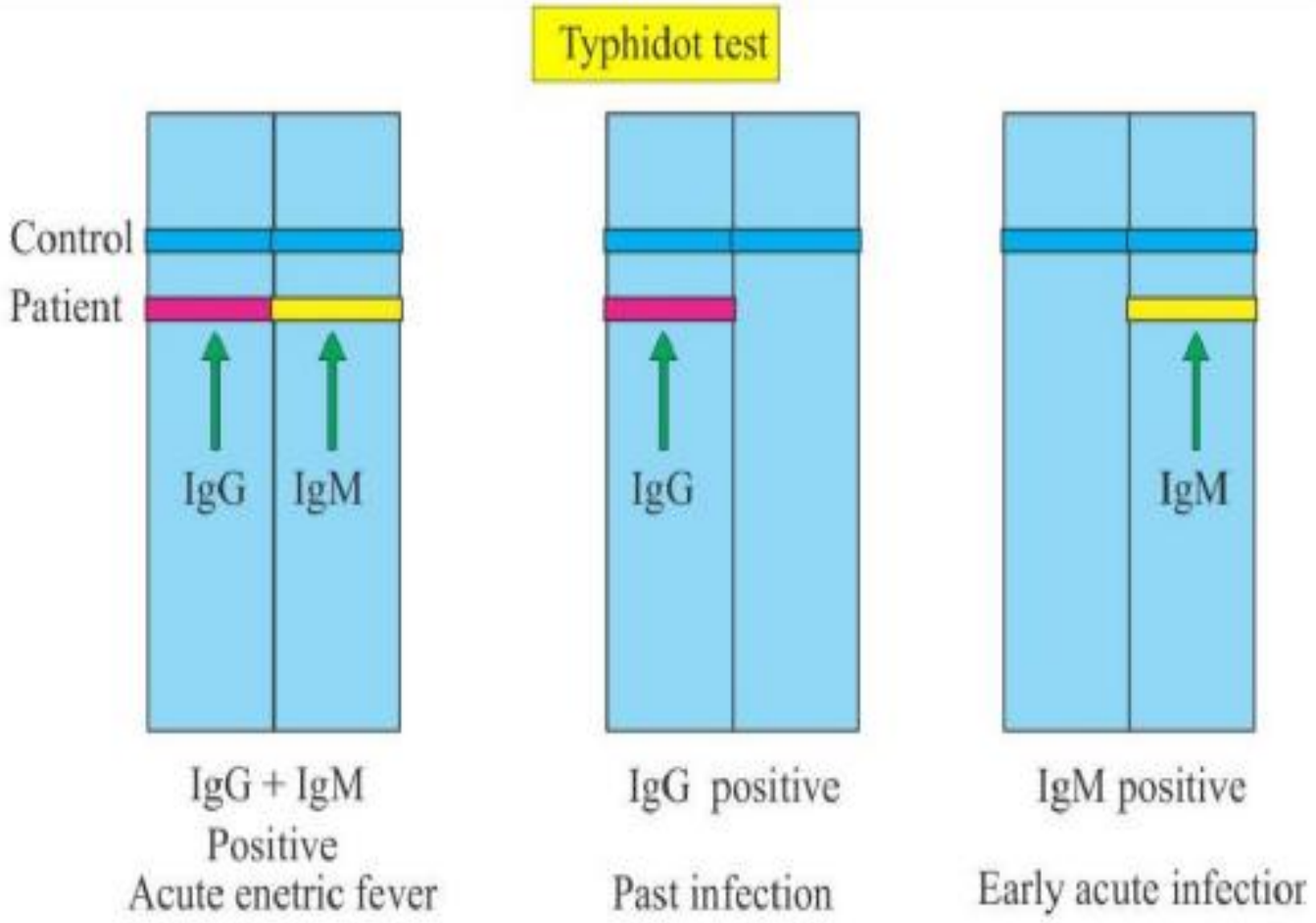
- **O agglutinins** appear **early and disappear early** → Recent infection.
- **H agglutinins** appear **late and disappear late.**

- **O antibodies** are serotype **nonspecific** (raised in all infections, i.e. *S.* Typhi, *S.* Paratyphi A and B)
- H antibodies are specific. TH, AH and BH antibodies are raised in *S.* Typhi, *S.* Paratyphi A and B infections respectively.

Other Antibody Detection Tests - commercial methods

- **Tyhidot test:** 50 kDa OMP antigen is used; it uses a dot ELISA format to detect both IgM and IgG separately after 2-3 days of infection
- **IDLTubex test:** O9 antigen is used, detects only IgM antibodies against *S.* Typhi by a semiquantitative colorimetric method
- **IgM dip stick test and ELISA** detect anti-LPS IgM antibodies
- **Dot blot assay:** Flagellar antigen is used, detects only IgG antibodies.

TYPHIDOT TEST



- **Demonstration of serum antigens –** ELISA
- **Molecular methods-** PCR (*flagellin* gene, *Iro B* and *fliC* gene)
- **Other non specific tests –** neutropenia, LFT moderately changes, muscle enzymes moderately elevated

Detection of carriers

- **Culture:** By stool and bile culture (fecal carriers) & urine culture (urinary carriers)
- **Detection of Vi antibodies:** Tube agglutination test by using *S.* Typhi suspension carrying Vi antigen (Bhatnagar strains) → Titer of ≥1:10 considered as significant. (diagnosis should always be confirmed by culture)

Detection of carriers

- **Isolation of salmonellae from sewage**
  - Sewer–swab technique: Gauze pads left in sewers are cultured on highly selective media, such as Wilson and Blair media
  - Filtration: Sewage can be filtered through Millipore membranes and the membranes are cultured on highly selective media.

Drug Resistance in Typhoidal Salmonellae

- **Multidrug-resistant (MDR) *S.* Typhi** - resistant to chloramphenicol, ampicillin and cotrimoxazole.
- **Fluoroquinolone (FQ) resistance -** key mechanisms responsible - mutations in *gyrA* and *parC* genes.
- **Resistance to ceftriaxone -** very rare (<1%), both ESBLs and AmpC β-lactamase producing *S.* Typhi have been detected

Prophylaxis

- Control of Reservoir
- Sanitation Measures
- Vaccine

Vaccines for Typhoid Fever

1. Parenteral Vi polysaccharide vaccine:

- Purified Vi capsular polysaccharide antigen derived from *S.* Typhi strain *Ty2*
- Dosage: Single dose given IM or subcutaneously
- Protection for 2 years, Booster every 2 years
- Age: >2 years of age
- **Vi-rEPA:** Vi antigen is conjugated with recombinant *Pseudomonas aeruginosa Exotoxin A*
- Can be given to children less than two years

2. Typhoral (oral live attenuated S. Typhi Ty21a vaccine):

- Stable live attenuated mutant of *S. Typhi strain* Ty21a
- Gal E mutant - lacks the enzyme UDP-galactose-4-epimerase
- Multiplies for some time initiates the immune response but self-destructs after 4–5 divisions
- Indicated only after 6 years of age

- Enteric coated capsules
- Before food on alternate days - 1, 3, 5, 7 with booster every 5 years
- Protective immunity starts after 7 days of the last dose and lasts for 4 years

3. Parenteral TAB vaccine

- It is a heat-killed whole cell *S.* Typhi/*S.* Paratyphi A and B vaccine
- It is no longer in use because of significant side effects.

NON - TYPHOIDAL SALMONELLA

- Non-typhoidal salmonellae cause mainly gastrointestinal manifestations.
- However, upto 8% of patients with NTS gastroenteritis develop into bacteremia - lead to either endovascular infection or seedling to various organs leading to metastatic infections.
- *S*. Choleraesuis (source-pig) and *S*. Dublin (source—cattle).

THANK YOU