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ZAPOROZHYE STATE MEDICAL UNIVERSITY

THE CHAIR OF MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY

Intestinal group of diseases

Practicum on Microbiology, Virology and Immunology

for foreign students
of III course of the medical faculty,
specialty "Medicine"

Zaporozhye - 2019

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МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ

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Кишкова група захворювань

Практикум з мікробіології, вірусології та імунології

для іноземних студентів
III курсу медичного факультету,
спеціальність «Медицина»

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Практикум з мікробіології, вірусології та імунології для іноземних студентів III курсу медичного факультету, спеціальність «Медицина».

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INTESTINAL INFECTIONS

The predominant aerobic bacterial flora the larger intestines of humans and animals is composed of nonsporing, nonacid fast, Gram negative bacilli. They exhibit general morphological and biochemical similarities and are grouped together in the larger and complex family ***Enterobacteriaceae.*** Members of this family may or may not be capsulated and are motile by peritrchate flagella, or are nonmotile.

Within the family, they exhibit very wide biochemical and antigenic heterogeneity.

The Enterobacteriaceae are a large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals. The family includes many genera (eg, Escherichia, Shigella, Salmonella, Proteus, and others). Some enteric organisms, eg, Escherichia coli are part of the normal flora and incidentally cause disease, while others, the salmonella and shigellae, are regularly pathogenic for humans. The Enterobacteriaceae are facultative anaerobes or aerobes, ferment a wide range of carbohydrates, prossess a complex antigenic structure, and produce a variety of toxins and other virulence factors.

They are Gram negative rods, either motile with peritrichous flagella or nonmotile; they grow on peptone or meat extract media without the addition of sodium chloride or other supplements; grow well on Endo medium. Many complex media have been devised to help in identification of the enteric bacteria.

Enterobacteriaceae have a complex antigenic structure. It is heat-stable somatic O (lipopolysaccharide antigens) heat-labile K (capsular) antigens and H (flagellar) antigens.

Most gram-negative bacteria possess complex lipopolysaccharides in their cell walls. Many Gram negative enteric bacteria also produce exotoxins of clinical importance.

Escherichia coli is member of the normal intestinal flora. Other enteric bacteria (Proteus, Enterobacter, Klebsiella, Serratia species) are also found as members of the normal intestinal flora but are considerably less common than E.coli.

The enteric bacteria generally do not cause disease, and in the intestine they may even contribute to normal function and nutrition. Some of the enteric bacteria (eg, Serratia, Enterobacter) are opportunistic pathogens.

LABORATORY DIAGNOSIS OF INFECTIONS CAUSED BY ESCHERICHIA COLI

Theme topicality. In spite of definite achievements in the fight against acute intestinal infection diseases, they continue to take considerable place in the infectious pathology of a human. People of different age have the intestinal disease, but children of preschool age fall ill more frequently. The highest morbidity is registered in the age group of 0–2 years.

E. coli are not only agents of diarrhoea, but also cause pyoinflammatory processes (e.g., meningitis, cystitis, peritonitis, sepsis) of different localization, and also are indicators of fecal contamination of environment, therefore the knowledge of biological features of agents of such diseases and microbiological diagnostics are necessary for doctors of various specialisations.

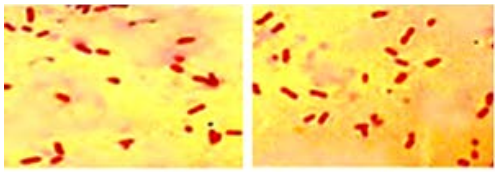
Primary objective: to be able to conduct and evaluate the microbiological diagnostics of intestinal and parenteral Escherichiosis.

QUESTIONS FOR STUDYIN

1. General description of the *Enterobacteriaceae* family bacteria.
2. Biological properties of *E. coli*, antigenic structure and classification of pathogenic *E. coli.*
3. Microbiological diagnosis of *E. coli* associated with diarrhoeal diseases and colibacillosis.
4. Epidemiology and pathogenesis of diarrhoea-causing *E. coli*. Specific features of the immunity in diarrhoea caused by *E. coli.*
5. Principles of prophylaxis and medical treatment of such type of diarrhoea.

PROCEDURE OF PRACTICAL WORK

1. Study the preparation of *E. coli* pure cultures microscopically; draw them in the protocol.



E. coli O_1:K_1 *E. coli* O_{55}:K_{59}:H_1

***E. coli*, Gram staining**

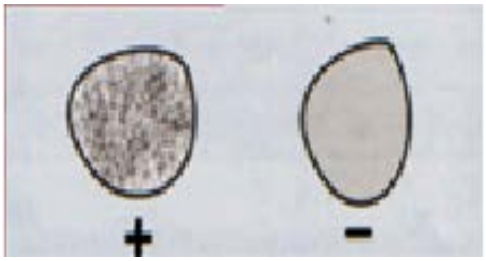
All types of *E. coli*. have identical staining and morphological properties. Therefore, Gram staining of pure culture of *E. coli* in preparation makes it possible to see gram-negative monobacteria of medium size. Morphological and tinctorial properties of cultures of pathogenic (*E. coli* O_{55}:K_{59}:H_1) and opportunistic pathogenic (*E. coli* O_1:K_1) *E. coli* do not differ.

2. Study cultural properties of causative agents on Endo agar plate: sketch colonies, make conclusions, and mark the plan of the further researches.

Cultural properties of *E. coli*

Endo and EMB agars are special media capable to expose the ability of bacteria to form pigment. All colonies of *E.coli* on the Endo agar are convex, rounded with the even edges, with medium size and crimson with the the metallic sheen. On the EMB agar plate *E.coli* formed dark blue-violet colonies with the tint of green.

3. **Study the antigenic structure of Escherichia and estimate the result of slide agglutination test with Escherichia polyvalent agglutination OK- and monovalent group sera.**

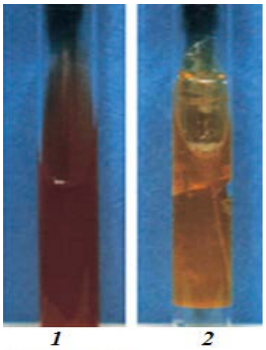


Slide agglutination test

The antigenic structure of *E. coli* in the relation of microorganism to the pathogenic serogroup is set, because colonies of pathogenic and opportunistic pathogenic *E. coli* on the Endo and EMB agars do not differ.

For this purpose put slide agglutination test with the Escherichia polivalent agglutinating OKA-serum (contains agglutinins for 22 O and K antigen of *E. coli*), and further – with OKB-, OKC-, OKD- or OKE-sera (slide agglutination test is performed according to the general method).

4. **Study the growth of *E. coli* on the triple sugar iron (TSI) agar.**

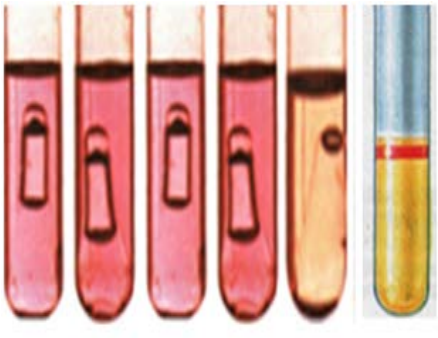


1. Control
2. Growth of E. coli

Triple sugar iron agar with *E. coli*

Agglutinable *E. coli* ferments glucose and lactose, which are the components of the medium with formation of acid and gas. There is discolouration of all medium volume in comparison with initial colour (rose colour of medium changes to yellow).

5. **Study the biochemical properties of *E. coli* on the Hiss medium.**

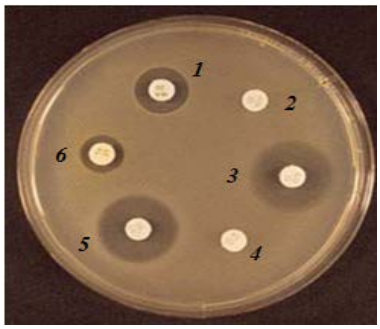


Biochemical properties of *E. coli*

on the Hiss medium

Agglutinable *E. coli* ferments the Hiss media with glucose, lactose, mannitol, and maltose with formation of acid and gas. The proof of the fermentation of sugars is the discoloration of the media, and of gasification – formation of blebs. *E. coli* does not ferment sucrose, and does not form sulphuretted hydrogen at the cultivation of it on the peptone water, but the reaction to the indol is positive.

6. Study the sensitivity of *E. coli* to antibacterial preparations.



Sensitivity of *E.coli* to antibacterial preparations

The size of the growth retardation zones of microorganisms around the disks depends on sensitivity of the causative agent to the given antibiotic.

The strain is considered to be stable if the diameter of the zone is less than 10 mm, weakly sensitive if it averages 11–15 mm, and sensitive if it reaches 15–25 mm. Zones exceeding 25 mm in diameter indicate high sensitivity of the microorganism to the given antibiotic.

6. Study the main antimicrobial drugs used for treatment, prevention, and diagnosis of suppurative diseases. Write them in your copybook.

Escherichia agglutinating polyvalent OKA-serum is a diagnostic preparation, which contains specific antibody to the superficial (K-) and somatic (O-) antigens of *E. coli*, which together with the proper antigens of *E. coli* cause their agglutination. Preparation is obtained from the serum of the rabbit, hyperimmunized by the mixture of corpuscular antigens of *E. coli* (O_{18}:K_{84}, O_{23}: K_{11}, O_{75}:K, etc.). Preparation is used for the determination of the pathogenic *E. coli* serogroup in slide agglutination test.

Preparation is preserved by chloroform, packed up and lyophilized. At the reaction of agglutination it is necessary to make the working solution of the serum 1:10.

Escherichia agglutinating adsorbed monovalent serum O_{55} is a diagnostic preparation, which contains specific antibody to the somatic (O_{55}) antigen of *E. coli*, which together with O_{55} antigen of *E. coli* causes their agglutination. Preparation is obtained from the serum of the rabbit, hyperimmunized by a corpuscular O_{55} antigen of *E. coli* with the subsequent adsorption of unspecific antibodies. Preparation is used for the slide agglutination test (with the heated culture) to determine O_{55} antigen of *E. coli*, the serogroup of *E. coli*.

Colibacterin is a therapeutic and prophylactic preparation, which contains lyophilical cells of the *E. coli* M17 stain, which express antagonistic characteristics in relation to the definite pathogenic intestinal bacteria. Preparation is used for prevention and treatment of overgrowth in children. Colibacterin is administered to children from the 6-month age. While using this remedy the simultaneous use of vitamins is necessary.

Bifidumbacterin is a therapeutic and prophylactic preparation, which contains lyophilical cells of *Bifidobacterium bifidum.* It is used for prevention and treatment of overgrowth in children.

Bificol is a therapeutic and prophylactic preparation, which contains lyophilical cells of the *E. coli* M17 stain and *B. bifidum.* It is used for normalization of the intestinal microflora at overgrowth .

Lactobacterin is a therapeutic preparation, containing obligate for the human lactic-acid bacteria and their waste products, which show antagonistic activity in relation to the pathogenic and opportunistic pathogenic enterobacteria.

It is produced in the form of tablets and is used for the medical treatment of overgrowth in children.

Laboratory diagnosis of the infection caused by *Escherichia coli*

Notion	Definition/explanation
Morphological, physiological and tinctorial features of *Enterobacterium*	Rod-shaped asporogenic capsular or acapsular peritrichous or immobile gram-negative monobacteria of *Enterobacteriaceae* family: aerobes or facultative anaerobes
Bacteria from *Enterobacteriaceae* family, that are of high importance for human	*Escherichia, Shigella, Salmonella, Citrobacter, Enterobacter, Klebsiella, Yersinia, Proteus*
Diseases caused by *Enterobacteriaceae*	1. Intestinal infections (colienteritis, shigellosis, typhoid fever, food toxic infections, and yersiniosis) which are caused by the representatives of genus of *Escherichia, Shigella, Salmonella, Yersinia*. They are characterized by the faecal-oral or oral mechanism of transmission via water, food or contact and house routes of the infection transmission. 2. Opportunistic (extraintestinal) infections (bacteriemia, meningitis of newborn, traumatic, genito-urinary, and respiratory infections) which are caused by the opportunistic pathogenic bacteria of *Escherichia, Klebsiella, Citrobacter, Enterobacter, Proteus* genus on the background of immunodeficiency
Systematic position of colibacillosis causative agents (family, genus,	Family: *Enterobacteriaceae.* Genus: *Escherichia.* Species: *Escherichia coli*

Morphological and tinctorial features of Escherichia	Straight asporogenic gram-negative rods with rounded ends, which have capsule or microcapsule, peritrichous
Respiration of *E. Coli*	Aerobes and facultative anaerobes
Antigens of *E. coli* and their value	Somatic O antigen (lipopolysaccharide of external membrane) determines the serogroup of *Escherichia* and provides primary differentiation of diarrhoeal *E. coli* from opportunistic pathogenic one. Surface (capsular) K antigen (polysaccharide of capsule and outer membrane) determines the antigen features of strains. Flagellar H antigens (flagellin protein) determine a bacteria type-specificity. O and H antigens of *E. coli* are basic antigens which determine a bacterium serovar
Denomination of the antigenic structure of *E. coli* (serogroup, serovar)	Formula of serogroup is O:K (O_{125}:K_{70}), formula of serovar is O:K:H (O_{142}:K_{76}:H_{18}, O_{124}:K_{72}:H-, O_{75}:K-:H_7)

Serovar of microorganism	Serological variant, which has a certain antigenic structure by O, K and H antigens or O and H antigens
E. coli according to antigenic and pathogenic properties	1. Opportunistic pathogenic bacteria. 2. Pathogenic (diarrhoeal)
Value of opportunistic pathogenic *E. coli*	1. It is found in the normal microflora of small and large intestines and vagina. 2. It is basic intestinal microflora of mammals, birds, reptiles, and fish. 3. There are agents of opportunistic infections (parenteral colibacillosis). 4. It is sanitary representative model of microorganisms (indicators of faecal environmental contamination)
Parenteral infection caused by *Escherichia coli*	Opportunistic infections which are caused by different serogroups (O_1:K_1, O_2:K_5, O_8:K_1, O_{18}:K_{23}) of endogenic opportunistic pathogenic *E. coli.* It causes pyoinflammatory processes of different localization: peritonitis, cystitis, cholecystitis, meningitis of newborn, sepsis on a background of immunodeficiency
Enteral (diarrhoeal, intestinal) infection caused by *E. Coli*	Infections caused by the agents of the different exogenous pathogenic *E. coli* serotypes

Table continuation

Notion	**Definition/explanation**
Source of diarrhoeal infection caused by *E. Coli*	Patient, bacteria carrier, animals (at enterohaemorrhagic *Escherichia coli* gastrointestinal disease)
Types of *E. coli*, which cause gastrointestinal disease	EPEC – enteropathogenic *E. coli* EIEC – enteroinvasive *E. coli* ETEC – enterotoxigenic *E. coli* EHEC – enterohaemorrhagic *E. Coli*
Enterotoxigenic *E. coli* and mechanism of pathogenic action of ETEC on epithelium	ETEC causes cholera-like gastrointestinal disease in children
Enteropathogenic *E. coli* and mechanism of pathogenic action of EPEC on intestinal cells	EPEC are agents of colienteritis (salmonella-like infection) mainly in children of the first year of life with the house, contact, and food routes of infection transmission. EPEC propagates on the surface of epithelium of small intestines, stipulating thinning, fragmentation, and tearing away of microvilli, damage of apical surface of epithelium, resulting in moderate inflammation and appearance of shallow erosions
Enterohaemorrhagic	EHEC are agents of haemorrhagic diarrhoea (haemorrhagic

E. coli, mechanism of pathogenic action of EHEC on epithelium and endothelium	colitis) in adults and in new-born can predetermine necrotic enterocolitis with the high percentage of lethal consequencies with the damage of sigmoideus, ascending and transversal large intestines; alimentary route of infection transmission. EHEC propagates oneself on the surface of epithelium of colon with destruction of microvilli, damage of apical surface of epithelium.
Method of microbiological diagnosis of gastrointestinal infection caused by *E. coli* and its features	Bacteriological. Identification of agents is based on the primary determination of *E. coli* antigenic structure. The study of biochemical properties of bacteria allows to confirm genus and species of culture, and to set its biovar
Material for microbiological diagnosis of gastrointestinal infection caused by *E. coli*	Faeces

Material for microbiological diagnosis of colibacillosis	Blood (at bacteriemia), urine (at diseases of urinogenital system), cerebrospinal fluid (at meningitis of newborns), sputum (at respiratory infections)
Features of Endo and EMB agar and purpose of their application	– Selective and differential diagnostic media for isolation of *Escherichia* and *Shigella*. Components of media are inhibitors for gram-positive microflora. The media are used for differentiation of bacteria on: – lactose-positive (lac^+ bacteria ferment lactose): strains of opportunistic pathogenic *E. coli* (ETEC, EPEC, EHEC) – lactose-negative (lac^- bacteria don't ferment lactose): strains of EIEC, *Shigella*, *Salmonella* –
Purpose and components of slide agglutination test	Determination of serogroupe of *E. coli* pathogenic strains. Material of colony, *Escherichia* agglutinating polyvalent OK-serum, 0.9% sodium chloride
Properties are necessary for *E. coli* identification	Antigenic structure (in slide agglutination test and tube agglutination test with groupspecific and monovalent escherichiosis sera – basic) and morphological, tinctorial, cultural, and biochemical properties
Immunity after colibacillosis	Humoral, untense type-specific (IgM appears only to that serovar, which has entailed a disease). Local immunity (slgA production by lymphoid cells of the intestine)
Drugs used for intestinal microflora correction	Colibacterin, bificol, bifidumbacterin, lactobacterin

Scheme of diarrhoea-causing *E. coli* laboratory diagnosis

Faeces, rectal swab

Stage 1

Primary growth in Endo and EMB (Levin's) differential diagnostic media in order to receive isolated colonies

Stage 2 **Estimation of the growth result**

Type and colour of colonies	Preparation, Gram staining	Serotyping: reference slide agglutination tests with the material of lactose-positive colonies (10) with polyvalent OK-antisera against pathogenic serotypes of Escherichia

Preliminary result

Reinoculation of agglutinable colonies	
on triple sugar iron agar (TSI)	on nutrient agar (pure culture obtaining)

Stage 3 **Identification of the pure culture**

Biochemical properties: growth on Hiss media and peptone water	Study of sensitivity to antibiotics	**Serotyping:** Reference slide agglutination tests with polyvalent OK-antisera. Reference slide agglutination tests with monovalent OK-antisera. Comprehensive agglutination reaction with monovalent OK-antiserum

END RESULT

LABORATORY DIAGNOSIS OF SHIGELLOSIS

Theme topicality For many years both in Ukraine and in the whole world,

shigellosis has been a major infection among acute intestinal infections. Especially tight epidemiologic situation is found in countries with low level of development of economy, culture, food industry, and infrastructure of the inhabited cities.

Shigellosis (bacillary dysentery) is on acute anthroponosis disease with faecal-oral mechanism of the causative agent transmission, which is caused by bacteria of *Shigella* genus and characterized by enteropathy (mainly distal section of the large intestine), general intoxication, and frequent watery stool with a mixture of mucus and blood, and tenesmus.

Shigellosis control is difficult through polymorphism of clinical displays, variety of factors of causative agent transmission, general receptivity in connection with short duration of species-specific immunity after the illness, and through high adaptation ability of *Shigella* which forms fast high resistance to antibacterial properties.

Knowledge of microbiology of shigellosis is important for doctors; it will help them to diagnose correctly and give proper medication.

Primary objective: to be able to conduct and evaluate the microbiological diagnostics of shigellosis.

QUESTIONS FOR STUDYIN

1. Biological features of *Shigella spp*.
2. Microbiological diagnostics of shigellosis.
3. Epidemiology and pathogenesis of shigellosis. Features of shigellosis immunity.
4. Basic measures for prophylaxis and treatment of shigellosis.

PROCEDURE OF PRACTICAL WORK

1. **Study the slide mounts of *S. flexneri 2a, S. sonnei* and *E. coli* O_{55}:K_{59}:H_1; compare results of microscopy, make conclusions, and sketch the results of the microscopy.**

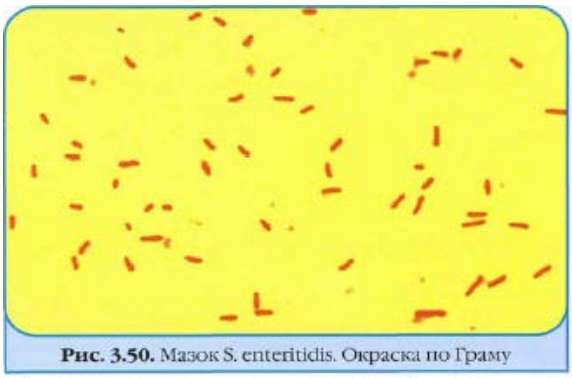


Рис. 3.50. Мазок S. enteritidis. Окраска по Граму

Smear from pure culture of Shigella.
Staining by Gram

Gram stained *S. flexneri 2a* and *S. sonnei* are lines of gram-negative monobacteria which have similar morphological and tinctorial properties.

Classification. Shigellae are classified into 4 species or subgroups based on a combination of biochemical and serological characteristics. Serotypes are distinguished within the species.

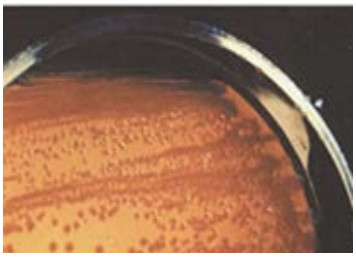
S. dysenteriae (subgroup A). This species of mannitol nonfermenting bacilli consists of 12 serotypes. Type 1 is the bacillus originally described by Shiga (*S. shigae).*

S. flexnery (subgroup B). This group is named after Flexner, who described the 1st of the mannitol fermenting shigellae from Philippines (1900). This group is biochemically heterogeneous and antigenically the most complex among shigellae. Based on type specific and group specific antigens, they have been classified into 6 serotypes (1-6) and several subtypes.

S. boydii (subgroup C). This group consists of dysentery bacilli that resemble S.flexneri biochemically but not antigenically. The group is named after Boyd, who 1st described these strains from India (1931). 18 serotypes have been identified, the last 3 having been described only in 1985. *S. boydii* are isolated least frequently from cases of bacillary dysentery.

S. sonnei (subgroup D). This bacillus 1st described by Sonne (1915) in Denmark, ferments lactose and sucrose late. It is indole negative. It is antigenically distinct and homogeneous but may occur in 2 forms- phase I and phase II-the latter forming colonies that are larger, flatter and more irregular.

Task 2. Study cultural properties of bacteria of *Shigella* genus on EMB agar and Hektoen enteric agar, sketch colonies, make conclusions, and mark the plan of the further investigation.



On EMB and Hektoen enteric media microorganisms form colourless (lactose-negative) shallow (1–1½ mm) convex, circular transparent smooth colonies with intact edges.

Cultural properties of *Shigella spp.*

2. **Study antigenic structure of the causative agent in slide agglutination test with shigellosis agglutination polyvalent serum for identification of the causative agent.**

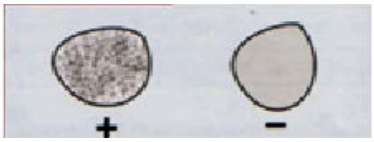


Figure 3.2.3 – Slide agglutination test with shigellosis polyvalent agglutination serum

Serologic identification of the isolated culture is conducted by the slide agglutination test at first with polyvalent and further with monospecific (for determination of the causative agent type), typical (for determination of the causative agent serotype) and group (for determination of causative agent subserotype) agglutination serum.

The results of serologic identification of the causative agent have a decisive value for determination of the serotype (subserotype) of the microorganism, in comparison with the investigation of the biochemical properties.

Task 4. Study the biochemical properties of *Shigella* on the triple sugar iron agar (TSI) and Hiss medium.

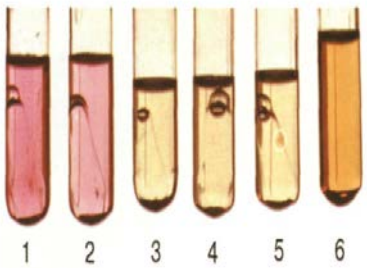
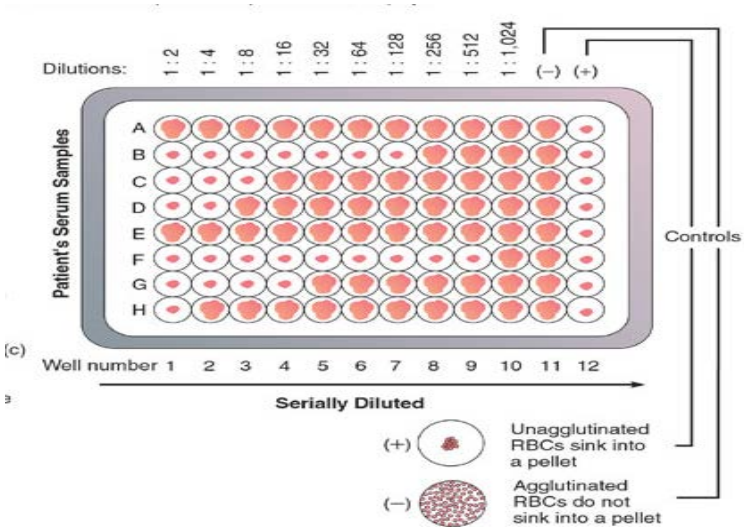


Figure 3.2.4 – Biochemical properties of *Shigella* on Hiss medium

Shigella flexneri ferments Hiss medium with glucose, and mannitol with formation of acid and gas.

The proof of the fermentation of sugars is discolouration of the medium. *Shigella flexneri* does not ferment sucrose, lactose, maltose, gives the negative reaction on the sulphuretted hydrogen at the cultivation of it on the peptone water, but the reaction on the indol is positive.

Task 5. Make the consideration of PHAT with erythrocyte diagnosticum of Flexneri for serological diagnostics of shigellosis.



PHAT for serological diagnostics of shigellosis

Task 6. Characterize diagnostic, therapeutic, and prophylactic preparations.

Shigellosis adsorbed agglutinating polyvalent serum is a diagnostic preparation which contains specific antibodies to the antigens of *S. flexneri 1–6* and *S. sonnei;* when mixing the specific antibodies with the proper antigens of shigella there is formation of agglutination. Agglutinating serum is obtained from hyperimmunization of rabbit, mixture of serotype of *S. flexneri 1–6* and *S. sonnei* with the adsorption of intergroup and other nonspecific antibodies.
It is used for slide agglutination test at the bacteriological method of culture investigation with the purpose of identification of *Shigella* from *Salmonella* and EIEC, which also form lactose-negative colonies on EMB agar and Hektoen enteric medium.

Adsorbed agglutinating typical (serospecific) serum for *Shigella flexneri* (type II) is a diagnostic preparation, which contains specific antibodies to typical antigen of *S. flexneri*. Agglutinating serum is obtained from hyperimmunization of rabbits by *S. flexneri type II* antigen with adsorption of other antibodies. It is used for slide aglutination test at the bacteriological method of culture investigation with the purpose of *S. flexneri* serotype determination.

Adsorbed agglutinating group 3, 4 *Shigella flexneri* serum is a diagnostic preparation which contains specific antibodies to the group of antigen 3, 4 *S. flexneri*. Agglutinating serum is obtained from hyperimmunization of rabbits, the antigen of *S. flexneri* of the groups 3, 4 with adsorption of other antibodies. It is used for slide aglutination test at the bacteriological method of culture investigation with the purpose of *S. flexneri* subserotype determination (*S. flexneri 2a* subserotype).

Shigellosis luminescent serum is a diagnostic preparation for express diagnosis of the shigellosis causative agents in the material of the patient, foodstuff and water (method of express diagnosis) or at the bacteriological method of causative agents investigation of shigellosis (with a culture, cultivated in enrichment medium). It contains known antibody that is labeled by fluorochrome. At formation of complex of shigellae specific antibody with causative agents of shigellosis, that labeled fluorochrome, there is luminescence on the luminescent microscope.

Shigellosis Sonnei diagnosticum is a diagnostic preparation which contains suspension of inactivated *Shigellae sonnei*. Agglutinate forms after mixing of the preparation with patient serum. Preparation is used in tube agglutination reaction to determine titre of specific antibodies in the patient with Sonnei shigellosis (serological method of diagnostics).

Shigellosis erythrocyte Sonnei diagnosticum is a diagnostic preparation that contains sheep erythrocyte with *S. sonnei* adsorbed on their surface. Preparation is used for the exposure of specific antibodies to *S. sonnei* in the patient with shigellosis and persons with the atypical form of shigellosis (serological method of diagnostics) by the reaction of indirect haemagglutination (PHAT) with paired sera. *S. sonnei* agglutinate with specific antibodies in blood serum of patient and form "friable" agglutination with unequal edge (inverted umbrella) at the bottom of test tubes or disk caps.

Polyvalent shigellosis (liquid) bacteriophage is diagnostic preparation which contains the sterile filtrate of bacteriophages, that lyse *S. flexneri* and *S. sonnei.* It is used for phagotyping of the isolated pure culture of *Shigellae* (to confirm isolated pure culture of *Shigellae* to *Shigellae* genus).

Polyvalent shigellosis (tableted) bacteriophage is a therapeutic and prophylactic preparation that contains the sterile filtrate of bacteriophages, that lyse *S. flexneri of 1–6 serotype* and *S. sonnei*. It is produced as tablets with acid-resisting coverage and used for the emergency prophylaxis of shigellosis and treatment of acute shigellosis.

Lactoglobulin is a preparation for treatment of patients with the subclinical form of shigellosis and its for prophylaxis. It contains sIgA from milk of cows hyperimmunized by shigellae.

Eubiotics: colibacterin, bifidumbacterin, bificol, lactobacterin are preparations for treatment and prophylaxis.

Laboratory diagnostics of shigellosis

Notion	Definition/explanation
Shigellosis (bacillary dysentery)	Acute antroponosis disease with faecal-oral mechanism of the causative agents transmission, which is caused by bacteria of *Shigella* genus. It is characterized by enteropathy (mainly distal section of large intestine), general intoxication, frequent watery stool with

	admixture of mucus and blood, and tenesmus
Taxonomic position of the shigellosis causative	*Enterobacteriaceae* family. *Shigella* genus

Table 3.2.1 continuation

Notion	Definition/explanation
agents	Species: *S. dysenteriae, S. flexneri, S. boydii, S. sonnei*
Features of shigellosis	The heaviest course of shigellosis is caused by *S. dysenteriae,* which produces Shiga toxin (exotoxin), less difficult course of shigellosis is caused by *S. sonnei*
Morphological and tinctorial features of the shigellosis causative agents	Short lines with rounded ends gram-negative immobile monobacteria. Spores and capsules are not formed
Respirstory type of shigellae	Facultative anaerobes
Biological properties of *Shigellae*, describing their pathogenicity	**1.** Adhesiveness, penetration into the epithelium of mucous membrane of the large intestine and intracellular duplication in them and in macrophages without their destruction **2.** External membrane of bacteria protects it from the action of gastric juice. **3.** K antigen, antigens 3, 4, and lipopolysaccharides protect bacteria from phagocytosis. **4.** Lipid A endotoxin of shigellae has immunosupressive action (represses activity of cells of immune memory)
Basic biotope of causative agents	Sigmoid colon and rectum
Factors of *Shigellae* pathogenicity	1. Toxins and toxic substances providing development of pathological process (basic factor of pathogenicity). 2. Factors of adhesion and colonization (provide interaction with epithelium). 3. Factors of invasion (resistance to the humoral and cellular defence mechanisms of macroorganism and productive capacity in cells of the macroorganism)
Factors providing the cooperation of the shigellae with epitheliocytes	Pili, lipopolysaccharides, enzymes: mucinaze, plasmocoagulaze, hyaluronidase, fibrinolysin
Invasive factors of shigellae, essence invasion, and its consequences	Proteins of external membrane (invasines) and proteins of endocellular distribution provide penetration of *Shigella* in enterocyte, reproduction in enterocytes and macrophages, cause apoptosis of macrophages, lysis of cell membranes, providing endocellular and intercellular distribution of shigella
Toxins of shigella	Exotoxins are Shiga toxin and Shiga-like toxins, enterotoxin (LT), endotoxin (lipopolysaccharide which is linked with O antigen of the cell wall)
Features of the Shiga toxin which is synthesized by *S. dysenteriae 1* (*Shigella* of Grigoriev-Shiga)	Neurotoxic property. Enterotoxic (aggravation of diarrhoeal syndrome is a result of adenylate cyclase activation, increase of water excretion in the intestine cavity) and cytotoxic (deranged synthesis of the proteins, absorption of Na^+ and water causing death of enterocyte and increases liquid in the center of an inflammation) activities. Toxins strike endothelium of submucous membrane of intestine (causes diarrhoea with blood), glomeruli of kidney (resulting in the haemolytic syndrome of uremia with development of kidney

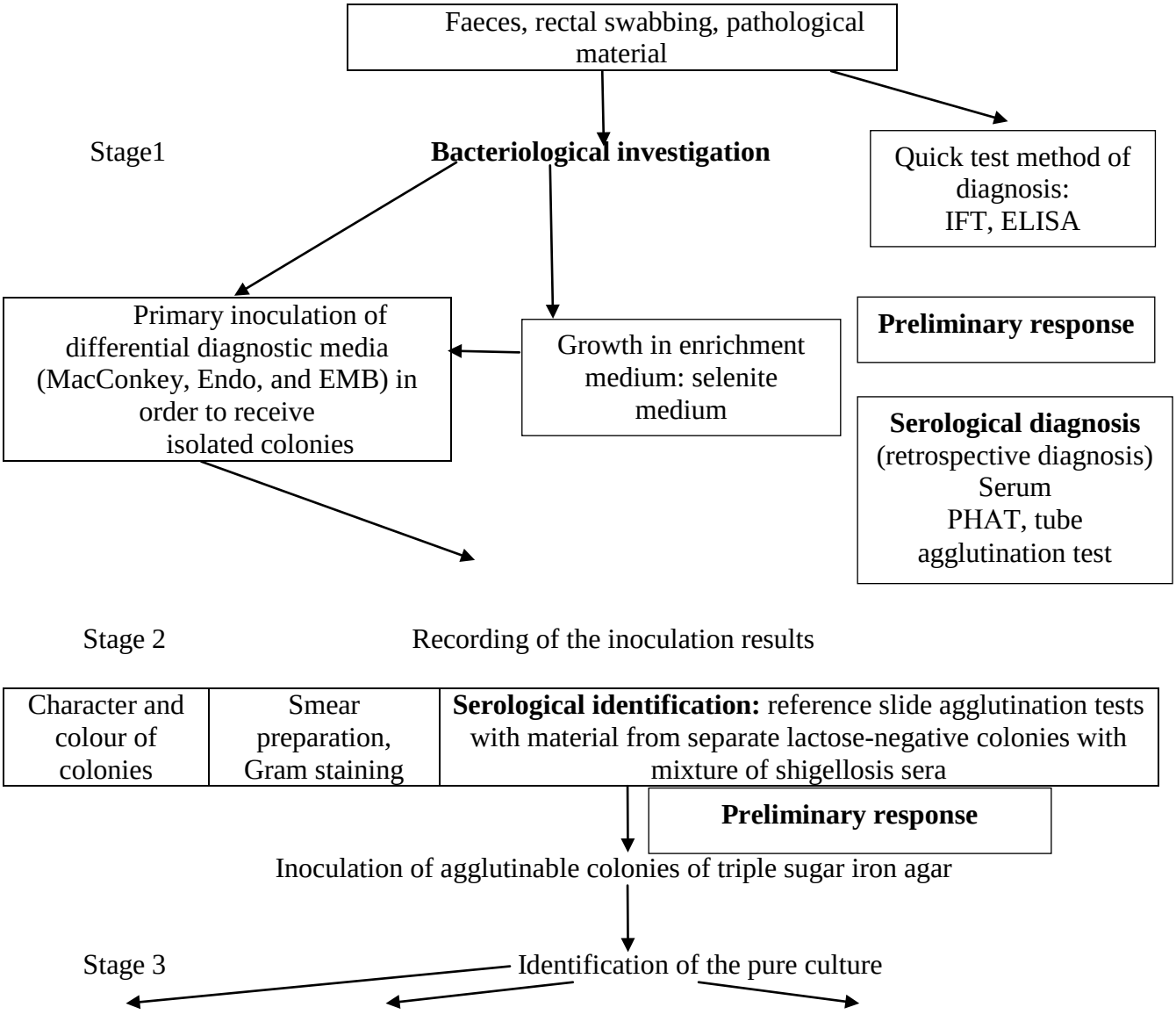
	insufficiency)

Table continuation

Notion	Definition/explanation
Features of shigella endotoxin	1. It has special tropism to the epithelium of mucous membrane of large intestine. 2. It protects shigella from action of bile. 3. Lipid A has immunosupressive activity (represses activity of cells of immune memory)
Stages of acute shigellosis pathogenicity	Adhesion, colonization, inversion of shigella into the cytoplasm of enterocyte, their endocellular reproduction, destruction of enterocyte and tearing away of epithelium, output of causative agents into the gut, forming of delayed hypersensitivity
A source of infection of shigellosis	Patients with clinical manifestations (especially in the first 3 days of disease), subclinical signs of diseases, infected workers or products of food retail industries, public food consumption, equipments for water supply
Transmission of the infection	Through contaminated water (prevails at shigellosis Flexneri), contaminated food: especially through milk and milk products (*S. sonnei* is kept and propagated for a long time), contact route of infection (especially for *S. dysenteriae* species). Insect vectors (flies, cockroaches) transfer causative agents on foodstuffs
Features of the shigellosis epidemiology	Change of specific composition of causative agents, biotype of *S. sonnei* and serotype of *S. flexneri* in certain regions, probably, related to the change of immunity and change of shigella properties
Reasons of shigellosis causative agents species composition change	Changes of collective immunity and properties of shigella
Terms of transition from acute to chronic shigellosis	Overgrowth syndrome, metabolic disturbance in an organism, hypovitaminosis. Weak immunogenicity of causative agents. Development of partial tolerance to the causative agents. Acquired insufficient activity of immune cells. Delayed hypersensitivity. Endocellular parasitism
Features of shigellosis immunity	Typespecific cellular and humoral (conditioned by increase of macrophages and T-lymphocytes activity, antimicrobial antibodies, and antitoxins). Cross immunity does not appear. Local immunity of mucous membrane of the intestine (sIgA)
Mechanism of local immunity	Prevention of shigella adhesion on epithelium, penetration of shigellae in a cell, prevention of immunological damage of the intestine
Material investigated at shigellosis	Bacteriological method: faeces (basic), foodstuffs (especially milk, cheese, sour cream) at outbreaks of shigellosis, autopsy material (fragment of large colon, pieces of parenchymatous organs, mesenterial lymphatic nodes), blood and urine (at suspicion on bacteriemia). Serological method: blood analysis
Methods of shigellosis microbiological diagnosis	The basic method is bacteriological, additional – serological (tube agglutination test, PHAT), express-methods of antigens diagnosis: IFT (investigation of faeces and urine), ELISA (investigation of blood serum), molecular and biological: PCR
Purpose of the bacteriological	Diagnosis of shigellosis; control of etiotropic treatment; detection of persons with subclinical forms of disease; contact

Notion	Definition/explanation
	Colocytes have receptors for adhesion and invasion of shigella. 5. Adhesion of shigella on enterocytes of the small intestine is repressed due to destruction of their superficial cytotoxic proteins by parenchymatous enzymes. In the large intestine of shigella cytotoxin regenerates. 6. The overwhelming defect of distal parts of the large intestine is conditioned by the protracted stay of intestinal contents, toxins, and bacteria in it. It creates favourable conditions for the massive invasion of causative agents into colocyte

Scheme of shigellosis laboratory diagnosis



Characteristics of sugar fermentation: growth of Hiss medium	Study of resistance to antibiotics	**Serotyping:** Reference slide agglutination tests with mixture of shigellosis sera. Reference slide agglutination tests with monovalent species-, group-, and type-specific shigellosis sera

END RESULT

LABORATORY DIAGNOSIS OF TYPHOID FEVER, A AND B PARATYPHOID

Theme topicality. Nowadays diseases caused by salmonellas (typhoid fever, A and B paratyphoid) still remain to be dangerous infections and are characterized by incidence rate affecting people of various ages, appearing as epidemic outbreaks or sporadic cases, causing substantial expenses. One of the major causes of typhoid fever, A and B paratyphoid incidence rate is the problem of bacteria carrying. A carrier can remain the source of infection for the whole life.

Knowledge of biological properties of typhoid fever, A and B paratyphoid causative agents is the key to understanding and solving of certain aspects of this problem.

Primary objective: to be able to conduct and evaluate the microbiological diagnostics of typhoid fever, A and B paratyphoid.

QUESTIONS FOR STUDYIN

1. General characteristics of the bacteria genus *Salmonella.* Classification of *Salmonella* according to biochemical properties and antigenic structure. Kauffmann-White classification. Pathogenesis of salmonellosis in human and animals.
2. Biological properties of typhoid fever, A and B paratyphoid.
3. Pathogenesis and features of immunity of the typhoid fever, A and B paratyphoid.
4. Microbiological diagnostics of typhoid fever, A and B paratyphoid.
5. Basic measures of specific prophylaxis and medical treatment of typhoid fever, A and B paratyphoid.

PROCEDURE OF PRACTICAL WORK

Task 1. Study slide mounts of *S. typhi, E. coli,* draw them in the protocol.

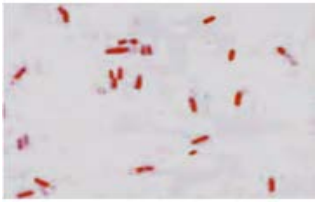
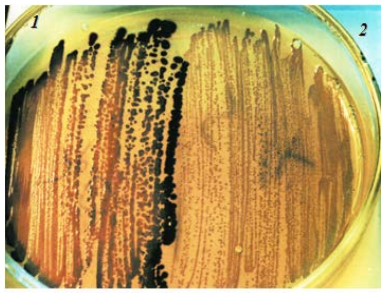


Figure 3.3.1 – *S. typhi,* Gram staining

S. typhi are short straight gram-negative monobacteria which by morphological and tinctorial properties don't differ from *E. coli*.

Task 2. Study cultural properties of causative agents on bismuth-sulphite agar and on nutrient agar with bile (Ploskirev's) medium, sketch colonies, make conclusions, and mark the plan of the further investigations



1 – Salmonella paratyphi B
2 – Salmonella paratyphi A

Cultural properties of *Salmonella spp.* on bismuth-sulphite agar

Bismuth-sulphite agar and Ploskirev's medium are differential diagnosis media. Salmonellas are lactose-negative microorganisms evolving hydrogen sulfide. On bismuth-sulfite agar *S. typhi* and *S. schottmuelleri (S. paratyphi B)* create black colonies with metallic sheen and when this colony is removed from media, black trace remains on it, and the other types of *S. paratyphi A* create brown-greenish colonies. On Ploskirev's medium colonies of different salmonella types are colourless and do not differ. An exception is *S. paratyphi B*, which on Ploskirev's medium also create colourless colonies, but has mucous bead around the circumference.

3. Record the results of the primary growth of investigated material in Rapoport medium (nutrient agar with bile).

Rapoport's medium is a highly selective liquid medium and it is used to inoculate blood (haemoculture investigation) and bone marrow content (myeloculture investigation) for separation and primary identification of *S. typhi* from *S. paratyphi A* and *S. paratyphi B*. In material inoculation to Rapoport medium components correlation 1:10 is to be kept (5 ml blood in 50 ml medium). If the patient has typhoid fever, causative agent changes medium colour from yellow to red and becomes muddy, and in case A and B paratyphoid is cultivated, gases generation also occurs.

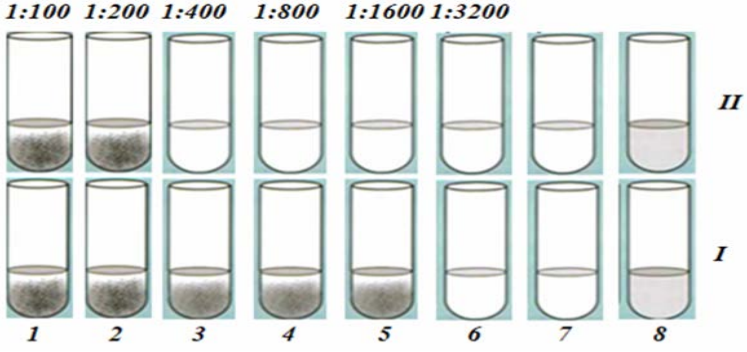
4. Study the antigenic structure of salmonella and estimate the slide agglutination test with salmonellosis polyvalent O serum.

Causative agent's relation to *Salmonella* genus is identified with salmonellosis polyvalent agglutinating O-serum in slide agglutination test. The aim of causative agent's serodiagnosis next stage is to determine its serogroup with salmonellosis polyvalent group – specific O-agglutinating serum and salmonellosis polyvalent group – specific monovalent O-agglutinating sera.

5. Study the results of Widal's test, make a conclusion.

Widal's test is made simultaneously with O-, H-typhoid fever and A and B paratyphoid diagnosticums. Antibodies to O antigen appear on the 1st week of the disease, accumulate in the height of disease and disappear fast at the moment of recovery. Antibodies to H antigen appear in the height of disease, accumulate at the end of disease and are kept in high titres for a long period after patient's recovery.

Serial (two-fold) dilutions of unknown serum are tested



Test tube 7 – serum control
Test tube 8 – diagnosticum control
1st Row– with Typhoid fever O9- diagnosticum
2nd Row– with Typhoid fever Hd- diagnosticum

Widal's test.

against antigens from representative salmonellae.

The results are interpreted as follows: (1) High or rising titre of O (> 1:160) suggests that active infection is present. (2) High titre of H (> 1:160) suggests past immunization or past infection. (3) High titre of antibody to the Vi antigen occurs in some carriers.

Results of serologic tests for salmonella infection must be interpreted cautiously. The possible presence of cross-reactive antibodies limits the use of serology in the diagnosis of salmonella infections.

Lately Widal's test is not considered to be a specific one as it can be positive at other diseases which are characterized with fever and in case of previous illness. Besides that in early antibiotics administration to treat sufferer antibody titre is low and can not be considered diagnostic. The results of Widal's test make it possible to confirm or reject typhoid fever, A paratyphoid, and B paratyphoid diagnosis.

Widal's test is made in accordance with general method. Blood was taken from patient on the 9th day of disease. Agglutinate forms in test tubes contain typhoid fever O9-diagnosticum.

Reaction must be registered in each test tube of the first and second rows, starting from control tubes. Widal's test should be made dynamically.

5. Study the results of PHAT with erythrocyte Vi diagnosticum.

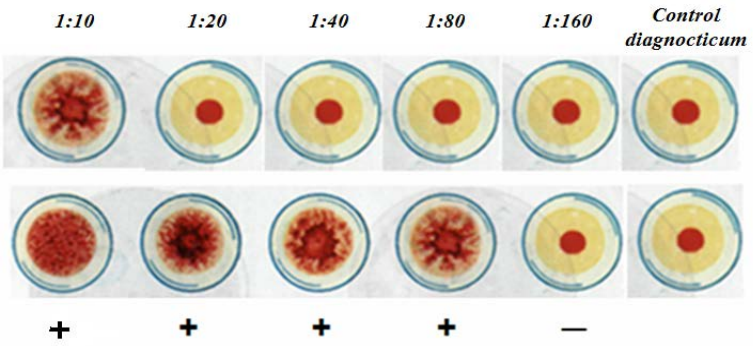


Figure 3.3.4 – PHAT with erythrocyte Vi diagnosticum

Antibodies to Vi antigen at typhoid fever do not have significant diagnostic or prognostic value. Antibodies to Vi antigen detection is important to detect bacteria carrying. To control survivors passive haemagglutination test (PHAT) is made dynamically, and to identify *S. typhi* bacteria carriers', passive Vi-haemagglutination test is to be made and evaluated. IgG diagnostic titre to confirm *S. typhi* bacteria carrying is 1:40 and higher titre, but to finalize "bacteria carrying" diagnosis it is necessary to detach causative agent from stool, bile or urine cultures.

PHAT with typhoid fever erythrocyte Vi-diagnosticum in the first turn is made to identify bacteria carriers; test also can be made to control survivors, blood of which will contain Vi antigen antibodies. At survivors' control the test is made dynamically. Positive test likes a disk with serrated edges and negative test is a disk with smooth edges.

Task 7. Study the diagnostic, therapeutic, and prophylactic preparations.

Adsorbed agglutinating polyvalent O serum is a diagnostic preparation containing specific antibodies to O antigen of basic (A, B, C, D, E) and rare (F, G, H) salmonellas' groups. When specific antibodies interact with corresponding antigens, agglutinate is generated. Agglutinating serum is obtained by hyperimmunization of rabbits with basic and rare salmonellas' groups O antigens mixture and with further adsorption of nonspecific antibodies. It is used to make slide agglutination test at bacteriological method of culture diagnosis to identify salmonellas from shigellas and EIEC, which on Ploskirev's and Endo media also form lactose-negative colonies.

Adsorbed salmonellosis agglutinating diagnostic sera: polyvalent group O (O1, O4, O5, O12) agglutinating diagnostic serum and monovalent O_4 agglutinating diagnostic serum. Diagnostic preparations contain specific antibodies to O1, O4, O5, O12 antigens of salmonellas – O antigens group and only O4 antigen salmonellas of B serogroup (according to Kauffmann-White classification). Agglutinating serum is obtained by rabbits' hyperimmunization with mixture of group O antigens or with group O4 antigen of salmonellas with further adsorption of nonspecific antibodies. It is used to make slide agglutination test to identify salmonella serogroup.

Adsorbed typhoid fever agglutinating monovalent Hd-serum is diagnostic preparation that contains specific antibodies to Hd antigen of typhoid fever causative agent. Agglutinating serum is obtained by rabbits' hyperimmunization with *S. typhi* Hd antigen with further adsorption of nonspecific antibodies. It is used for slide agglutination test to determine salmonella's serotype.

Luminescent typhoid fever serum is a diagnostic preparation. It contains antibody which has luminescent marker (marked with isothiocyanate fluorescein). At salmonella-specific antibody complex formation its luminescence is observed when luminescent microscopy is used.

Monovalent typhoid fever O9, paratyphoid O2, O4 diagnosticums are diagnostic preparations which contain *S. typhi*, *S. paratyphi A*, *S. schottmuelleri* are killed with boiling for 1½-2 hours period (flagellar antigens have been destructed). At their interaction with specific antibodies in patient's blood serum agglutinate is generated. Preparation is used in Widal's test to identify specific antibody titre in serum of patient with typhoid fever, A and B paratyphoid (serologycal diagnostic method).

Monovalent typhoid fever Vi diagnosticum is a diagnostic preparation which contains *S. typhi* strain, including Vi antigen, treated with alcohol. Preparation is used in Widal's test to identify specific antibodies titre to Vi antigen (serologycal diagnostic method). Antibodies to Vi antigen accumulate in reconvalestent and are detected with bacteria carriers.

Salmonellosis complex erythrocyte A, B, C, D, E diagnosticum is a diagnostic preparation containing sheep erythrocytes with A, B, C, D, E groups' salmonellas O antigens adsorbed on their surface. Preparation is used to identify specific antibodies to salmonellas O antigens (serological diagnostic method) in indirect haemagglutination test with paired sera. If the test is positive you will see "umbrella-like" agglutinate. Antibody titre 2–4 and over times increase is an evidence of the fact that person is infected with salmonella infection.

Typhoid fever erythrocyte O_9 diagnosticum is a diagnostic preparation containing sheep erythrocytes with salmonellas O_9 antigens adsorbed on them. Preparation is used to identify specific antibodies to salmonellas O_9 antigens with patient ill with typhoid fever (serological diagnostic method) in indirect haemagglutination test (PHAT) with paired sera. Salmonellas O_9 antigen that is fixing on sheep erythrocytes agglutinate with specific antibodies in patient's blood serum and form uneven-bordered agglutinate on lunulas bottom „like umbrella upside down". Antibody titre 2–4 and over times increase is an evidence of the fact that person is ill with typhoid fever. This test is more sensitive in comparison with Widal's test.

Typhoid fever erythrocyte Vi diagnosticum is a diagnostic preparation containing I (O) blood group human erythrocytes binding with *S. typhi* Vi antigen. Preparation is used to identify specific antibodies to typhoid fever causative agent's Vi antigen in indirect Vi haemagglutination test (serologycal detection of bacteria carriers and reconvalestents examination). Erythrocytes loaded with Vi antigen with specific antibodies being present in blood serum form uneven-boarded agglutinate "like umbrella upside down". Test is made in a plate.

Polyvalent salmonellosis A, B, C, D, E bacteriophage (tableted) contains sterile filtrate of phages lysing *S. paratyphi A, S. schottmuelleri, S. typhimurium, S. heidelberg, S. newport, S. infantis, S. choleraesuis, S. oranienburg, S. dublin, S.enteritidis, S. gallinarum, S. anatum, S. newlands.* It is produced in tablets with acid resistance coverage. It is used for salmonella infection urgent prophylaxis and treatment.

Typhoid fever bacteriophage (tableted) contains sterile filtrate of phages lysing *S. typhi*. It is produced in tablets with acid-resistant coverage. It is prescribed to contact persons to provide active prophylaxis and to patients ill with typhoid fever.

Alcohol typhoid fever vaccine enriched with Vi antigen is used for typhoid fever specific prophylaxis according to epidemiological indications. It is chemical vaccine in which Vi antigen concentration is increased in the result of certain part of liposoluble substances alcohol wash-cleaning, which are an integral part of *S. typhi* surface antigens.

Chemical absorption of typhoid-paratyphoid-tetanus vaccine contains full-value typhoid fever, A and B paratyphoid bacteria full-value antigens and tetanus toxoid. It is used to provide typhoid fever, A and B paratyphoid, and tetanus specific prophylaxis according to epidemiological indications.

Typhoid fever vaccine with sixtoxoid contains typhoid fever causative agent's O and Vi antigens and cleaned concentrated toxoids of tetanus (*Clostridium tetani*), botulism (*Clostridium botulinum* A, B, E types), gas gangrene (*Clostridium perfringens* type A and *C. novyi*), causative agents absorbed on $Al(OH)_3$. It is used for specific prophylaxis according to epidemiological indications.

Laboratory diagnostics of typhoid fever, A and B paratyphoid

Notion	Definition/explanation
Typhoid fever	It is acute anthroponosis infection systemic disease with faecal-oral transmission mechanism, that is caused by *Salmonella typhi*, characterized by small bowel lymphatic apparatus lesion, bacteraemia, fever, typhoid maculopapular rash, organism intoxication and cyclic course

Table 3.3.1 continuation

Notion	Definition/explanation

Taxonomic classification of typhoid fever, A and B paratyphoid causative agents	*Enterobacteriaceae* family. *Salmonella* genus. Species: 1. *Salmonella enterica* subspecies *choleraesuis* serotype *typhi* – causative agent of typhoid fever. 2. *Salmonella enterica* subspecies *choleraesuis* serotype *paratyphi A* – causative agent of A paratyphoid. 3. *Salmonella enterica* subspecies *choleraesuis* serotype *paratyphi B (S. schottmuelleri*) – causative agent of B paratyphoid
Morphological and tinctorial features of the causative agent	Short straight with rounded ends gram-negative mostly movable (peritrichaetes) monobacteria. They do not generate spores or capsules. Various types of salmonellas do not differ from one another morphologically
Causative agent respiration type	Facultative anaerobe
Salmonella serological classification according to Kauffmann-White	Referring to O antigenic structure, salmonellas are classified in serum groups, and inside serum group – in serovars in conformance with difference in H antigenic structure
Typhoid fever causative agent antigenic structure	D serogroup. O antigens: O9, O12, H antigen of the phase 1 – Hd, Vi antigen
A paratyphoid causative agents antigenic structure	A serogroup. O antigens: O1, O2, O12 and H antigen of the phase 1 – Ha
B paratyphoid causative agent antigenic structure	B serogroup. O antigens: O1, O4, O5, O12 and H antigens: phase 1 – Hb, phase 2 – 1, 2, M antigen
Biological features of typhoid fever, A and B paratyphoid causative agents	They are able to resist phagocytosis and propagate in lymphatic system's cells
Reasons of bacteria carrying at typhoid fever, A and B paratyphoid	1. Local inflammatory processes in bile passages and bone marrow. 2. L-transformation of causative agents (they are situated in bone marrow macrophages, are unavailable for chemicals and antibodies and persist for a long time in human body) and reversal into typical forms. 3. Macrophages functional insufficiency. 4. Mononuclear phagocytes system imperfection. 5. Macroglobulin O antibodies – IgM deficiency
Virulent factors of typhoid fever causative agent	Factors of adhesion, colonization, invasion (invasine), Vi antigen, endotoxin, DNAse enzyme. Causative agent does not generate exotoxin
Source of typhoid fever, A and B paratyphoid infection	At typhoid fever and A paratyphoid – patient and bacteria carrier (employees of catering facilities, water supply objects – anthroponosis infection). B paratyphoid – patients, bacteria carriers, and animals (cattle stock, poultry) – zooanthroponosis infection
Routes of typhoid fever, A and B paratyphoid causative agents transmission	The basic routes are: water-born (is the most widely spread and causes epidemic outbreaks); food-born (primarily through milk, diary and meat stuff in which causative agent is able to propagate), rare route is contact and house (contagion in domestic conditions when transmission factor is not identified)

Table continuation

Notion	**Definition/explanation**
Methods of typhoid fever, A and B	**1.** Bacteriological (basic): diseases diagnosis, contact persons examination, bacteria carriers identification and infection source

paratyphoid microbiological diagnosis	detection. **2.** Serological method. **3.** Express method
Material for typhoid fever, A and B paratyphoid bacterial diagnostics	Basic material is blood (haemoculture), faeces (stool culture), urine (urine culture), bile (bile culture), bone marrow aspirates (myeloculture). Additional material is roseolas content (roseola culture), autopsy material, and drinking water
Media used for determination of cultural properties of causative agents	Differential diagnostic media are bismuth-sulfite agar, Ploskirev's, Endo, and EMB agars (for causative agent's primary inoculation and reinoculation from enrichment medium). Bismuth-sulfite agar is highly selective and differential diagnostic medium for salmonella cultivation. Ploskirev's medium is used for differentiation of lactose-negative microorganisms (salmonellas, shigellas, EIEC). Endo and EMB agars are of less selective properties for salmonella cultivation. Rapoport's medium is a selective medium. Enrichment medium is a selenite medium
Cultural properties of the causative agents	Colonies are small (2–4 mm), transparent (muddy in case m/o has Vi antigen), smooth, gentle. *S. paratyphi B* colonies are rougher; mucous. On Endo agar colonies are colourless or whitish-pink, on Ploskirev medium they are colourless, on bismuth-sulfite agar typhoid fever and B paratyphoid causative agent's colonies are black with metallic sheen with medium under colony coloured black, and A paratyphoid colonies are greenish. Selenite medium becomes muddy, while salmonellas are growing
Comparison of *Salmonella, Escherichia,* and *Shigella* biochemical properties	Hydrogen sulphide generation; indole is not generated
The aim of haemoculture and myeloculture test at typhoid fever, terms of blood sampling and its volume	Causative agent isolation from blood and bone marrow is absolute confirmation of typhoid fever diagnosis. Blood should be taken from the first day of fever period and untill this period is finished. On the first week of disease – 5–10 ml from median ulnar vein (adult), 2 ml from the lobule of the ear, heel or finger (small children). On the second week of disease (in adults) – 15–20 ml (there is a causative agent in the blood for the whole fever period, but its quantity comes down gradually). Blood is inoculated in Rapoport medium at patient's bed in 1:10 rate (such rate of blood and medium is necessary to suppress blood proteins bactericidal effect)
Terms and features of haemoculture test at typhoid fever, A and B paratyphoid	Inoculation is to be incubated for 3–4 weeks (taking into account possibility of L-forms creation), but at least 10 days. Growth character should be studied in 24 hours, and in case no growth was observed – in 48, 72 hours, 5 and 10 days. Typhoid fever causative agent causes change of medium colour, A and B paratyphoid causative agents are additionally characterized with gas generation. In case gram-negative movable bacilli are detected, Rapoport medium colour changes or gas is present the first preliminary answer is given

Table continuation

Notion	**Definition/explanation**
Terms and aim of other material investigation at typhoid fever	Probability of diagnosis confirmation while testing stool culture, urine culture and bile culture starts from the 2nd week of disease. Causative agent detachment in these cultures taken from patient with fever does not give the right for final diagnosis "typhoid

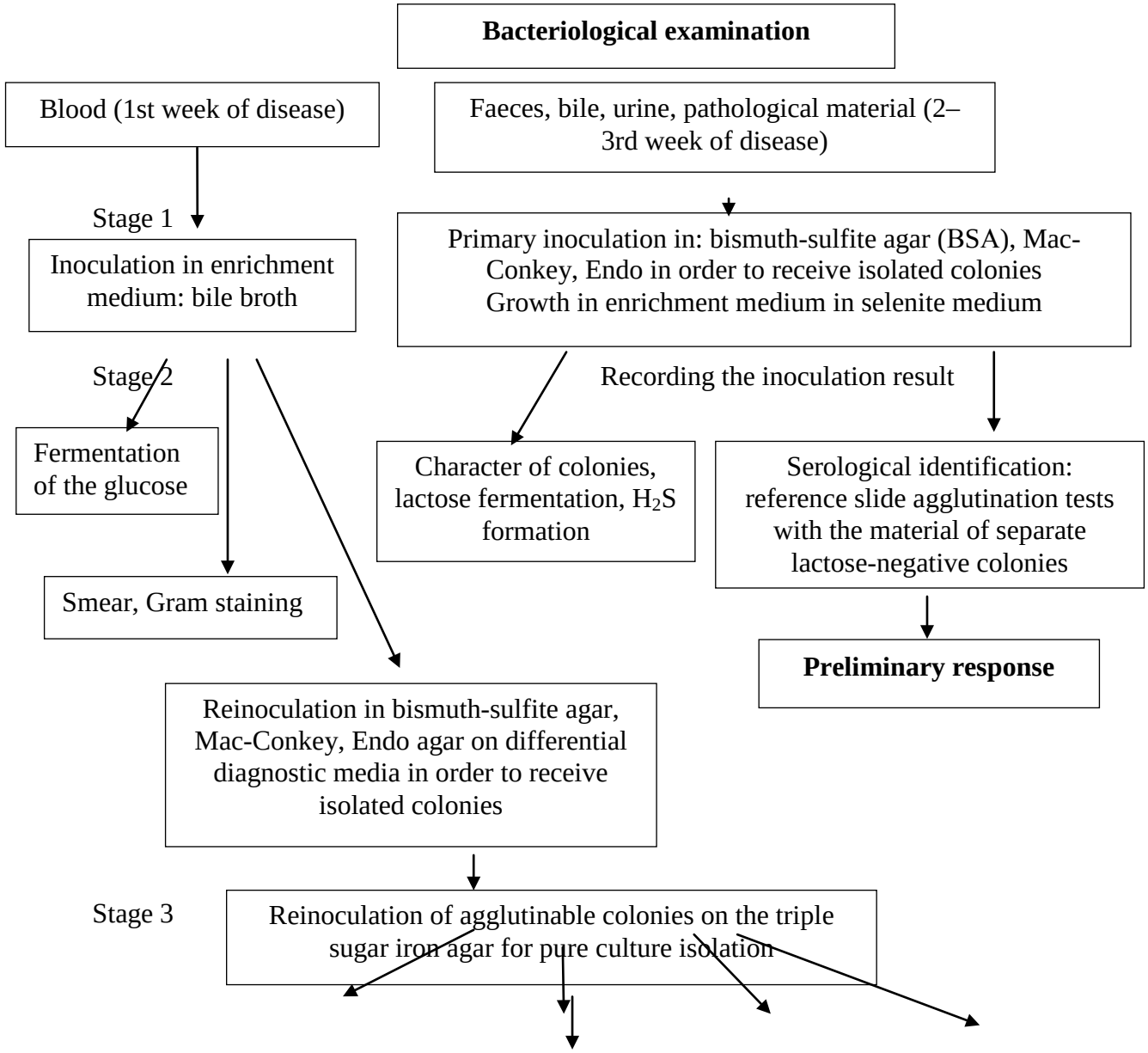
	fever". The results of serum diagnosis are necessary to do so
The aim of blood, faeces, urine and bile bacteriologic test at typhoid fever, A and B paratyphoid	Haemoculture, urine culture, and bile culture tests are used to confirm the diagnosis in the height of disease, treatment effectiveness control and bacteria carriers identification
Features used to differ typhoid fever causative agents of A and B paratyphoid causative agents	According to biochemical properties and antigenic structure peculiarities at bacteriological method of material investigation
Typhoid fever causative agent biochemical features	It ferments glucose, maltose, mannitol with acid generation only, generates hydrogen sulphide
A and B paratyphoid causative agents biochemical features	It ferments glucose, maltose, mannitol with acid and gas generation
Aim and terms of Widal's test. Widal's test components	It is used for diagnosis of typhoid fever, A and B paratyphoid from the end of the 1st week of disease. Antibodies to O antigen appear in the1st week of disease, accumulate in the height of disease and disappear fast at recovery. Antibodies to H antigen appear in the height of disease, accumulate at the end of disease and are kept in high titres after patient's recovery or immunization for a long period. Patient's blood serum (on the 7th–9th days of disease and in 10–12 days), typhoid fever O (O9) and H (Hd) monodiagnosticums, paratyphoid O (O2, O4) and H (Ha, Hb) monodiagnosticums, physiologic saline are needed for test. The test is done in test tubes. Diagnostic titre of immunoglobulin to O and H antigens equals 1:200. In case the test is done dynamically, antibody titre increase in patient's serum 2–4 and more times is a diagnosis confirmation. Test specificity is not sufficiently high
Aim and terms of passive haemagglutination test (PHAT) making, its components and features	Diagnosis of typhoid fever, A and B paratyphoid from the end of the 1st week of disease and confirmation of bacteria carrying. Blood serum (in case disease test is made with paired sera, first portion of blood is to be taken on the 7th–9th day, second one – in 10–12 days), erythrocyte typhoid fever (A paratyphoid, B paratyphoid) O diagnosticums, physiologic saline. Test is done in polystirol sheet. Antibody titre 2–4 and more times increase is an evidence of corresponding infection. To control survivors and to confirm bacteria carrying, the test with cysteic specimen (serum is preliminary treated with cysteine) is made to determine IgG titre. Test is of high specificity
Antibodies identified in height of disease	Antibodies to O antigen – IgM
Antibodies identified in	Antibodies to H antigen – IgG

Table continuation

Notion	**Definition/explanation**
blood serum of survivors and vaccinated	
Bacteria carrying	The major proof is causative agent identification. Material to be

diagnosis	tested: duodenal content, excrements, urine (at typhoid fever, A and B paratyphoid), excrements. Auxiliary data – simultaneous detection of O, H, Vi or H, Vi antibodies
Post-infection immunity peculiarities	Tense, type-specific. Cellular. Local (mediated with secretion of IgA, which prevent process of salmonellas penetration into small intestine mucous membrane). Humoral immunity (antibodies to Vi, O, H antigens) is not of protective activity, it is infection process confirmation
Typhoid fever specific prophylaxis	Chemical adsorbed *Vi*-typhoid fever monovaccine administration according to epidemiological indications
Postvaccinal immunity peculiarities	Unexpressed (≈ 12 months)

Scheme of typhoid fever, A and B paratyphoid laboratory diagnosis



Study the biochemical signs: inoculation on Hiss medium	Serotyping	Phagotyping	Study of antibiotics resistance

Serological examination →	Widal's test, PHAT

END RESULT

LABORATORY DIAGNOSIS OF CHOLERA

Theme topicality. Vibrios are among the most common bacteria in surface waters worldwide. They are curved aerobic rods and are motile, possessing a polar flagellum. *V. cholerae* serogroups O1 and O139 cause cholera in humans, while other vibrios may cause sepsis or enteritis. The epidemiology of cholera closely parallels the recognition of *V. cholerae* transmission in water and the development of sanitary water systems.

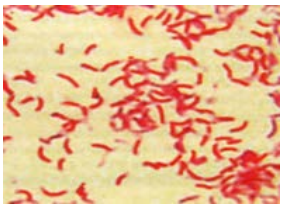
Primary objective: to be able to conduct and evaluate the microbiological diagnostics of cholera.

QUESTIONS FOR STUDYING

1. Biological properties of *Cholerae spp.*
2. Epidemiology and pathogenesis of the diseases caused by *Cholerae spp*. Specific features of immunity in such cases.
3. Microbiological diagnostics of cholera.
4. Basic measures of prophylaxis and treatment of cholera.

PROCEDURE OF PRACTICAL WORK

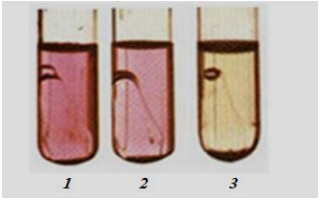
1. Study the preparation of the pure cultures of *V. cholerae* microscopically; draw them in the protocol.



V. cholerae, Gram staining

At the microscopy of the *V. cholerae* preparations there is a comma-shaped, curved rod 2–4 μm long. It is actively motile by means of a polar flagellum. On prolonged cultivation, vibrios may become straight rods that resemble the gram-negative enteric bacteria.

Task 2. Study biochemical properties of the causative agents on Hiss media, make conclusions and mark the plan of the further investigations.



V. cholera biochemical properties

In relation to manose, sucrose, and arabinose (Hayberh's triad) all vibrios are divided into 8 groups. The result of fermentation of carbohydrates has diagnostic value. Causative agent of cholera belongs to the group 1 of the Hayberh's triad, that is *V. cholerae* ferments manose and sucrose to acid, it does not ferment arabinose. Other groups consist of non-haemolytic vibrios.

4. Determine the biovar of the *V. cholerae* according with table 1. Make the conclusion.

Test for differentiation of the *V. Cholerae*

Test	*V. cholerae* serogroup O1	*V. cholerae* O1 El Tor	*V. cholerae* O139	Non-01 /non-O139 *V. cholerae*
Agglutination by cholera *O1*-serum	agglutinates	agglutinates	does not agglutinate	does not agglutinate
Agglutination by Ogáva and Inába serum	agglutinates	agglutinates	does not agglutinate	does not agglutinate
The main tests				

Table 3.4.1 continuation

Lysis by El-Tor bacteriophage	lyses	does not lyse	does not lyse	lyses
Test	*V. cholerae* serogroup Ol	*V. cholerae* O1 El Tor	*V. cholerae* O139	Non-01 /non-O139 *V. cholerae*
Lysis by Cholera monophagous C IV	does not lyse	lyses	does not lyse	lyses
Resistance to the action of polymyxin B	sensitive (growth is absent)	resistant (growth is present)	resistant (growth is present)	resistant (growth is present)
Additional test				
Haemolysis of sheep erythrocytes	–	+	–	+
Hexsamine test	–	+	–	+
Voges-Proskauer test	–	+	+	+

5. Study the main antimicrobial preparations used for diagnosis, treatment, and prevention of suppurative diseases. Write them in your copybook.

Cholera O1 agglutinating serum is a diagnostic preparation, containing specific antibodies to O antigens of cholera vibrios belonging to the O1 serogroup. The interaction of the specific antibodies with the corresponding antigens is formed agglutinates. Agglutinating serum obtained by hyperimmunisation of the rabbits with a mixture of G1-cholera antigens, followed by adsorption of

nonspecific antibodies. It is used for setting agglutination test for bacteriological study of antigenic structure of the parasite and reaction of immobilization of vibrios.

Cholera H-agglutinating serum is a diagnostic preparation, containing specific antibodies to H antigens of vibrio cholerae. Agglutination anti-H cholera serum obtained by hyperimmunisation of the rabbits with using *V. cholera*. It is followed by adsorption of nonspecific antibodies. It is used for setting agglutination test for bacteriological study of antigenic structure of the parasite.

Agglutinating Ogáva serum is a diagnostic preparation, containing specific antibodies to O1-cholera antigens serovar Ogawa. Agglutinating serum obtained by hyperimmunisation of the rabbits with using *V. cholera* serovar Ogawa, followed by adsorption of nonspecific antibodies. It is used for setting agglutination test for bacteriological study of antigenic structure of the parasite

Cholera bacteriophage El Tor (liquid) is a diagnostic preparation, containing a sterile filtrate of *V. cholerae* biovar eltor bacteriophages. It is used for phagotyping of the cholera vibrio biovar El Tor.

Cholera fluorescent serum is a diagnostic preparation, containing specific antibody labeled with fluorochrome (fluorescein isothiocyanates). At the formation of the complex of vibrio cholerae-specific antibodies, a glow with fluorescent microscopy is observed. A positive result is the identification of the even single vibrios and can be obtained within 1–2 hours from the start of the study with a content of not less than 10^6 vibrios in 1 ml of the material. The preparation is used for the rapid identification of the causative agent in the material of the patient, in drinking water or in pure culture in bacteriological diagnosis of cholera.

Vibrio polyvalent bacteriophage (tablets) is a preventive preparation containing a sterile filtrate of the bacteriophages that lyses *V. cholerae*. Available in the form of acid-coated tablets. It is administered to contact persons for prevention.

Corpuscular inactivated dry cholera vaccine. The preparation is used for specific prevention of the cholera. It is a suspension of equal amounts of pure cultures of *V. cholerae* O1 biovar eltor, Ogawa and Inaba serovars.

Cholera vaccine (choleragen-anatoxin + O antigen) is a preventive preparation. It is obtained by purification of broth culture of the cholera vibrio of serovar Inaba, inactivated by formalin. It contains choleragen-toxoid and somatic O antigen. It is used for prevention of cholera by epidemiological indications.

Bivalent cholera chemical vaccine (pelletized) contains choleragen-anatoxin of Ogawa and Inába serovars and somatic O antigen Inaba and Ogawa serovars. It is used for prevention of epidemiological indications.

Choleragen-toxoid is used for specific prevention of cholera at epidemiological indications (creation of active expressed antitoxic immunity). It is a suspension of inactivated cholera vibrios, cultivated in liquid nutrient medium. The drug is purified from the ballast proteins. It is administered subcutaneously.

Laboratory Diagnostics of Cholera

Notion	Definition/explanation
Cholera	Acute extremely dangerous anthroponosis quarantine disease caused by *Vibrio cholerae* O1 and O139 serogroups, which is characterized as the spread of epidemics, profuse watery diarrhoea, vomiting, dehydration, severe intoxication, faecal-oral mechanism of transmission and high death-rate

Taxonomic position of cholera germs	*Vibrionaceae* family. *Vibrio* genus. *V. cholerae* type. Serogroup O1, serotype Ogáva (AB), Inába (AU), Hikodzhúma (FAA), biovar: classic – *V. cholerae cholerae* and El Tor – *V. cholerae* eltor serogroup *V. cholerae* O139 (Bengál)
Clinical signs of cholera	Tenesmus, vomiting, diarrhoea (patient is losing up to 30 liters of liquid a day); faeces in the form of "rice-water" (colourless, odourless heavy faeces containing mucus and epithelial cells), dehydration, renal failure, aphonia, haemoconcentration, anoxia, cardiac insufficiency, hypothermia (cholera algid). Characteristic sign of dehydration is Hippocratic face: hollow eyes, sharp facial features, and sharp protrudent cheekbones. In addition, convulsions, loses of consciousness, and high mortality are characteristic
Features of immunity in cholera	Humoral cellular, intense, short-lived, antitoxic, antimicrobial. Cross immunity is absent. Postvaccinal immunity continues for 6–8 months
Morphological and staining properties of the cholera causative agents	Short slightly curved or straight gram-negative rods are of medium size (1.5–4 × 0.2–0.4 mm), they form clusters in the form of “fish shoats”, mobile (monotrixous). Spore and capsules are not forming
Types of *V. cholerae* respiration	Aerobic, facultative anaerobic
Biovar of the bacteria	It is a type of bacteria, which differ in certain biological and biochemical characteristics
Antigenic structure of *V. cholerae*	O and H antigens. According to the structure of O antigen, 139 serogroups are distinguished. The causative agent of cholera belongs to O1 and O139 (Bengal) serogroups; vibrios of other serogroups are not agents of NAG vibrios cholera. Antigens of O1 serogroup are A, B, C subunits: AB – Ogawa serovar, AC – Inaba serovar, ABC – Hikodzhúma serovar; R-shape – lose O antigen, M-shape – change the structure of O antigen. H antigen is total for the *Vibrio* genus

Table continuation

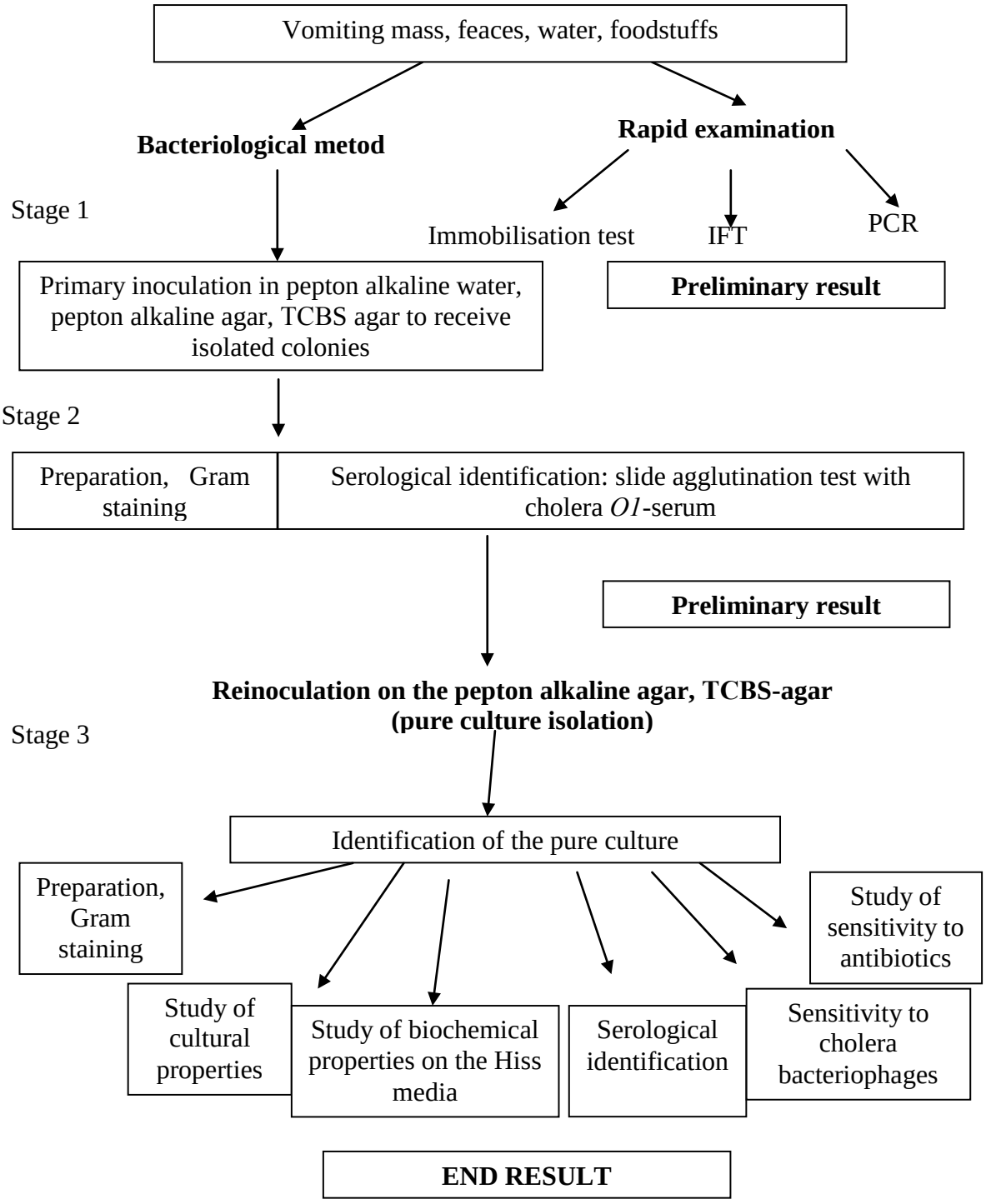
Notion	**Definition/explanation**
Cholera NAG-nonagglutinating) vibrios	*Vibrio* having similar with *cholera* pathogen morphological, cultural, and biochemical characteristics, vibrio of not O1 serogroup (not agglutinated O1 serum), cause acute intestinal infections
Virulence factors of *V. cholerae*. Biological action of virulence factors	Exotoxins: choleragen, LT, ST, SLT; endotoxin; pili adgesion to the glycocalyx of the epithelium of the mucous membrane of the small intestine; fibrinolysin and hyaluronidase – aggression enzymes, enzymes of mucynase and neuraminidase (increases binding of cholera exotoxin with epithelium of the small intestine)
V. cholerae action	It consists of A and B subunits in monosial gangliozid interacts with the receptor and forms hydrophobic intramembrane channel for the penetration of A subunit in the epithelium of the small intestine and provides the antigenic specificity of the toxin. A subunit activates intracellular adenylate cyclase, a rise of cAMP content and the excretion of electrolyte solution in the intestine. Sodium-dependent chloride secretion is increased, and absorption of sodium and chloride is inhibited. Diarrhoea occurs with resulting dehydration, shock, acidosis, and death

Action of the *V. cholerae* endotoxin	Stimulation of the prostaglandins synthesis, causing contraction of the smooth muscles of the small intestine; inhibition of phagocytosis. It causes general intoxication, tenesmus, and diarrhoea, lower blood pressure, infectious and toxic effects
Source of infection of the cholera causative agents	The source of infection is patient and carrier. The patient excretes from 100 mln to 1 billion vibrios in 1 ml of excreta. The reservoir of infection is also water
Resistance of the cholera causative agents	It is sensitive to heating (at 56 ºC is killed in 30 minutes, at 100 ºC – instantly), the action of direct sun, under the action of disinfectants dies in 5–15 minutes, is sensitive to acids. In stagnant water containing organic substances, it is viable for 2–3 weeks. It persists for 2–3 days in fresh faeces and 5 days in milk. Vibrio biovar El Tor is more resistant in the environment
Routes of *V. cholerae* transmission	The principal routes are through water, milk, vegetables, fruits, often – food or water. All epidemics and pandemics of cholera have water in nature. Some role is played by flies
Distribution of vibrios in nature	The agent is able to multiply in the presence of organic substances in fresh and seawater at t > 12 °C, growing in aquatic organisms (molluscs, crustaceans, fish, and frogs)
Material taken from the contact persons with cholera	Faeces after the use of the laxatives (25–30 g of magnesium sulphate). The upper part of faeces is taken
Methods of cholera diagnosis	Bacteriological. Method of rapid diagnosis (IFT, reaction of immobilization of vibrios, PCR). Microscopic. Serological (for retrospective diagnosis) – ELISA, IFT, PHAT (definition of anti-cholera Ig titres and antitoxin)
Purpose of PCR use	1. Rapid identification of the small number of cholera in the test material. 2. Identifation of *V. cholerae* forms, which are not cultivated on the nutrient media

Table continuation

Notion	**Definition/explanation**
Natural protective mechanism in the penetration of cholera	Normal acidity of gastric juice. Infective dose is 1 million vibrios
Preparation for nonspecific prevention of cholera	Preparation of the tetracycline series
Cholera bacteriophages application	For diagnosis and prevention of cholera (contact persons)

Scheme of cholera laboratory diagnosis



LABORATORY DIAGNOSIS OF CAMPYLOBACTERIOSIS AND HELICOBACTERIOSIS

Theme topicality. Presently a considerable place in aetiology of acute intestinal infections is occupied by "new" causative agents, one of which it is the bacterium of *Campylobacter* family. The causative agent is widely spread in an external environment and among animals. In a number of countries of the world, the epidemic (food and water) flashes of campylobacteriosis are registered.

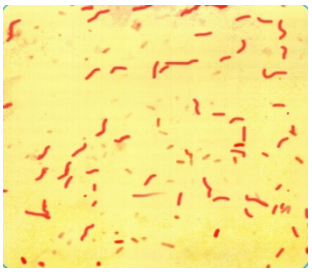
Primary objective: to be able to conduct and evaluate the microbiological diagnostics of campylobacteriosis and helicobacteriosis.

QUESTIONS FOR

1. Biological features of campylobacteriosis and helicobacteriosis causative agents.
2. Microbiological diagnostics of campylobacteriosis and helicobacteriosis
3. Epidemiology and pathogens of campylobacteriosis and helicobacteriosis. Features of the immunity.
4. Principles of campylobacteriosis and helicobacteriosis prophylaxis and treatment

PROCEDURE OF PRACTICAL WORK

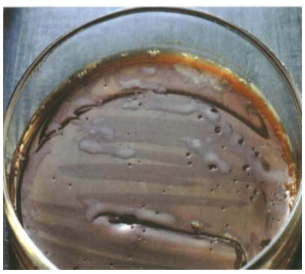
1. Study the preparation of the pure cultures of *C. jejuni* microscopically; draw it in the protocol.



C. jejuni, Gram staining

Campylobacter jejuni is a gram-negative, thin, spiral rod. Have one complete turn of spiral, can be of C- or S-form or reminds a "flying gull" at connection of two cages in a short chain. Spores and capsules are not forming, have one or two polar flagellums.

Task 2. Study the growth of *Campylobacter spp.* and *Helicobacter spp.* on the special medium. Fill in the protocol with their cultural properties.



Growth of *Campylobacter spp*. on chocolate agar

Preston agar, Skirrow agar, Butzler agar, and charcoal-based solid media are the special media. In chocolate agar *Campylobacter spp*. forms mucoid, transparent, nonhaemolytic, flat, very small, convex, opaque, colorless or grayish colonies.

Kery-Bler medium is a transport medium for the *Campylobacter spp.* Medium is spilled in centrifuge test tubes for diminishing of volume of air; a test tube is closed with a rubber cork for creation of microairphillic terms, which are needed for cultivation of campylobacter.

Laboratory diagnostics of campylobacteriosis and helicobacteriosis

Notion	Definition/explanation
Campylobacteriosis	Antropozoonotic bacterial infection, that is characterized by acute course, primary involvement of digestive tract (gastroenteritis, diarrhoea) with acute onset, fever, and development of arthritis, septic tromboflebia, septicemiae, meningitis, involvement of the urinary system. For pregnant abortions, premature births, and newborn infecting

Table 5.1 continuation

Notion	Definition/explanation
	during births are possible
Systematic position of causative agents of the	*Campylobacteriaceae* family. *Campylobacter* genus.
campylobacteriosis	Species: *C. jejuni, C. coli, C. fetus, C. lari*
Morphological features of bacteria of *Campylobacter* genus	Gram-negative thin flexi bacteria (have one complete turn and more), C- S-like form. In smears from pathological material, it is disposed in pairs in the form of “flying gull”. One or two polar flagellums. Spore is absent. Capsules are not formed
Factors of the *Campylobacter spp.* pathogenicity	Endotoxin, adhesions. Some strains produce cholera-like enterotoxin and cytotoxin
Pathogenesis of the campylobacteriosis	Onset of diseases is acute. Intoxication and diarrhea are expressed. The causative agent easily penetrates through the membrane of epithelium and intercellular spaces. Colonisation of small results in the development of inflammatory changes, oedema, hyperplasia of mucous membrane, appearance of erosions. Faeces contain blood
Antigens of the *Campylobacter spp.*	O, H antigenes
Type of bacteria respiration in *Campylobacter spp.*	Microaerophils (optimal concentration of O_2 is 3–15%) and capnophils (10–15% CO_2 is needed)
Source of infection	The basic source is domestic animals (cattle, sheep, pigs), poultry (chickens, parrots), rarely human
Route of the *Campylobacter spp.* transmission	Alimentary (food and water), contact and house (at violation of sanitary and hygienic norms at the care of patients and sick animals)
Material for microbiological diagnosis of campylobacteriosis	Faeces, rectum content taken with rectal tampon, vomit masses, drinking water, liquid from pregnant uterine, food products (milk), lavages from objects
Methods of microbiological diagnosis	Bacteriological is the basic method; bacterioscopical (microscopy preparation of the faeces, Gram staining, phase-contrast microscopy of faeces suspension in the liquid medium), serological (PHAT, CFT, ELISA), express diagnostics method (IFT)
Media and features of campylobacter cultivation	Butler’s medium, eritric agar, transport Kery-Bler and Miller-Hinton media. Inoculated materials are incubated in 10% CO_2 atmosphere at t=25 °C, t=37 °C, t=42 °C during 24–72 hours
Properties of campylobacteries culture	On liquid media, it forms diffuse opacity and sediment. On solid media with blood, campylobacter forms colourless transparent homogeneous convex lustrous colonies (in the form of water drops)
Specific prophylaxis	Is absents

Helicobacteriosis	Diseases are caused by *Helicobacter pylori*
Systematic position of *Helicobacter spp*.	*Helicobacteriaceae* family. *Helicobacter* genus. Species: *H. pylori* (typical species), *H. heilmannii*, *H. cinaedi*, *H. fennelliae*
Helicobacter spp. in nature	It is isolated from the mucous membrane of stomach of monkeys and rodents

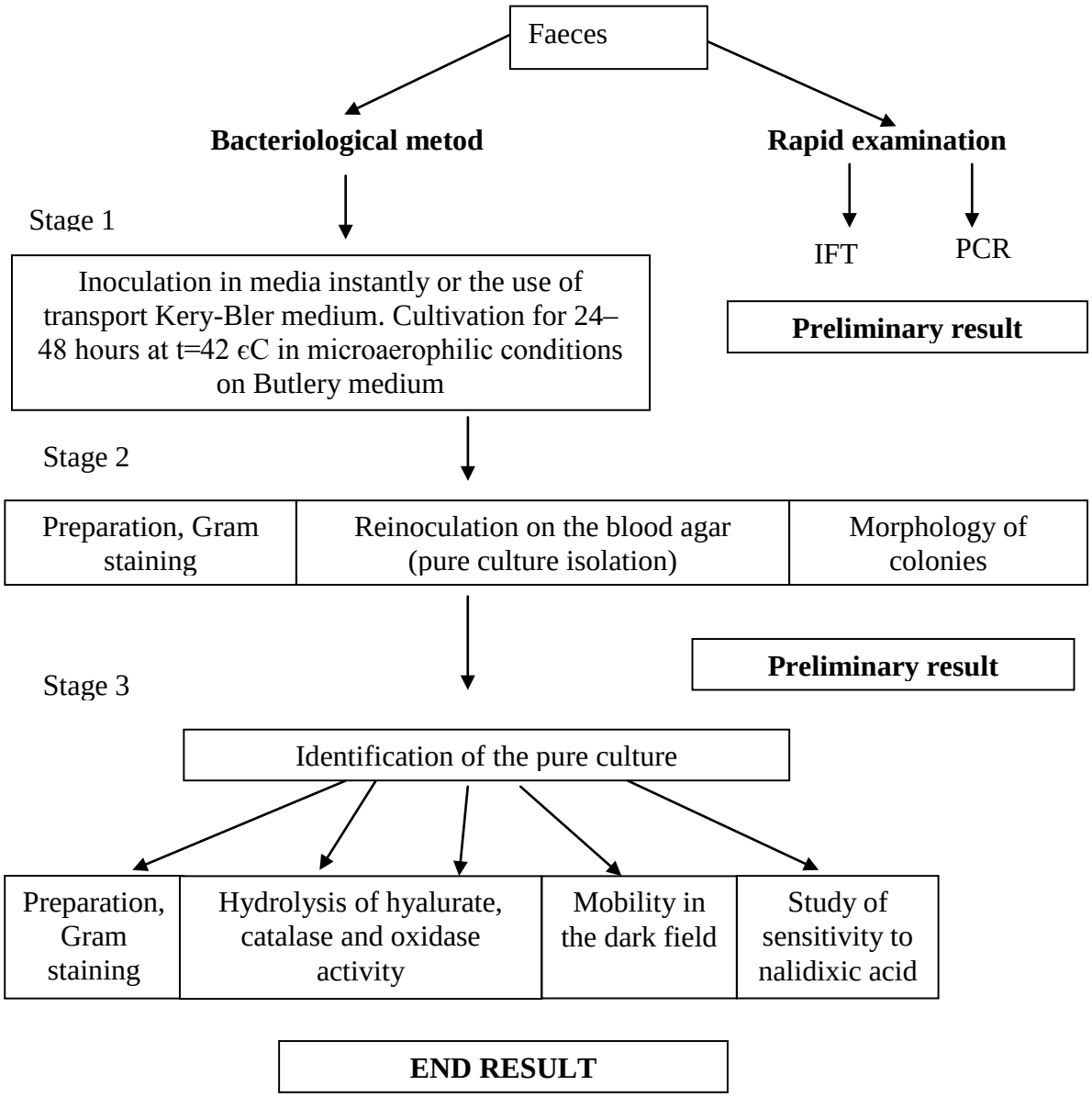
Table continuation

Notion	Definition/explanation
Diseases caused by *Helicobacter pylori*	Chronic gastritis, ulcerous illness of stomach and duodenum, gastric adenocarcinoma, lymphoma of stomach
Morphology of *H. pylori*	Small, short gram-negative nonspore-forming bacteria, S-like rods. Mobile lophotrichous (flagella 4–5) microaerophils, have retort-like bulges on ends
H. pylori antigenic structure	It is O, H, and surface albuminous antigens
Distribution of *H. pylori* in the human	It colonizes the mucous membrane of stomach in 95% of patients, suffering from ulcerous illness of duodenum, in 70–80% of patients with ulcerous illness of stomach, in 60–70% with adenocarcinome of stomach, in 8–10% of healthy people
Routes of infection transmission	Alimentary (through the infected drinking water and vegetables), contact (with saliva); transmission is possible at endoscopy or intubation of stomach (iatrogenic)
Methods of helicobacteriosis diagnostics	Ureatic test with biopsy material of mucous membrane of stomach, bacteriological, serological (ELISA, CFT), express-method (PCR)
Media for *H. pylori* cultivation	Blood agar, chocolate agar
Cultural properties of *H. pylori*	Shallow (about 1 mm) transparent lustrous colonies (in 48–72 hours), haemolysis on blood agar is caused. In liquid media it forms superficial blue-grey colonies and insignificant dimness of medium

Factors of *Helicobacter pylori* virulence

Factor of virulence	Biological effect
Urease	Slitting of urea into the ammonia and carbon dioxide
Protein	Inhibitor of muriatic acid secretion
Glucophosphotase	Destruction of protective sulphomucopolysaccharide of mucous membrane
Protease and phospholipase	Violation of epithelium layer integrity, providing penetration of causative agents in intercellular space
Adgesines	Attaching of bacteria to fabrics
Catalase and alcoholhydrolase	Formation of peroxide radicals, damage of epithelium and protection of bacterium from phagositosis
Cytotoxins	Vacuolization and damage of stomach epithelium cells

Scheme of campylobacteriosis laboratory diagnosis



LABORATORY DIAGNOSIS OF PSEUDOTUBERCULOSIS AND INTESTINAL YERSINIOSIS

Theme topicality. The cause of acute intestinal infections may be *Y. pseudotuberculosis* and *Y. enterocolitica* belonging to the *Yersinia* genus. The highest incidences of these infections occur among people with lowered resistance, children and the elderly. The peculiarity of these agents is that they constitute about 30% of the total incidence of acute intestinal infection, and their aetiological identification cannot hold traditional microbiological methods, because such infections are not covered by preventive measures.

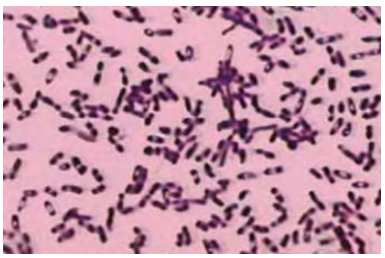
Primary objective: to be able to conduct and evaluate microbiological diagnosis of pseudotuberculosis and intestinal yersiniosis.

QUESTIONS FOR STUDYING

1. Pseudotuberulosis and intestinal yersiniosis: epidemiology, pathogenesis, immunity.
2. Microbiological diagnosis of pseudotuberulosis and intestinal yersiniosis.
3. Preparations for the diagnosis, prevention and treatment of pseudotuberulosis and intestinal yersiniosis.

PROCEDURE OF PRACTICAL WORK

Task 1. Study the preparation of the pure cultures of *Yersinia pseudotuberculosis* and *Y. enterocolitica* microscopically; draw them in the protocol.



Y. pseudotuberculosis, Gram staining

All types of *Yersinia spp*. have identical staining and morphological properties. They are gram-negative rods that exhibit striking bipolar staining with special stains. They are nonmotile.

2. Study the results of indirect haemagglutination test with erythrocyte O9- intestinal yersiniosis diagnosticum, draw it in the protocol.

PHAT is a reaction that is set in plate. Components of the reaction are patient's serum, erythrocytic O9 intestinal yersiniosis diagnosticum. The positive agglutination test is like disk with jagged edges, negative test is like disk with equal edges. The titre of the specific antibody increases 4 times and more.

4. **Study the preparations for the diagnosis of intestinal yersiniosis and pseudotuberculosis (according to the characteristics of the drug). Fill the results in the protocol.**

Laboratory diagnosis of pseudotuberulosis and intestinal yersiniosis

Notion	Definition/explanation
Systematic position of intestinal yersiniosis and pseudotuberculosis	*Enterobacteriaceae* family. *Yersinia* genus. Species: *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*
Differentiation of *Y. enterocolitica* from *Y. pseudotuberculosis*	It is differentiated by biochemical properties and antigenic structure
Intestinal yersiniosis	Infectious disease is characterized by diarrhoea, enteritis, pseudoappendicitis, ileitis, and lymphadenitis of the mesentery

Table continuation

Notion	Definition/explanation
Tinctorial and morphological features of the *Yersinia enterocolitica*	It is polymorphic, gram-negative bacteria: rods with rounded or ovoid end and bipolar staining. It is mobile (peritrichous), does not form spores
Features of the antigenic structure	It has O antigen (O3, O5, O8, O9 bacteria serogroups cause disease in humans)
Type of the *Y. enterocolitica* respiration	Facultative anaerobe
Pathogenicity factors of *Y.enterocolitica*	Endotoxin, cytotoxin, enterotoxin
Distribution of pathogen of intestinal yersiniosis in nature	It is distributed in the soil, water, infected plants. With water and plants yersinia is distributed among animals (rats, mice, birds and farm animals)
Route of the intestinal yersiniosis pathogen transmission	The main route is alimentary: through water and food (meat, milk, vegetables)
Source of the intestinal yersiniosis	Patients with intestinal yersiniosis, synantropic rodents and animals (cows, pigs, goats, horses). It can spread from person to person, causing sometimes hospital infections
Material for microbiological diagnosis of intestinal yersiniosis	Faeces, urine, blood, liquor, bile, appendix biopcy matherial
Methods for microbiological diagnosis of intestinal yersiniosis	Bacteriological (basic) and serological – PHAT, ELISA
Media for *Y. enterocolitica* cultivation	Endo, Ploskirev, and selenit media
Cultural properties of *Y. enterocolitica* in Endo medium	It forms S-convex colonies, round, shiny, pink with straight edge
Pseudotuberculosis	Acute infectious zoonotic disease characterized by fever, polymorphic rash, lymphadenitis of the mesentery, chronic

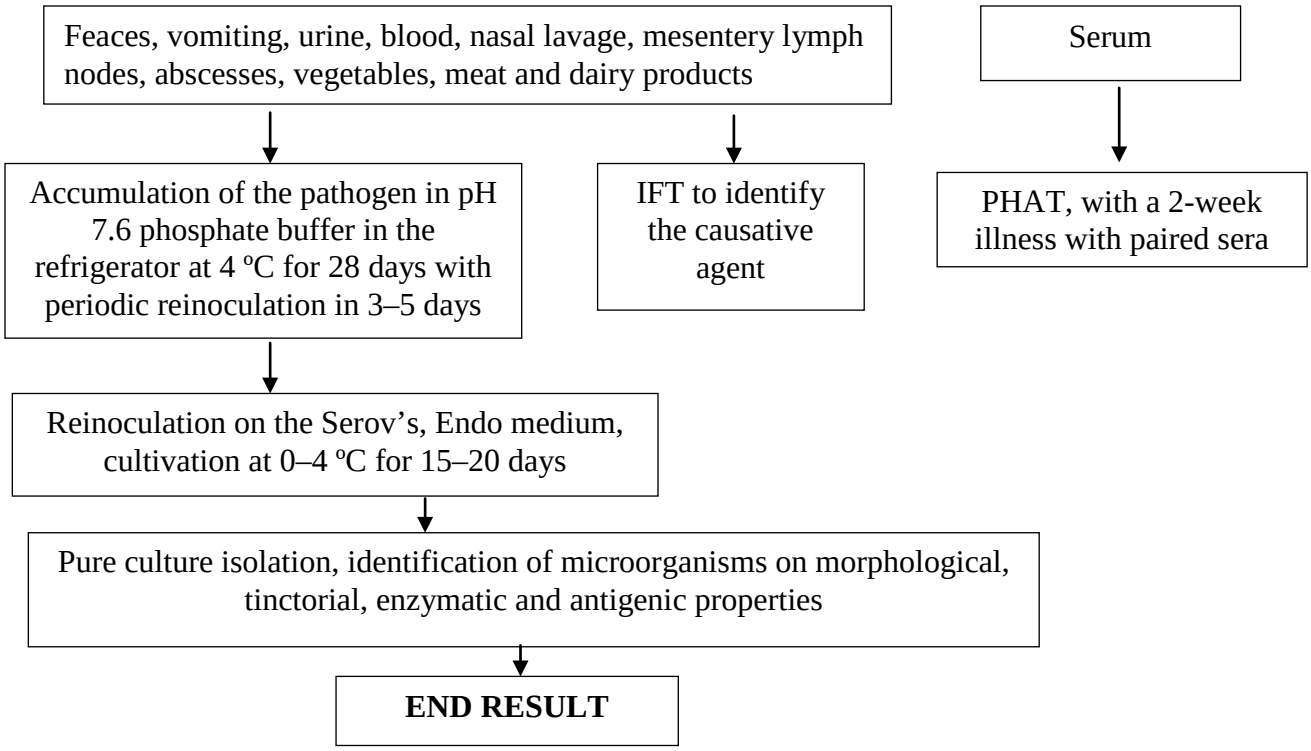
	diarrhoea, septicemia, body allergysation, joint disease, prolonged course
Morphological and tinctorial features of pseudotuberculosis causative agents	Gram-negative bacillus with bipolar ovoid staining, forms a capsule. It has flagella (peritrichous) and does not form spores
Pathogenesis of the pseudotuberculosis	Causative agent moves through M cells after invasion of intestinal mucosa. Bacterium enters the lymph nodes of the mesentery, causing lymphadenitis (typical epigastric pain, symptoms of acute appendicitis are). After overcoming the barrier of the lymph bacteremia is observed, this leads to the formation of granules in the macrophages of liver, spleen, lungs, and joints. There is allergysation of the body and incomplete phagocytosis
Antigenic structure of *Y. pseudotuberculosis*	O, H antigens (bacteria subdivide into 8 serovars)
Pathogenic factors	Enterotoxin, cytotoxin, endotoxin

Table continuation

Notion	**Definition/explanation**
Reservoir and source of pseudotuberculosis for humans	Mammals (cattle, cats, mice, rats), birds that excrete the causative agent of faeces and urine, as well as water, soil, which accumulates parasite
Route of the pseudotuberculosis pathogen transmission	Alimentary: water, fruits and vegetables that are contaminated by the urine of the sick animals, which were keeping at low temperatures (4–12 ° C) because parasite can multiply in food substrates. *Y. pseudotuberculosis* from human to human does not transmit
Material for microbiological diagnosis of pseudotuberculosis	Faeaces, bile, lymphoid nodes, blood
Methods for microbiological diagnosis of pseudotuberculosis	Bacteriological, serological – PHAT (titre 1:400), ELISA (2–5 weeks), biological, skin-allergic test
Features of bacteriological investigation *Y. pseudotuberculosis*	The material inoculated in the Serov's media is kept for 15–20 days in the refrigerator (t = 4 °C) with the following inoculation of it on differential media
Cultural properties of *Y. pseudotuberculosis*	Bacteria are cultivated well on universal media by t = 22–28 °C. It forms S-colonies (small, round, cloudy, discoloured); R-colonies – bulging, lumpy. There is a film or turbidity in liquid medium
Purpose, method and essense of skin-allergic tests	It is made for detection of *Y. pseudotuberculosis* patient sensitization. Skin-allergic test is made on the second week of disease (sensitization develops in 7–20 day and is stored for 2–5 years). Accounting for the reaction conducted in 48 hours. If there is redness positivity and infiltration movements that are painful and itchy
Features of immunity at pseudotuberculosis	It is cellular (delayed type hypersensitivity), unbending
Nonspecific prevention of	1. Permanent sanitary control of water supply system.

pseudotuberculosis	2. Control of technological modes of processing and storage of food. 3. Combat rodents

Scheme of pseudotuberculosis and intestinal yersiniosis laboratory diagnosis



LABORATORY DIAGNOSIS OF FOOD POISONING, TOXIC INFECTIONS, AND ACUTE INTESTINAL INFECTIONS

Theme topicality. Salmonellosis continues to be not only one of the most hygienic and epidemiological problems, but also it is gaining in importance due to environmental and economic distress in most regions, intensive migration, widespread use of antibiotics for treatment and the addition of antibiotics in foods for preventive purposes. At present there is unsolved issue of Ukrainian population incidence of acute intestinal infections (AII) caused by opportunistic pathogenic bacteria and toxic infections of bacterial aetiology. Despite positive results in dealing with acute intestinal infection morbidity remains high, so knowledge of microbiology agents of acute intestinal infections is necessary for the physician regardless of speciality.

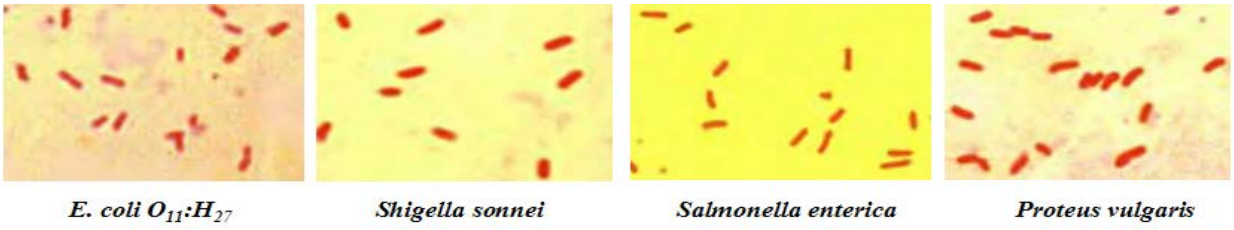
Primary objective: to study the microbiology of salmonella and causative agents of acute intestinal infections caused by opportunistic pathogenic bacteria and food poisoning and toxic infection bacterial aetiology, to be able to evaluate the results.

QUESTIONS FOR STUDYING

1. Biological properties of salmonella, their antigenic structure and classification.
2. Biological properties of acute intestinal infections, food poisoning and toxic infection agents: ETEC, *S. sonnei, Salmonella typhimurium, Salmonella enterica, Klebsiella pneumoniae, Proteus vulgaris, Proteus mirabilis, Citrobacter freundii, Citrobacter diversus*.
3. Epidemiology and pathogenesis of salmonellosis, acute intestinal infection caused by opportunistic pathogenic bacteria and toxic infections, features of the immunity.
4. Microbiological diagnosis of salmonellosis and food poisoning.
5. Features of microbiological diagnosis of acute intestinal infection and toxic infection, caused by opportunistic pathogenic bacteria.
6. The principles of prevention and treatment of salmonellosis, acute intestinal infection caused by opportunistic pathogenic bacteria, food poisoning and toxic infections bacterial aetiology.

PROCEDURE OF PRACTICAL WORK

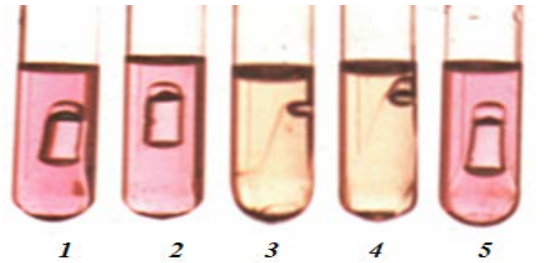
1. **Study the preparation of the pure cultures of *E. coli* O1:H27, *S. sonnei, S. enterica*, and *P. vulgaris* microscopically; draw them in the protocol.**



E. coli O1:H27, *S. sonnei, S. enterica, P. vulgaris,* Gram staining.

E. coli O11:H27, *S. sonnei*, *S. enterica*, and *P. vulgaris* are gram-negative monobacteria of the medium size.

2. Study the biochemical activity of salmonella in Hiss medium, draw it in the protocol.



Biochemical activity of salmonella in Hiss medium

Hiss media showed that salmonella ferments glucose, mannitol, and dulcyt. It forms acid and gas and does not ferment lactose (test tube 3) and sucrose (test tube 4).

The examination of the bacteria antigenic structure in slide agglutination test is necessary for final identification of the causative agent.

2. **Study the results of PHAT with salmonellosis polyvalent (A, B, C, D, E) erythrocyte diagnosticum. Fill the results in the protocol.**

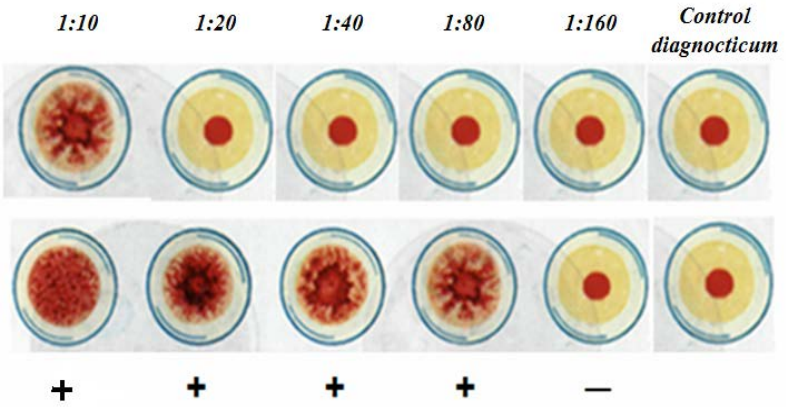
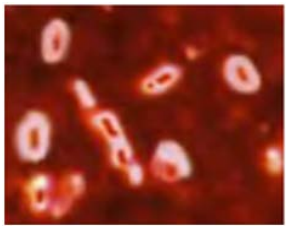


Figure 3.7.3 – PHAT with salmonellosis polyvalent (A, B, C, D, E) erythrocyte diagnosticum

Serological metod is a subsidiary method in salmonellosis investigation, as the family unites more than 2500 Salmonella serotypes. Doctors prescribe serological testing in cases there are clinical symptoms of salmonellosis infection, but biological investigation of the material was not held, the causative agents is not detected, and for retrospective epidemiological analysis of outbreaks of salmonellosis in public catering and organized groups.

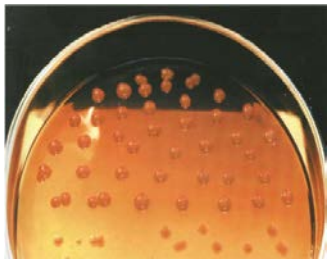
For PHAT bacteriologist used salmonellosis polyvalent (A, B, C, D, E) erythrocyte diagnosticum and paired sera of the patient (the first serum of the patient was in the first days of illness, and the second – in 8–10 days after onset). There is agglutinin titre 4 times increase; it proves the existence of salmonellosis in a patient.

Study the morphological and tinctorial properties of *Klebsiella pneumoniae*.


Klebsiella pneumonia, Gram staining

Klebsiella pneumoniae is pathogenic bacteria that alone or in association with other opportunistic pathogenic bacteria cause acute intestinal infection. It is gram-negative straight single rods, in pairs or short chains. Bacteria form a capsule, which is detected after Burri-Gins staining.

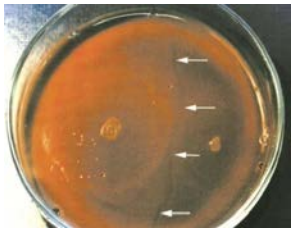
5. Study the cultural properties of *Klebsiella pneumoniae* on Ploskirev's medium.



FCultural properties of *Klebsiella pneumonia,* on Ploskirev's medium

The feature of klebsiella is formation of large, moist, excessively mucous, convex, lactose-positive colonies. Mucilaginous nature of the colonies is explained by the presence of bacteria in the large caps. Pink colour of the colonies is caused by the ability of bacteria to ferment lactose. Generic accessory is definited by bacteriologist at investigation of antigenic structure in slide agglutination test with klebsiellosis diagnostic antisera.

Task 6. Study the growth of *Proteus vulgaris* on nutrient agar.



Growth of *P. vulgaris* on nutrient agar

The feature of proteus is its ability to creeping growth after innoculation by Shukhevych (in liquid condensation on the nutrient agar slant). Growth of bacteria is observed as veiled incrustation on the surface of media. This feature is typical for growth H-form proteus inoculation.

Task 7. Study the biochemical activity of *Proteus mirabilis* in the Hiss medium.

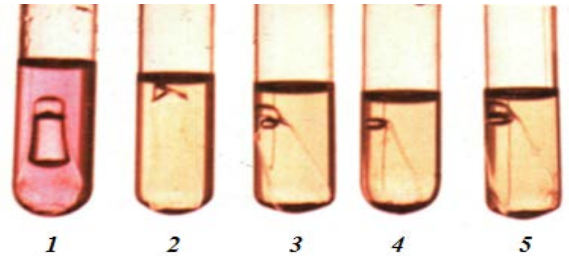


Figure 3.7.7 – Biochemical activity of *P. mirabilis* in the Hiss medium

Proteus mirabilis ferments only glucose (test tube 1) to form acid and gas and does not ferment lactose, maltose, mannitol and sucrose (test tubes 2, 3, 4, and 5) so that the colour of the original environment does not change, gas formation is absent. The results of biochemical activity of the culture are important for identification of the causative agent, but for its final identification it is necessary to examine antigenic structure of the causative agents.

7. Make quantitative accounting of the opportunistic pathogenic microorganisms in the medium (inoculation of the faeces of healthy and sick person) and analyze the results.

At bacteriological investigation of the material bacteriologist uses quantitative method of sectoral inoculation, which allows to determine the number of bacteria in 1 g of the investigated material –CFU/g.

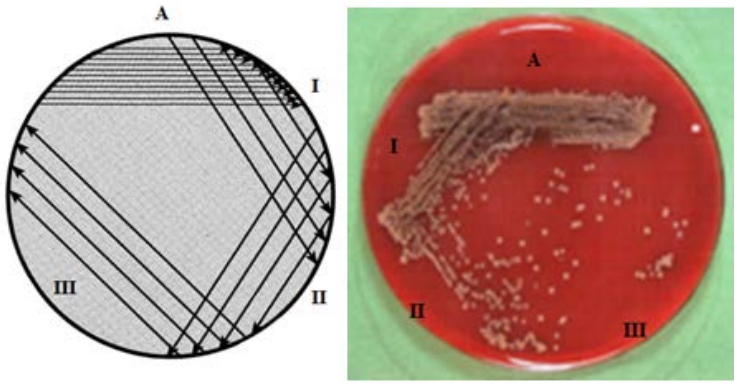
Bacteriologist prepares faeces dilution 1:10. The nutrient medium surface is divided into 4 sectors: A, 1, 2, 3. Platinum loop (0.1 ml of the investigated material) inoculates the material in section A, making 40 streaks.

After the loop is sterilized in a flame of spirit lamp and four innoculations are made from sector A, in sector 1, from sector 1 in sector 2, and from sector 2 in sector 3 (each time the loop is sterilized).

Culture is incubated in the thermostat at t = 37 ºC for 18–24 hours, then the number of colonies that have grown up is counted up, and sets the number of bacteria in 1 g of the investigated material is determined (according to table).

Table – Bacteria count according to the number of colonies in the sectors

Bacteria count in 1 ml of the investigated material	Number of colonies in the sector			
	A	**1**	**2**	**3**
Less than 1 thousand	1–6	–	–	–
1 thousand	8–20	–	–	–
5 thousand	20–30	–	–	–
10 thousand	30–60	–	–	–
50 thousand	70–80	–	–	–
100 thousand	100–150	5–10	–	–
500 thousand	Innumerable	20–30	–	–
1 million	Innumerable	40–60	–	–
5 millions	Innumerable	100–14	–	–
10 millions	Innumerable	Innumerable	10–20	–
50 millions	Innumerable	Innumerable	60–80	Single growth



Inoculation of the investigated material for to determine the number of bacteria in 1 g

The results show evidence that in the patient's material bacteria grew in all sectors. In sector 3 there grew a very large number of colonies of bacteria that is > 108 CFU/g of the investigated material.

In the material of healthy people, bacteria grew only in the sector A and the number of bacteria was 5, that according to this table 2 is 1×103 CFU/g of the investigated material. The number of opportunistic pathogenic microorganisms in microbiocenosis is in the normal range.

8. Study the preparations for the diagnostic, therapeutic, and preventive purposes of the acute intestinal infections, food poisoning and toxic infection.

Monovalent antitoxic antibotulinum serum (A, B, C, E, F). It is obtained by hyperimmunization of the horses by toxoid A, B, and E types. Serum is purified and concentrated by means of enzymatic peptic digestion. The measurement unit is IU. Application in treatment and urgent prophylaxis of botulism. Method and doses of its administration is subcutaneous, intramuscular injection. For urgent prophylaxis: $(1–2)\times10^4$ IU of each serotypes. For treatment: type B – $(5–30)\times10^3$ IU, type A and E – $(1–6)\times10^4$ IU. This serum is injected by Bezredko's method.

Coliproteus lactoglobulin is obtained by hyperimmunization of cows by *E. coli* and *Proteus spp*. It contains secretory IgA from milk. Application is in treatment of overgrowth syndrome.

Coliproteus bacteriophage contains the bacteriophages of patogenic serovars of *E.coli* and *Proteus spp.* Application in treatment of overgrowth syndrome.

Colibactein, lactobacterin, and simbiter are eubiotics. Application is in treatment and prophylaxis of overgrowth.

Dry diagnostic botulinic sera of A, B, C, E, F type is used for determination of botulotoxin in NT. The method of obtaining is hyperimmunization of animals by toxoid. Content is antitoxin. The measurement unit is IU/ml.

Agglutinating adsorbed salmonella O- and H-sera (dry) is used for determination of species and type of salmonella in slide agglutinatin test. The method of obtaining is hyperimmunization of rabbits. The measurement unit is antibody titre.

Intesti bacteriophage (liquid) is a mixture of sterile filtrate of *S. flexneri* 1, 2, 3, 4, 6 serovars, *S. sonnei, S. enterica, S. typhimurium, S. choleraesuis, S. infantis, S. paratyphi A, S. oranieburg, E. coli* O18, O20, O26, O76, O111, O114, O128, O142, O154, *P. vulgaris, P.mirabilis, S. aureus, Pseudomonas aeruginosa* bacteriophages. It is used for treatment of intestinal infections.

Salmonellosis coliproteus lactoglobulin is a purified globulin fraction of colostrum of immunized cows. It is antibodies to *S. enterisa, S. typhimurium, P. vulgaris, P. mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa*. It is used for treatment of diarrhoeal diseases and pyoinflammatory diseases in children.

Laboratory diagnosis of acute intestinal infections, food poisoning, and toxic infection

Notion	Definition/explanation
Salmonellosis	Acute intestinal antropozoonosis infection caused by the numerous salmonella serovars, transmitted through food, mainly characterized by gastrointestinal disorders, often proceeds in the form of gastroenteritis, at least in the form of generalized forms: typhoid or septic
Major causative agents of salmonellosis	*S. enterica* and *S. typhimurium*
Taxonomic position of salmonella causative agent	*Enterobacteriaceae* family. *Salmonella* genus. Species: *S. typhimurium, S. enterica, S. choleraesuis, S. salamae, S.heidelberg, S. derby, S. anatum, S. newport*. Diseases caused by salmonella: typhoid, paratyphoid A, paratyphoid B, food poisoning salmonellosis aetiology, hospital (nosocomial) salmonellosis
Clinical forms of salmonellosis	Gastrointestinal, generalized (typhoid and septic), subclinical (asymptomatic), bacteria carrying (acute, chronic, transitory). *S. anatum* causes mostly subclinical form of the disease, at least

Table continuation

Notion	Definition/explanation
	moderate diarrhoea. *S. choleraesuis* – generalized form, diarrhoea is rare
Cause of bacteria carrying	High resistance to antibiotics of salmonella, development of overgrowth syndrome after antibiotics use (leads to severe and prolonged disease course, increases the probability of process generalization).
Features of the immunity	Cellular, humoral type-specific (5–7 months), local (sIgA prevent penetration of salmonella into intestinal mucous membrane)
Terms of bacteriological investigation at different forms of salmonellosis	At gastrointestinal form (investigation of the basic material) first days of disease. At generalized form (blood analysis) the end of the second or the beginning of the third week
Source of infection at salmonellosis	Birds, animals, patients, bacteria carriers. Poultry (ducks and other water fowl, chickens) and eggs (salmonella infect shell and penetrate the egg). Animals (cows, pigs, sheep, horses) with clinically symptomatic or asymptomatic typical infection, animals emit causative agents in the environment with faeces, urine, milk and saliva. Dangerous patients with salmonellosis (especially decree groups – the workers of public catering establishments; secrete causative agent from 3 days to 1 year)
Route of salmonellosis transmission	Enteral (mainly through food, at least – through water), contact and house (rare)
Routes of meat infection	1. Infection of the live animal (the use of natural and artificial feeds that are infected with salmonella, causative agents is carried in muscles with blood.

	2. During slaughter of cattle and carcass development. 3. At transportation and storage of meat. 4. At cooking meat
Routes of eggs infection	1. When passing eggs in oviduct; 2. Through the intact shell at improper storage of eggs (in egg causative agents is contained in the yolk, where lysozyme is absent)
The most infected poultry eggs	Especially dangerous are duck eggs and other water birds (chickens infected with precarious conditions of chickens detention)
The causes of food infection with salmonella	Meat of poultry and animals – 70–75%, milk – 10%, eggs – 10%, liver (beef and pork), fish and fish products – 3–5%, ready meals without heat treatment: salads, beetroot salad, confectionery
Case water is the route of *Salmonella* infection	At sewage water polution containing salmonella
The case a person is infected through contact and house route	In nursing and congestion of people: preschool establishments, children's hospitals (disease causes *S. typhimurium*, contagiousness, and fast-spreading are characteristic for outbreaks)

Table continuation

Notion	**Definition/explanation**
	causing diarrhoea. Thermostable (ST) enterotoxin inhibits the biosynthesis of protein and activates the formation of prostaglandins
Basic materials for bacteriological investigation at salmonellosis	Faeces, vomit masess, lavage fluid of stomach, bile, blood (at generalized form), autopsy material (the content of the stomach and intestine, parts of parenchymatous organs, mesenteric lymph nodes), food scraps, foodstaffs
Diagnostic value of the investigated material at salmonellosis	Isolation of pathogens from vomit masess, lavage fluid of stomach and blood is the confirmation of the diagnosis ,,salmonellosis", isolation of causative agent from the faeces, and bile may be conditioned by bacteria carrying
Methods for microbiological diagnosis of salmonellosis	1. Bacteriological (main) method. 2. For rapid diagnosis: IFT 3. Additional: - serological: ELISA, PHAT (from the first days of illness and after 8–10 days); - biological
The aim of biological investigation	Diagnosis of salmonellosis, detection of bactera carriers, contamination of food and other objects of the environment
The purpose of serological investigations	Detection of specific antibodies, if: 1. Biological investigation of the material was not carried out. 2. Parasite in the material has not been detected. 3. Retrospective analysis of mass disorders in public catering and in organized groups
The medium for primary cultivation of salmonella	Differential diagnostic MacConkey's, Levin's, Ploskyrev's, and bismuth sulfite agar (inoculation of any material other than blood), Rapoport's medium (for blood inoculation)
Cultural properties of salmonella	Colonies are lactosonegative, small (2–4 mm) clear (turbid, if bacteria have Vi antigen), smooth tender; liquid media with turbidity

The features of biochemical properties in comparison with salmonella and shigella	Formation of hydrogen sulfide and absence of indole
Nonspecific salmonellosis prevention	Health supervision, maintenance of farm animals, conditions of cattle slaughter and transport of carcasses, compliance with food storage, technology implementation and cooking. Scheduled examination of catering staff (detection of patients and bacteria carriers). Control of water supply source
Specific prevention of salmonellosis	It is not conducted
Pathogenesis of botulism	During the growth *C. botulinum* toxin is released into the environment. The toxins are neurotoxic proteins of similar structure and action. They are made up of heavy and light chain linked by disulfide bond. The light chain blocks the calcium. Toxin acts by blocking the transmission of nerve signals to the muscles, producing paralysis. *C. botulinum* can also colonize intestine or wounds, and cause generalized weakness or paralysis.
Typical clinical manifestations of botulism	Symptoms develop in 18–24 hours after ingestion of the toxic food, with visual disturbances (incoordination of eye muscles, double vision), inability to swallow, speech difficulty, signs of bulbar

Tablecontinuation

Notion	**Definition/explanation**
	paralysis are progressive, death occurs from respiratory paralysis or cardiac arrest. Gastrointestinal symptoms are not regularly prominent. There is no fever
Main potential pathogenic bacteria	*Proteus vulgaris, Proteus mirabilis, Klebsiella pneumoniae, Citrobacter freundii, Citrobacter diversus*
Basic method of microbiological diagnosis and its features	Bacteriological. Qualitative and quantitative determination of the causative agents
Essence of *P. vulgaris* and *P. mirabilis*	They are potential pathogenic bacteria of normal intestinal flora of humans and animals that cause toxic food, and pyoinflammatory diseases of the urogenital system, acute purulent affection of wounds and burns, osteomyelitis, meningitis, sepsis
Taxonomic position of proteus	*Enterobacteriaceae* family. *Proteus* genus (includes 4 species). Spesies (causing disease in humans): *P. vulgaris, P. mirabilis*
Morphological and tinctorial features of *Proteus spp*.	Gram-negative bacillus, peritrichous, does not form spores and capsules
Type of *Proteus spp.* respiration	Facultative anaerobes
Virulence factors of *Proteus spp.*	Endotoxin, haemolisine, urease, pili
Features of immunity	Non protective

after proteus infection	
Method of microbiological diagnosis of the infection caused by *Proteus spp.*	Bacteriological
Features of bacteriological investigation of *Proteus spp*.	Occurence of "creeping" culture growth at inoculation by Shukevich. Bacteria give Diens' phenomenon (formation of concentric rings on the periphery of the central colony) at inoculation on the solid media. Growth of bacteria is accompanied by unpleasant smell
Specific prevention of diseases caused by *Proteus spp*.	Is not developed
Drugs for treatment of disease caused by *Proteus spp*.	Coliproteus bacteriophage, intesti-bacteriophage, antibiotics
Taxonomic position of *Citrobacter spp*.	*Enterobacteriaceae* family. *Citrobacter* genus. Species: *C. freundii* (standard type), *C. diversus*
Antigenic structure of the *Citrobacter spp*.	It has O, K, Vi, H antigens. The antigenic structure is very similar to salmonella
Morphological and tinctorial features of *Citrobacter spp*.	It is short, small, motile, gram-negative bacillus, does not form spores, capsules and arranged singly or in pairs
Type of *Citrobacter spp.* respiration	Facultative anaerobes

Table continuation

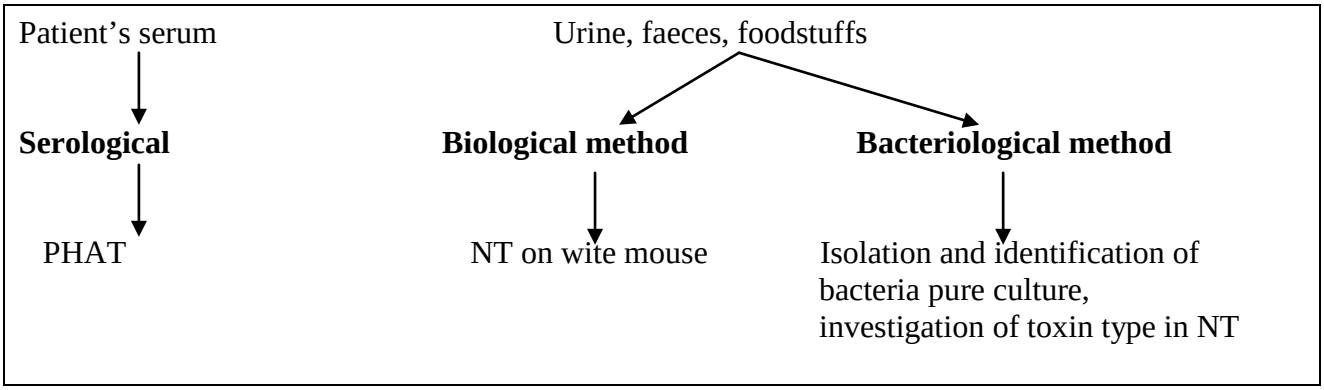
Notion	**Definition/explanation**
Virulence factors	Endotoxin, enzymes of virulence, surface adgesion protein; pili, flagella (factors of invasion)
Distribution of *Citrobacter spp*. in nature	In soil, sewage, in the stool of healthy people and patients with acute intestinal infections
Diseases caused by *Citrobacter spp*.	Acute intestinal infections, toxic infection, diseases of bile and urinary tracts, endocarditis, otitis, osteomyelitis, meningitis, CNS abscess
Immunity at disease caused by *Citrobacter spp.*	Cellular, humoral, type-specific, nonprotective
Taxonomic position of the *Klebsiella pneumonia*	*Enterobacteriaceae* family. *Klebsiella* genus *Klebsiella pneumonia* species. Subtype *Klebsiella pneumoniae, pneumoniae*
Klebsiella spp. antigenic structure	Somatic O antigens (> 10 serogroups), K antigens (> 80 serotypes)
Morphological and tinctorial features of the *Klebsiella spp.*	Straight short thick immobile gram-negative bacilli, placed singly, in pairs or short chains. Capsules are formed; spores don't form

The type of respiration	Facultative anaerobes
Virulence factors	Thermostable (increases output of fluid in the small intestine cavity) and termolabile is (cytotoxicity is typical, penetration of bacteria in the blood system is mediated), enterotoxins and endotoxin, enzymes (DNAse, neuraminidase, phosphatase)
Distribution of *Klebsiella spp.*in nature	Widely spread in soil, water, flowers, fruits, vegetables, and industrial waste waters
Diseases caused by *K. pneumoniae subsp. pneumoniae*	Bronchitis, pneumonia, toxic infection, diseases of excretory system, purulent post-natal complications, neonatal infection (pneumonia, intestinal infection, toxic and septic conditions), sepsis, acute postoperative complications, hospital infections
Features of immunity at disease caused by *Klebsiella spp.*	Cellular (DHT), humoral (antibodies are not protective)
Routes of *Klebsiella spp.* human infection	Exogenous, endogenous, contact and house
Methods of the diagnosis of the disease caused by *K. pneumoniae*	Bacteriological (basic), microscopic, serological (CFT with O antigen, agglutination test in tubes, PHAT)
Media used for cultivation of *K. pneumoniae*	Nutrient agar, Endo, Levin's, Ploskirev's media
Cultural properties of *K. pneumoniae*	It forms lactose-positive large mucous colonies with metallic sheen (in Ploskirev's medium) and uniform turbidity in nutrient broth
Specific prevention of klebsielosis	Is not developed
Nonspecific prevention of diseases caused by	Food storage abidance, aseptics and antiseptics in hospitals, personal hygiene

Table continuation

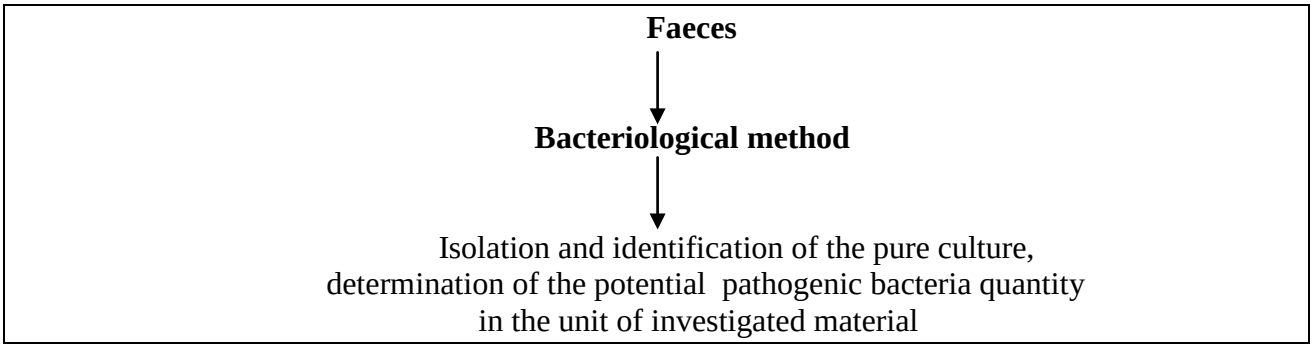
Notion	**Definition/explanation**
Klebsiella spp.	
Essence of toxic infection	Group of acute infectious diseases is associated with foodstaffs that contain high amounts of bacteria (105–106 CFU/ml), contains toxins and is characterized by intoxication, clinical gastroenteritis, violation of water-salt metabolism
The main types of bacteria causing toxic infection	*S. typhimurium, S. enterica*, ETEC, *S. sonnei, Proteus vulgaris, Proteus mirabilis, Klebsiella pneumoniae, V. parahaemolyticus, V. vulnificus, V. anginolyticus*, NAG-vibrio (nonaglutinating vibrios)
Features of toxic infection pathogenesis	It has a short incubation period, acute severe transient disease course, infectious process continues after the removal of toxins from the body of the patient, because the bacteria retain their toxic properties, often group diseases, contagious cases are absent
Material for investigatin of toxic infection	Faeces, vomit masses, lavage fluid of stomach, suspicious remains of food, food, washouts from the surface of tables and hands of staff

Scheme of botulism laboratory diagnosis



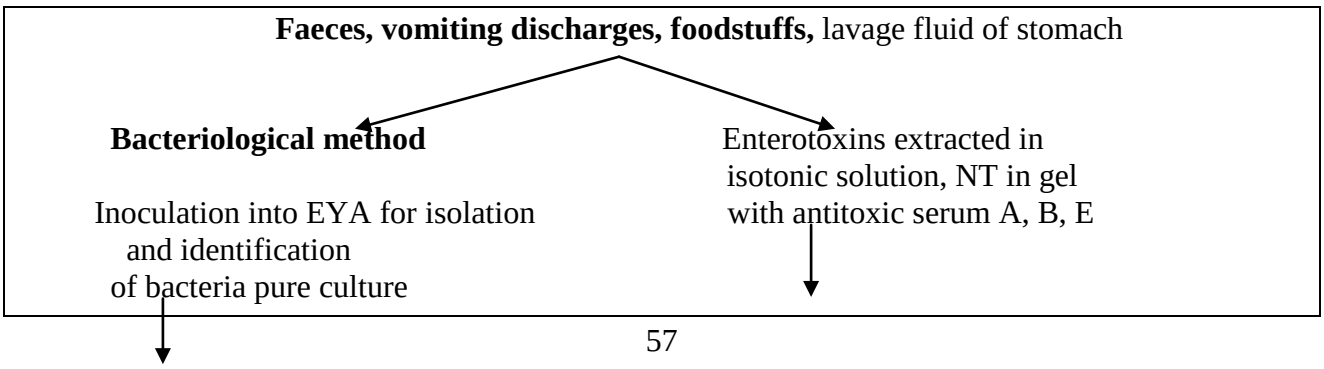
END RESULT

Scheme of acute intestinal infections, which are caused by nonpathogenic bacteria, laboratory diagnosis



END RESULT

Scheme of *Staphylococcus aureus* food poisoning laboratory diagnosis



Biological method

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Study of enterotoxigenic properties in PT in gel with antitoxic serum A, B, C; phagotyping test with the epidemiology object

Infection of new-born cats by investigated material

END RESULT

PATOGENESIS OF FOOD TOXIC INFECTION

↓

Penetration in the alimentary canal
(infected product, pathogen toxins in the product)

↓

(Incubation period is 1–12 hours)

↓

Stomach

↓

Some bacteria die:
– nausea, consumptive vomiting (local effect of endotoxin);
– general intoxication of the body (generalized effect of endotoxin).

↓

Small Intestine

↓

Effects of pathogen and enterotoxins on the mucous membrane
– exhausting vomiting;
– violation of electrolytic homeostasis – watery diarrhoea and vomiting (cAMP increase);
– inflammation of the mucous membrane (the action of histamine and serotonine).

↓

– congestion, swelling of the mucous membrane;
– haemorrhages in the mucous membrane of small and large intestines;
– formation of erosions and ulcers (*C. perfringens*);
– absorption of toxins in the blood;
– changes in nerve function and blood circulation.

↓

At grave course:
– dehydration;
– violations of haemodynamics;
– hypoxia;
– metabolic acidosis;
infectious toxic shock with lethal outcome.

QUIZZES

1. The confirming of diagnosis of an intestinal disease demands using of bacteriological method based on culturing of examined material on nutritional media. What's its aim?

A. Revealing of specific antibodies
B. Revealing of specific antigen
C. Isolation of pure culture of an agent
D. Revealing infections allergy
E. Revealing immune reconstruction of an organism

2. An intestinal bacillus was isolated from faeces of a sick child; it was attributed to serological group O26. What diagnosis can be made in this case?

A. Escherichioses
B. Salmonelloses
C. Cholera
D. Dysentery
E. Yersiniosis

3. A large number of colonies of dark-red color grew on Endo medium at the study of defecation of 5-month-old child with the symptoms of acute intestinal infection. What microorganisms could cause the disease?

A. Salmonella
B. Escherichia
C. Shigella
D. Streptococcus
E. Staphylococcus

1. The main method of diagnostics of escherichioses is a bacteriological method. What is the purpose of this method of investigation?

A. Revealing of specific antibodies
B. Revealing of specific antigen
C. Isolation of pure culture of an agent
D. Making the preparation – smear of examiner material
E. Reproduction of infection in susceptible laboratory animals

5. On suspicion of colienteritis, the faeces of an examined person is inoculated on Endo medium. What groups of nutritional media according to its function the medium belong to?

A. Selective medium
B. Differential diagnostic medium
C. Special medium
D. Enrichment medium
E. Liquid agar

6. Doctor diagnosed that the patient has enteric fever. What microorganism causes this infection?

A. Shigella dysenteria
B. Staphylococcus aureus
C. Escherichia coli
D. Salmonella enteritidis
E. Salmonella typhi

7. The blood of patient with the diagnosis of enteric fever was sent to the laboratory. Which nutrition medium can the bacteriologist use for the isolation of pure culture of Salmonella typhi?

A. Endo medium
B. Blood Agar
C. Hiss medium
D. Chocolate medium
E. Bile broth

8. For treatment of the patient with enteric fever, doctor prescribed levomycetin. In the next day, the patient's health got worse with increase in temperature to 39ºC. Why did his condition get worse?

A. Reinfection
B. Action of endotoxin of salmonella
C. Insensitivity of salmonella to levomycetin
D. Allergic reaction
E. Secondary infection

9. Bacteriologist sourced the feces of patient with enteric fever on the selective nutrition medium. Within 24 hrs on the surface of the medium, grew black colonies. Which nutrition medium did the bacteriologist use for sourcing?

A. Endo medium
B. Blood agar
C. Bismuth – sulphite agar
D. Hiss medium
E. Bile broth

10. The doctor used Vidal's reaction for revealing the antibodies in the patient's serum. Which reaction is used?

A. Reaction of agglutination
B. Complement fixation test
C. Reaction of neutralization
D. Hemagglutination test
E. Precipitation reaction

11. The clinical symptoms of the patient showed high temperature for 8 days. The doctor diagnosed it to be enteric fever. What material for investigation can be used in this stage of infectious disease?

A. Serum
B. Feces
C. Urine
D. Bile
E. Blood

12. The patient had enteric fever. He continued shedding typhoid bacilli in feces for more than a year .What is he considered as?

A. Convalescent carriers
B. Chronic carriers
C. Transient carriers
D. Permanent carriers
E. Suspected carriers

13. Salmonella typhi differs from other species of Salmonella in:

A. Is found mainly in animals.
B. Produces enteritis in small intestine
C. Causes acute dysentery
D. Causes inflammation in the lymphatic nodes in the small intestine
E. Causes food poisoning infection.

14. Which one of the following organisms causes bloody diarrhea, is non motile, does not ferment lactose and does not produce H2S:

A. Salmonella typhi
B. Shigella flexneri
C. Salmonella enteritidis
D. Escherichia coli
E. All of them

15. Which one of the following exotoxins can stimulate hypersecretion of fluids and electrolytes?

A. Shiga-toxin of Shigella dysenteriae

B. Toxic shock syndrome toxin of S. aureus
C. Endotoxin of all Enterobacteriaceae
D. Enterotoxin of Salmonella typhi
E. Heat-labile and heat-stable enterotoxin of E. coli

16. Which one of the following exotoxins can kill Vero cells in culture?
A. Heat-stable enterotoxin of E. coli
B. Shiga-toxin of Shigella dysenteriae
C. Heat-labile enterotoxin of E. coli
D. Endotoxin of all Enterobacteriaceae
E. Toxic shock syndrome toxin of S. aureus

17. Which one of the following organisms is the most common cause of uncomplicated urinary tract infections in humans?
A. Staphylococcus aureus
B. Pseudomonas aeruginosa
C. Streptococcus pyogenes
D. Escherichia coli
E. Klebsiella pneumonia

18. Factors responsible for the pathogenicity of E. coli include all of the following EXCEPT:
A. Heat-labile enterotoxin
B. Capsular (K) antigen
C. Lipoteichoic acid
D. Heat-stable enterotoxin
E. Endotoxin

19. 5-year-old girl develops bloody diarrhea and no vomiting 3 days after visiting her friend's birthday. Stool culture on Endo agar grows both lactose-positive and lactose-negative rods with Gram-negative stain reaction. Which one of the following organisms is the most likely to be the cause?
A. Salmonella cholera-sues
B. Clostridium difficile
C. Klebsiella pneumonia
D. Shigella flexneri
E. Staphylococcus aureus

20. The virulence factor that can be responsible in part for all diseases caused by enteric pathogens is:
A. Endotoxin
B. Flagella
C. Capsule
D. Peptidoglycan
E. Vi - antigen

21. The unique surface antigen that is used in identification of particular enteric pathogen is:
A. M protein antigen
B. H flagella antigen
C. A protein antigen
D. Vi capsular antigen
E. Teichoic acid antigen

22. A key characteristic of the etiological agent of bacillary dysentery is:
A. Strict localization of the pathogen in the small intestine
B. The organism is found mainly in animals
C. Production of a potent cytotoxin responsible for invasiveness
D. The organism is transmitted by droplet aerosol
E. It is a member of normal flora of the human body

23. Blood and leukocytes are seen frequently in the stool during:

A. Salmonellae food poisoning
B. Staphylococcal food poisoning
C. Traveler's diarrhea
D. Shigellosis
E. Disbiosis

24. The predominant virulence factor of Shigella dysenteria is a (an):
A. Enterotoxin/cytotoxin
B. Potent endotoxin
C. Polysaccharide capsule
D. Flagella
E. Plasmid mediated antibiotic resistance factor

25. A 30-year-old woman developed watery diarrhea, little vomiting, nausea, and low-grade fever on the airplane when she was returning from vacation in the Middle East. A stool culture reveals only lactose-positive colonies on MacConkey's agar. Which one of the following organisms is the most likely to be the cause?
A. Escherichia coli
B. Salmonella paratyphi A
C. Klebsiella pneumonia
D. Salmonella enteritides
E. Shigella flexneri

26. The patient's feces has a lot of erythrocytes and mucous. What infection did the doctor think about?
A. Enteric fever
B. Cholera
C. Escheriosis
D. Dysentery
E. Salmonelloses

27. Cases of falling sick with dysentery are registered in a kinder garden. Name a possible way of transmission of this infection?
A. Aero droplets
B. Alimentary
C. Transplacental
D. Transmissive
E. Direct contact

28. Dysenterial bacteriophage was used by the doctor for a child infected with shigella dysentery. What was the doctor's intention in doing so?
A. Prophylaxis
B. Treatment
C. Phage typing
D. Specific prophylaxis
E. Specific treatment

29. From the feces of the patient, pure culture of shigella was isolated. After identification of microorganism, the bacteriologist started serological variant for shigella. By which particularities was the microorganism identified?
A. Biologic
B. Antigenic
C. Morphologic
D. Biochemical
E. Sensitive antibiotics

30. Dirty hands are the main factor of transmission of bacterial dysentery. What microbe from the family Enterobacteriaceae causes its infection?
A. Escherichia
B. Salmonella
C. Yersinia

D. Shigella
E. Pseudomonada

31. Dysentery is transmitted only between human to human. How do we call its infection?
A. Anthroponotic
B. Zoonotic
C. Anthropozoonotic
D. Mixed infection
E. Secondary infection

32. The main mechanism of transmission of dysentery is fecal-oral. Who might be the source of infection in this disease?
A. Infected person
B. Carrier
C. Acute carrier
D. Chronic carrier
E. Infected person and the carrier

33. A 2 year old girl was hospitalized in the infection department. The person has the following clinical symptoms: high temperature, abdominal pain, diarrhea with blood and mucus. The doctor diagnosed it to be dysentery. Which material for investigation is necessary to be taken?
A. Urine
B. Bile
C. Blood
D. Saliva
E. Feces

34. The case of bacterial dysentery is registered in the student's hostel. Which preparation should be used for immediate specific prevention of its infection?
A. Antibiotic
B. Interferon
C. Diagnostic
D. Serum
E. Bacteriophage

35. A key characteristic of the etiological agent of bacillary dysentery is:
A. Strict localization of the pathogen in the small intestine
B. The organism is found mainly in animals
C. Production of a potent cytotoxin responsible for invasiveness
D. The organism is transmitted by droplet aerosol
E. It is a member of normal flora of the human body

36. A 5-year-old girl develops bloody diarrhea and no vomiting 3 days after visiting her friend's birthday. Stool culture on Endo's agar grows both lactose-positive and lactose-negative rods with Gram-negative stain reaction. Which one of the following microorganisms is the most likely to be the cause?
A. Salmonella cholerae-suis
B. Clostridium difficile
C. Klebsiella pneumoniae
D. Shigella flexneri
E. Staphylococcus aureus

37. The case of bacterial dysentery is registered in the hostel. Which preparation should be used for immediate specific prevention of its infection?
A. Antibiotic
B. Bacteriophage
C. Interferon
D. Diagnostic
E. Serum

38. From the feces of the patient, pure culture of shigella was isolated. After identification of microorganism, the bacteriologist started serological variant for shigella. By which particularities was the microorganism identified?

A. Biologic
B. Antigenic
C. Morphologic
D. Biochemical
E. Sensitive antibiotics

39. The patient had shigellosis. He continued shedding bacilli in feces for more than a year. What is he considered as?

A. Convalescent carriers
B. Chronic carriers
C. Transient carriers
D. Permanent carriers
E. Suspected carriers

40. The best e nrichment medium for V. cholerae is:

A. Selenite broth
B. Peptone water
C. TCBS medium
D. Salt – yolk medium
E. Alkaline peptone water

41. The best selective medium for V. cholerae is:

A. Selenite broth
B. Mac s medium□Conkey
C. TCBS
D. Endo s medium□
E. Peptone water

42. Vibrio cholerae strains can be classified into how many serogroup on the base of O-Ag:

A. 3 (Ogava, Inaba, Hikojima)
B. 2 (El Tor and Classical)
C. It can not be classified because it is antigenically homogenous

43. Which one of the following exotoxins can stimulate hypersecretion of fluids and electrolytes?

A. Shiga-toxin of Shigella dysenteriae
B. Endotoxin of all gram-negative rods
C. Choleragen of V. cholerae
D. Shiga – like toxin of EHEC
E. Staphylococcal enterotoxin

44. Factors responsible for the pathogenicity of V. cholerae include all of the following EXCEPT:

A. Endotoxin
B. Mucinase
C. Flagellum
D. Heat – labile enterotoxin
E. Capsular (K) antigen

45. A 40-year-old man develops diarrhea, vomiting, abdominal pain, and fever after he ate some raw sea food at the party. Halophilic, hemolytic coma-shaped bacteria were isolated from the feces. Which one of the following organisms is the most likely to be the cause?

A. Salmonella typhimurium
B. Vibrio parahaemolyticus
C. Shigella flexneri
D. E. coli

E. Staphylococcus aureus

46. 50-year-old woman returned from India, where there was an epidemic of cholerae. She now has multiple episodes of diarrhea, characterized as:

A. Rice water stools
B. Watery diarrhea with blood
C. Diarrhea with blood and leucocytes
D. Mucous stools
E. All the options are right

47. A 15 year old boy is admitted to the hospital. He suffers from abdominal pain, vomiting, rice water stools ten times a day. What is the diagnosis?

A. Dysentery
B. Enteric fever
C. Cholerae
D. Escherichioses
E. Staphylococcal infection

48. A patient is a 20-year-old woman who has just returned from a trip to Indonesia. Now she has rice water stools, with mucus with 10 liters of liquid lost per day, nausea and marked dehydration. Which one of the following exotoxins can stimulate the hypersecretion of fluids and electrolytes?

A. Shiga toxin of shigella dysenteriae
B. Staphylococcal enterotoxin
C. Choleragen of V.cholerae
D. Salmonella endotoxin
E. Endotoxin of all gram negative rods.

49. Cholera belongs to the group of exceptional chorentinnal infections. What is the main mechanism of transmission for its infection?

A. Transmissive
B. Aerogenic
C. Fecal-oral
D. Direct contact
E. Transplacental

50. Cholera mostly affects during summer. What is the main factor of transmission of this infection?

A. Food
B. Water
C. Insects
D. Dirty hands
E. Private things

51. Cholera may be caused by two biological variants of V.cholerae. Which of them causes this infection in the recent years in the world?

A. Classical Vibrio cholerae
B. Vibrio El-Tor
C. Vibrio Al-Benzis
D. Vibrio proteus
E. Vibrio Mechnikovskii

52. There was an epidemic of cholerae in the city. Which method of expressed diagnostics can be used in this case?

A. Reaction of agglutination
B. Complement fixation test
C. Precipitation reaction
D. Reaction of neutralization
E. Immunofluorescence reaction

53. Bacteriologist prepared glass smear from patient's feces from cholera and stained according Gram. Which form has got Vibrio cholerae?

A. Short rod
B. Long rod
C. Cocci
D. Diplococci
E. Coma shaped

54. Specific prevention of cholerae in the epidemic regions is used. What immunobiological preparation is used for specific prophylaxis of this infection?
A. Serum
B. Vaccine
C. Gamma-globulin
D. Interferon
E. Tetracycline

55. There was an epidemic of cholera in the city. Cholera may be caused by two biological variants of V. cholerae. Who discovered classical V.cholerae?
A. Louie Pasteur
B. Robert Koch
C. Gram
D. I.I.Mechnikov
E. Neisser

56. There was an epidemic of cholerae in the city. What preparation is used for non specific prevention of this infection?
A. Serum
B. Vaccine
C. Gamma-globulin
D. Interferon
E.Tetracycline

57. Numerous red colonies grew on Endo medium at bacteriological study of feces in 4-month-old child with intestinal infection. What are these microorganisms?
A. Salmonella
B. Escherichia
C. Shigella
D. Streptococcus
E. Staphylococcus

58. The attachment of intestinal bacillus to epithelial cells with the help of special formations and their producing the adhesines are important at the first stage of pathogenesis of escherichioses. How do we call these formations?
A. Capsules
B. Spores
C. Flagella
D. Fimbrae
E. Grains of volutine

59. There is a natural immunity against the agents of coli-infection that is colienteritis in children of early age. It depends on some factors. Which of these factors is interconnecting and promotes the formation of intestinal biocenosis in the first days of a child's life?
A. Antibodies of mother**'s** milk
B. Colonization of epithelium of gastro-intestinal tract with bifidumbacteria
C. Fermentative activity of intestinal bacilli
D. A number of microorganisms in the intestine
E. The properties of intestinal flora

60. Feces of a child with colienteritis was inoculated on Endo medium. The growth of red colonies was observed on the surface of the medium 24 hours later. How to reveal the colonies containing enteropathogenic intestinal bacillus among grown colonies?
A. Determining the mobility
B. Making and staining the preparation smear according to Gram

C. Phage typing
D. Reaction of agglutination with polyvalent OB-serum
E. Re-inoculation of colonies on Ressel's medium

61. On suspicion of colienteritis, the faeces of an examined person is inoculated on Endo medium. What groups of nutritional media according to its function the medium belong to?
A. Selective medium
B. Differential diagnostic medium
C. Special medium
D. Enrichment medium
E. Liquid agar

62. Doctor diagnosed that the patient has enteric fever. What micro-organism causes this infection?
A. Shigella dysenteria
B. Staphylococcus aureus
C. Escherichia coli
D. Salmonella enteritidis
E. Salmonella typhi

63. . In the bacteriological laboratory investigated the feces of patient with intestinal yersiniosis. What is the causative agent have alike morphology?
A. Tularemia
B. Plague
C. Enteric fever
D. Brucellosis
E. Colienteritis

64. Yersinia enterocolica is causative agent of diseases characterised by defeat of digestive tract. Name the family of Y.enterocolitica.
A. Enterobacteriaceae
B. Chlamydiaceae
C. Mycoplasmaceae
D. Mycobacteriaceae
E. Neisseriaceae

65. The blood of patient with the diagnosis of enteric fever was sent to the laboratory. Which nutrition medium can the bacteriologist use for the isolation of pure culture of Salmonella typhi?
A. Endo medium
B. Blood Agar
C. Hiss medium
D. Chocolate medium
E. Bile broth

66. For treatment of the patient with enteric fever, doctor prescribed levomycetin. In the next day, the patient's health got worse with increase in temperature to 39ºC. Why did his condition get worse?
A. Reinfection
B. Action of endotoxin of salmonella
C. Insensitivity of salmonella to levomycetin
D. Allergic reaction
E. Secondary infection

67. Bacteriologist sourced the feces of patient with enteric fever on the selective nutrition medium. Within 24 hrs on the surface of the medium, grew black colonies. Which nutrition medium did the bacteriologist use for sourcing?
A. Endo medium
B. Blood agar
C. Bismuth sulphite agar
D. Hiss medium

E. Bile broth

68. The doctor used Vidal's reaction for revealing the antibodies in the patient's serum. Which reaction is used?

A. Reaction of agglutination
B. Complement fixation test
C. Reaction of neutralization
D. Hemagglutination test
E. Precipitation reaction

69. The clinical symptoms of the patient showed high temperature for 8 days. The doctor diagnosed it to be enteric fever. What material for investigation can be used in this stage of infectious disease?

A. Serum
B. Faeces
C. Urine
D. Bile
E. Blood

70. Salmonella typhi differs from other species of Salmonella in:

A. Is found mainly in animals.
B. Produces enteritis in small intestine
C. Causes acute dysentery
D. Causes inflammation in the lymphatic nodes in the small intestine
E. Causes food poisoning infection.

71. The most common infection caused by Salmonella in developed countries is:

A. Pseudomembranal colitis
B. Typhoid fever
C. Septicemia
D. Gastroenteritis
E. Enteric fever

72. Which one of the following organisms causes bloody diarrhea, is non-motile, does not ferment lactose and does not produce H_2S:

A. Salmonella typhi
B. Shigella flexneri
C. Salmonella enteritidis
D. Escherichia coli
E. All of them

73. Which one of the following exotoxins can stimulate hypersecretion of fluids and electrolytes?

A. Shiga-toxin of Shigella dysenteriae
B. Toxic shock syndrome toxin of S. aureus
C. Endotoxin of all Enterobacteriaceae
D. Enterotoxin of Salmonella typhi
E. Heat-labile and heat-stable enterotoxin of E. coli

74. Which one of the following exotoxins can kill Vero cells in culture?

A. Heat-stable enterotoxin of E. coli
B. Shiga-toxin of Shigella dysenteriae
C. Heat-labile enterotoxin of E. coli
D. Endotoxin of all Enterobacteriaceae
E. Toxic shock syndrome toxin of S. aureus

75. Which one of the following organisms is the most common cause of uncomplicated urinary tract infections in humans?

A. Staphylococcus aureus
B. Pseudomonas aeruginosa
C. Streptococcus pyogenes
D. Escherichia coli

E. Klebsiella pneumonia

76. Factors responsible for the pathogenicity of E. coli include all of the following EXCEPT:

A. Heat-labile enterotoxin
B. Capsular (K) antigen
C. Lipoteichoic acid
D. Heat-stable enterotoxin
E. Endotoxin

77. The most important source of Salmonella typhi infection is:

A. Poultry and wild birds
B. Typhoid bacilli carriers
C. Warm-blooded animals
D. Humans that develop typhoid fever
E. All of the above

78. The virulence factor that can be responsible in part for all diseases caused by enteric pathogens is:

A. Endotoxin
B. Flagella
C. Capsule
D. Peptidoglycan
E. Vi - antigen

79. Bacteriological diagnosis of typhoid fever can be developed BEST by culture of:

A. Feces taken during the course of the disease and during convalescence
B. Blood taken during the first week of the disease
C. Urine
D. Blood taken during the second or third week of the disease
E. Pus from suppurative lesions

80. Blood and leukocytes are seen frequently in the stool during:

A. Salmonellae food poisoning
B. Staphylococcal food poisoning
C. Traveler's diarrhea
D. Shigellosis
E. Disbiosis

81. The predominant virulence factor of V. cholerae is a (an):

A. Polysaccharide capsule
B. Phage-mediated exotoxin
C. Flagellum
D. Plasmid- mediated antibiotic resistance
E. Enterotoxin with cytotoxicity

82. 50-year-old woman returned from India, where there was an epidemic of cholerae. She now has multiple episodes of diarrhea, characterized as:

A. Watery diarrhea without blood, no PMNs in the stool, and growth of curved Gram-negative rods in the blood culture
B. Watery diarrhea without blood, no PMNs in the stool, and no organisms in the blood culture
C. Bloody diarrhea, PMNs in the stools, and growth of curved Gram-negative rods in the blood culture
D. Bloody diarrhea, PMNs in the stools, and no organisms in the blood culture
E. All of following episodes can occur

83. A 21 year old boy is admitted to the hospital. He suffers from abdominal pain, vomiting, rice water stools ten times a day. What is the diagnosis?

A. Dysentery
B. Enteric fever
C. Cholerae
D. Escherichioses
E. Staphylococcal infection

84. Name the serological reaction use in the laboratory diagnostics of salmonelosis.
A. Precepitation test
B. Vidal reaction
C. Coomb s reaction
D. Complement fixation test
E. Hemagglutination
85. Local immunity of food poisoning is provide …
A. By the lysozyme
B. By the secretory Ig A
C. By Ig G
D. By an interferon
E. By Ig M
86. A patient is a 40-year-old woman who has just returned from a trip to Indonesia. Now she has rice water stools, with mucus with 10 liters of liquid lost per day, nausea and marked dehydration. Which one of the following exotoxins can stimulate the hypersecretion of fluids and electrolytes?
A. Shiga toxin of shigella dysenteriae
B. Staphylococcal enterotoxin
C. Choleragen of V.cholerae
D. Salmonella endotoxin
E. Endotoxin of all gram negative rods.
87. Cholera mostly affects during summer. What is the main factor of transmission of this infection?
A. Food
B. Water
C. Insects
D. Dirty hands
E. Private things
88. Cholera may be caused by two biological variants of V.cholerae. Which of them causes this infection in the recent years in the world?
A. Classical Vibrio cholerae
B. Vibrio El-Tor
C. Vibrio Al-Benzis
D. Vibrio proteus
E. Vibrio Mechnikovskii
89. Blood and leukocytes are seen frequently in the stool during:
A. Salmonellae food poisoning
B. Staphylococcal food poisoning
C. Traveler's diarrhea
D. Shigellosis
E. Disbiosis
90. On bacteriological study of rinsing water of the patient with food poisoning, the pure bacterial culture was inoculated with the following properties: gram-negative motile bacillus in the Endo environment grows like achromic colony. Representative of what genus has caused the illness?
A. Yersinia
B. Citrobacter
C. Salmonella
D. Shigella
E. Escherihia.
91. A 19-year-old male student became ill with diarrhea within a day or two after eating a hamburger at a fast-food shop. Initial culture of his bloody stool revealed no Salmonella,

Shigella, Campylobacter, or Yersinia. A filtrate of the stool was put on Vero cells and cytotoxicity occurred within 24 hours. The most likely cause of the illness is…
A. Escherichia coli 0157/H7
B. E.coli LT toxin
C. E.coli endotoxin
D. Vibrio cholera
E. Clostridium difficile
92. From the defecation of a 6-year-old ill child, who has artificial feeding, the intestinal bacillus with antigen structure 0-111 is excreted. What is the diagnosis?
A. Food poisoning
B. Disentery-like diseases
C. Gastroenteritis
D. Coli-enteritis
E. Cholera-like diseases
93. *Salmonella* species can be differentiated from *Shigella* species in the diagnostic laboratory on the basis of:
A. Motility
B. Lactose fermentation
C. Gram's stain
D. Glucose fermentation
E. Tolerance to anaerobic conditions
94. Blood culture of causative agent of typhoid fever was isolated. Which of the following cultural properties has this bacterium?
A. Forming red colonies on the Endo agar.
B. Forming achromic colonies on Wilson and Blair bismuth sulphite agar.
C. Production of zone of hemolysis around colonies.
D. Forming colorless colonies on Endo agar.
E. Forming thin pellicle on base pepton water.
95. Patient was admitted to the infection unit with diagnosis of bacterial dysentery. On laboratory studies it was revealed that causative element is sensitive to many antimicrobial medicines, but patient had anemia. What medicine was contraindicated to the patient?
A. Enteroseptol
B. Ampicillin
C. Furazolidone
D. Levomycetin
E. Phthalazol
96. A 50-year-old patient with typhoid fever was treated with levomycetin, the next day his condition became worse, temperature rose to 39,6°C. What caused complication?
A. Reinfection.
B. Irresponsiveness of an agent to the levomycetin.
C. Allergic reaction.
D. The effect of endotoxin agent.
E. Secondary infection addition.
97. Cholera is a toxicogenic dysenteric disease common in many parts of the world. In the treatment of patients who have cholera, the use of a drug that inhibits adenyl cyclase would be expected to:
A. Kill the patient immediately.
B. Eradicate the organism.
C. Increase intestinal motility.
D. Block the action of cholera toxin.
98. A patient develops explosive, watery diarrhea 24 hours after eating seafood imported from South America. What bacterium is most likely involved?
A. Campylobacter fetus.

B. Salmonella typhimurium.
C. Shigella flexneri.
D. Vibrio cholerae.
E. Vibrio parahaemolyti.

99. The first seeding of water into the 1 % pepton base water the thin film appeared on the surface of the medium in 5 hours. Which of the following of the causative agent of the infectious diseases has such cultural properties?

A. Plaque.
B. Tuberculosis.
C. Cholera.
D. Dysentery
E. Salmonelosis.

100. Endo medium is used in the laboratory diagnostics of intestinal diseases. What the kind of carbohydrate is included into composition of this nutrient medium?

A. Glucose
B. Mannitol
C. Sucrose
D. Lactose
E. Maltose

101. Name the family of causative agent of dysentery.

A. Enterobacteriaceae
B. Neisseriaceae
C. Micrococcaceae
D. Yersinia
E. Streptococcaceae

102. The nutrient Endo medium is used for laboratory diagnostics of intestinal disease. What colonies of E.coli grow on this medium?

A. Large white
B. Small black
C. Red with metal shine
D. Colorless
E. Small white

103. For isolation of salmonella use bismuth susphite agar. Which colonies grow on this medium?

A. Black
B. Green
C. White
D. Brown
E.Yellow

104. Classification of salmonellae within the genus is on antigenic characterization based on …

A. Calmette-Guerin scheme
B. Vidal scheme
C. Koch classification
D. Kaufmann-White scheme
E. Salmon scheme

105. Name the family of the causative agent of salmonelosis (food poisoning).

A. Yersinia
B. Neisseria
C. Enterobacteriaceae
D. Mycobacteria
E. Vibrioceae

CORRECT ANSWERS

1	2	3	4	5	6	7	8	9	10	11	12	13	14
C	A	B	C	B	E	E	C	C	A	E	B	D	B
15	16	17	18	19	20	21	22	23	24	25	26	27	28
A	B	D	C	D	A	D	C	D	A	A	D	B	E
29	30	31	32	33	34	35	36	37	38	39	40	41	42
B	D	A	E	E	E	C	D	B	B	B	E	C	A
43	44	45	46	47	48	49	50	51	52	53	54	55	56
A	E	B	A	C	C	C	B	B	E	E	B	B	E
57	58	59	60	61	62	63	64	65	66	67	68	69	70
B	D	A	D	B	E	B	A	E	B	C	A	E	D
71	72	73	74	75	76	77	78	79	80	81	82	83	84
D	B	E	B	D	C	B	A	B	D	B	B	C	B
85	86	87	88	89	90	91	92	93	94	95	96	97	98
B	C	B	B	D	C	C	D	A	D	D	D	D	D
99	100	101	102	103	104	105							
C	D	A	C	A	D	C							

Recommended reading list

Main literature

1. Ananthanarayan R. Textbook of Microbiology / R. Ananthanarayana, Jayaram CK. Paniker ; ed. by.: A. Kapil. - 9th ed. - India : Universities Press (Verlag), 2015. - 710 p.
2. Gaidash I. Microbiology, Virology and Immunology. Vol. 1 / I. Gaidash, V. Flegontova; Ed. N. K. Kasimirko. - Lugansk : S. N., 2004. - 213 p.
3. Gaidash I. Microbiology, Virology and Immunology. Vol. 2 / I. Gaidash, V. Flegontova; Ed. N. K. Kasimirko. - Lugansk : S.N., 2004. - 226 p.
4. Jawetz, Melnik & Adelberg's Medical Microbiology : учебное пособие. - 22 Edition. - New York : Lange Medical Books/McGraw-Hill, 2001.

- 695 p.

5. Medical Microbiology : textbook / D. Greenwood [et al.]. - 17th ed. - Toronto : Churchill Livingstone, 2007. - 738 p.

Further Reading

1. Talaro K. Faundations in microbiology. Basic principles. - Talaro K., Talaro A. - Pasadena, 2005, by TMHE group.
2. Microbiology. A human perspective / M. T. Nester, E. V. Nester, C. E.Roberts. - 1995.
3. Levenson W. E. Medical microbiology and immunology / W. E. Levenson, E. Javetz. – Norwalk, 1994,
4. Krivoshein Yu. S. Handbook on microbiology / Yu. S. Krivoshein– Moscow : Mir Publishers,.1989
5. Tropical Diseases : A Practical Guide for Medical Practitioners and Students / Y.A. Meunier, M. Hole, T. Shumba, B. J. Swanner. - OUP USA, 2013.

Informational resourses:

1. American Society for Microbiology — http:// asm.org.;
2. http://journals.asm.org; (American Society for Microbiology) — http:// asm.org.;
3. http://www.news-medical.net/health/Virus-Microbiology-(Russian).aspx;
4. http://www.rusmedserv.com/microbiology; http://www. rusmedserv.com/
5. http://rji.ru/immweb.htm; http://www.rji.ru/ruimmr;
6. http://www. infections.ru/rus/all/mvb journals.shtml;
7. http://dronel.genebee.msu.su/journals/ microb-r.html.
8. http://commons.wikimedia.org/wiki/Category:Medical_illustrations_by_Patrick_Lynch.
9. http://www.nejm.org/doi/pdf/10.1056/nejmra064142
10. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3438653/
11. http://www.prb.org/pdf10/neglectedtropicaldiseases.pdf

http://www.who.int/neglected_diseases/diseases/NTD_Report_APPMG.pdf