Higher State Educational Establishment
Ukrainian Medical Stomatological Academy
Microbiology, Virology and Immunology Department

MANUAL

for Microbiology, Virology and Immunology practical lessons

with Dental faculty students

part III

dental faculty student

Poltava 2007

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The manual for practical lessons of the Microbiology, Virology and Immunology for dental faculty student is composed by an author collective:

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The manual for practical lessons of the Microbiology, Virology and Immunology can be used by dental faculty students for preparation to practical, control lessons, the final module of the subject.

Literature for self work:

Main sourses:

1. Levinson W., Jawetz E. Medical microbiology and immunology. -International edition. - 2003. – P.23-26.
2. Tsyganenko A. Ya., Dikiy I. L., Tkachenko V. L., Shevelyova N. Ya., Velika M. M., Vasilchenko V. N.. Microbiology handbook to laboratory classes in microbiology. – Kharkiv ‹‹ Osnova ››, 2005.

Additional ones:

1. Atlas R. M. Principles of microbiology. – McGraw-Hill; Boston, Massachusetts, 2001.
2. Barom S. (ed.). Medical microbiology. – Galveston, Texas, 1996
3. Donlan RM and Costerton JW (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. Clinical Microbiology Reviews, 15, 167-93.
4. Fields B. N., Knipe D.M. Field’s virology. – New-York, Raven Press, 1990.
5. Hart T., Shears P. Color atlas of medical microbiology. – Mosby-Wolf, 1996.

2. Moukowiak. PA The normal microbial flora, New Eng J \ted. 307. 83.- 1982.

3. Murray, PR Human Microflora. In Tapley & Wilson's Microbiology and Mit-robial Infections,1 edn. Vol. 2, London: Arnold.- 1998.

4. Skinner. FA and Carr. JG The Normal Microbial Flora of Man. London: Academic Press. -1974.

5. Lang NP, Mombelli A, Amtrom 11 . Dental Plaque and Calculus. In Lindhe J, Karring T, Lang NP (eds) Clinical Periodontology and Implant Dentistry, 4th edn, Chapter 23. Blackwell Munksgaard, Copenhagen.- (2003)

6. Marsh PD Dental plaque as a microbial biofilm. Caries Research. - 2004. - 38,204-11.

7. Marsh PD and Martin MV . Oral Microbiology, 4th edn. Butterworth Heinemann, London.- 1999

8. Marsh PD . Are dental diseases examples of ecological catastrophes? Microbiology, 149, 279-94.- 2003.

9. Ross P. W., Peutherer J. F. Clinical microbiology. – London – New-York, 1987.Sbordone L and Bortolaia . Oral microbial biofilms and plaque-related diseases: microbial communities and their role in the shift from health to disease. Clinical Oral Investigations, 1, 181-8,C 2003.

10. Schaechter M. et al. Mechanisms of Microbial Diseases. - Baltimore, W-W, 1993.

Microbiological metods of diagnostic of infection diseases

Microscopic (Bacterioscopic, viroscopic, protozoascpic).- Manufacturing and coloration of smears of the test material of the patient and studying it under a microscope. It allows to quickly identify the typical morphological features the causative agent and has a large importance in diagnostics of gonorrhea, meningococcal meningitis, tuberculosis, leprosy, syphilis, relapsing fever, smallpox, malaria, leishmaniasis, toxoplasmosis and more.

Bacteriological method is to crop material from the patient to the appropriate culture media, allotment of pure cultures of the pathogen and determine its type and, thus, the final diagnosis of the disease. It is critical to in the diagnosis of typhoid fever, dysentery, cholera, diphtheria, plague and other diseases.

Serological methods based on the detection of specific antibodies in the serum of patients with a particular pathogen. For this purpose, various immunological (serological) reaction: agglutination, precipitation, complement fixation and more. For example, on typhoid fever are often held Widal agglutination test, on brucellosis - the Wright reaction, on chronic gonorrhea - complement fixation reaction of Bordeaux - Zhang and others.

Biology (Experimental) method is the infection of susceptible laboratory animals a dedicated pure culture of the pathogen, studied material or introduction of bacterial toxins and reproducing the typical picture of the disease. To do this, use white mice, rats, guinea pigs, rabbits. This method determine the virulence of microbes. For the diagnostic biological sample often used for plague, anthrax, tularemia, tetanus, botulism, anaerobic gas infection, encephalitis, etc.

Allergic method allows to establish the diagnosis by intradermal allergic tests which detect the condition of hypersensitivity to the causative agent or the products of its life activity (allergens). This method is widely used on the diagnosis of tuberculosis (Mantoux test), brucellosis (sample Byurne), tularemia, and many other diseases.

Practical lesson №1

Theme: Methods of cultivation, indication and identification of viruses. Virological method of research

a) The list of issues that must be studied:

1. General characteristics of viruses. Classification.
2. Reproduction of viruses during their interaction with cells. The main stages of the interaction of viruses with cells for productive infection.
4. Integrative and abortive types of viruses interact with host cells. Persistence of the virus in cells. Interference ща virusб defective interfering particles. Viruses satellites.
5. Methods of culturing viruses in cell cultures in chicken embryos, in the body of laboratory animals. Classification of cell cultures used in virology, their characteristics.
6. Methods of detection (indication) of viral reproduction by cytopathogenic action , reactions of hemagglutination (RHA) hemadsorbtion (RHAds), viral inclusions.
7. Identification of viruses by the antigenic properties (IF, RIA, ELISA).
8. Genetic methods for determining the viruses and their nucleic acid components.

b) The list of practical skills that are necessary to master:

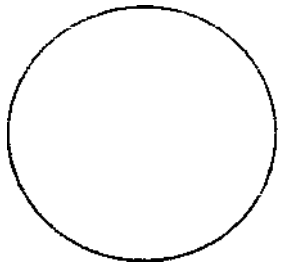
1. Microscope preparations in the light microscope with immersion lens..
2. Ability to identify the virus in chicken embryos for hemagglutination reaction in cell culture by cytopathogen action
3. Ability to set, conduct consideration and evaluate the results of serological tests used in virology (hemagglutination reaction).

Practical lesson's Protocol

Practical tasks should be done:

Task №1. To sketch the structure of chicken embryo.

Task №2. To sketch the cell culture after viral infection.



Task №3. To conduct consideration and estimate the results of hemagglutination reaction (HAR) for virus presence determination in a chicken embryo. To do a conclusion.

Solubilization / Ingredients	1:10	1:20	1:40	1:80	1:160	1:320	Control of red corpuscles
Alantois liquid (ml)	0,1	0,5	0,5	0,5	0,5		-
Ph.solution (ml)	0,5	0,5	0,5	0,5	0,5	0,5	-
1% red corpuscles(ml)	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Incubation 30 minutes at a room temperature							
Consideration							

Conclusion:

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Signature of teacher

Practical lesson N2
Topic: Laboratory diagnostics of Orthomyxoviral, Paramyxoviral and Rhabdoviral infections.

a) The list of issues that must be studied:

1. General characteristics and classification ortomyxsovirus.
2. Human influenza virus. Structure of the virion. Features of the genome. Cultivation. Sensitiveness to physical and chemical factors.
3. Characteristics of antigens of human influenza virus. Hemagglutinin, neuraminidase, functional activity. Classification of human influenza virus. Types of antigenic variability and its mechanisms.

4. Epidemiology and pathogenesis of influenza. The role of virus persistence in humans and animals in the preservation of important epidemic strains. Immunity.
5. Lesions of the oral mucosa under flu.
6. Methods of laboratory diagnostics of influenza.
7. Specific prophylaxis and treatment of influenza.
8. General characteristics and classification of paramyxovirus and rhabdovirus.
9. Paramyxovirus (parainfluenza viruses, measles, mumps, respiratory syncytial flu) and rhabdovirus (rabies virus). Structure of virions. Antigens.
10. Epidemiology and pathogenesis under the conditions of paramyxovirusand rhabdovirus infections.
11. Lesions of the oral mucosa under the chicken pox.
12. Immunity under the paramxovirus infections. Persistence paramyxovirus..
13. Methods of laboratory diagnostics and paramyxovirus and rhabdovirus infections.
14. Specific prophylaxis and treatment of paramyxovirus and rhabdovirus infections.

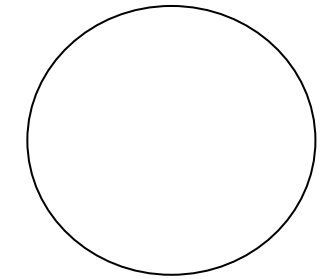
b) The list of practical skills that are necessary to master:

1 Microscope preparations in the light microscope with immersion lens..
2. Ability to identify the virus in chicken embryos for hemagglutination reaction in cell culture by cytopathogen action
3. Set, conduct consideration and evaluate the results of serological tests used in virology (hemagglutination reaction).

Practical lesson's Protocol

Practical tasks should be done:

Task №1. Microscope and sketch the flu virus inclusion stained by Romanovscy - Gimza.



To mark the inclusion

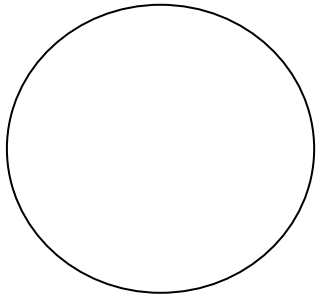
Task №2. To conduct consideration and estimate the results of the hemagglutination inhibition reaction (HAIR) with the pair examined serums and standard parotitis diagnosticum. To do a conclusion.

№ test tubes Ingredients	1	2	3	4	5	6	7	Control of serum	Control of red corpuscles
Solubilization of patient serum (ml)	1:10 0,25	1:20 0,25	1:40	1:80 0,25	1:160 0,25	1:320 0,25	1:640 0,25	1:10 0,25	-
Standard parotitis diagnosticum (ml)	0,25	0,25	0,25	0,25	0,25	0,25	0,25	-	-
Ph. sol.	-	-	-	-	-	-	-	0,25	0,25
Incubation 30 m at a room temperature									
1 % red corpuscles (ml)	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Incubation 30 m at a room temperature									

Consideration	Serum №1									
	Serum №2									

Conclusion: _____

Task №3. Microscope and sketch the inclusion at hydrophobia, stained by Turevich.



To mark the inclusion

Task: №4. To describe immunobiological preparations for a specific prophylaxis and treatment of Orthomyxoviral, Paramyxoviral and Rhabdoviral infections.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher

Practical lesson N3
Topic: Laboratory diagnostics of HIV

a) The list of issues that must be studied:

1. General characteristics of retroviruses. Classification. Representatives.
2. Human immunodeficiency virus (HIV). The structure and chemical composition.
3. Features of the HIV genome. Variability and its mechanisms. Types of HIV. Origin and Evolution.
4. Cultivation, stage of HIV interaction with sensitive cells.
5. The sensitiveness of HIV to the physical and chemical factors.
6. Epidemiology and pathogenesis of HIV infection. Target cells in humans, characteristics of surface receptors.
7. Mechanisms of HIV and AIDS - associated infections.
8. Lesions of the oral mucosa under conditions of HIV infection.
9. Classification of the manifestations of HIV infection in the mouth by the degree of likely connection because of this infection:
   a) The first group - lesions of the oral mucosa, which closely connected with HIV (candidiasis, HIV gingivitis, necrotizing ulcerative gingivitis-, HIV-paradontyt et al.)

b) The second group - lesions less connected with HIV infection (atypical ulcers, diseases of the salivary glands, viral infection, etc..)
c) The third group - destruction, which may be connected with HIV infection (bacterial infections, exacerbation of apical periodontitis, osteomyelitis, etc.).

10. Methods of laboratory diagnostics of HIV infection. PCR in the diagnosis of HIV infection and vesternblot (imunoblot) - test.
11. Treatment (causal, immunomodulating, imunozamisni means) of HIV. Prospects for a specific HIV prevention.
12. AIDS prophylaxis in dentistry.

b) The list of practical skills that are necessary to master:

1. Ability to conduct consideration and evaluate the results of serological tests used in virology (ELISA).

Practical lesson's Protocol

Practical tasks should be done:

Task №1. To estimate the results of ELISA with the examined serums . To do a conclusion.

	1	2	3	4	5	6	7	8	9	10	11	12	
AND	0.005 NCl	-0.005 neg	0.0120 neg	0.002 neg	0.006 neg	0.006 neg	0.000 neg	****	****	****	****	****	AND
B	00.96 COl	0,002 neg	0,004 neg	0,003 neg	0,002 neg	0,004 neg	0,005 neg	****	****	****	****	****	B
C	0.266 CO2	0,003 neg	0,003 neg	0,004 neg	0,002 neg	0,005 neg	****	****	****	****	****	****	C
D	0.209 CO3	0,000 neg	0,016 neg	0,000 neg	-0,001 neg	0,221 POS	0,004 neg	****	****	****	****	****	D
E	0.338 PC1	0,002 neg	0,007 neg	0,003 neg	0,270 POS	0,004 neg	0,002 neg	****	****	****	****	****	E
F	0,314 POS	-0,005 neg	0,003 neg	0,005 neg	0,002 neg	0,005 neg	0,003 neg	****	****	****	****	****	F
G	0,002 neg	0,002 neg	0,015 neg	0,001 neg	0,004 neg	0,007 neg	0,005 neg	****	****	****	****	****	G
H	0,017 neg	0,003 neg	0,005 neg	-0,004 neg	0,003 neg	0,003 neg	0,004 neg	****	****	****	****	****	H
	1	2	3	4	5	6	7	8	9	10	11	12	

*****INDICATES VALUE OUT RANGE
#####INDISATES SOVINED DATA
ROS INDISATES AND ROSITIVE REACTION

peg INDISATES And NEGATIVE REACTION
??? INDISATES EQUAL OR BETWEEN LIMITS
31. INDISATES VALUE OUT RANGE

Conclusion:_____
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Task №2. To estimate the results of chain polimerase reaction (CPR). To do a conclusion.
Conclusion:_____
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Task №3. To describe immunobiological preparations for a specific prophylaxis and medical treatment of HIV- infection.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher

Practical lesson №4

Theme: Laboratory diagnostics of Enteroviral, Flaviviral and Coronaviral infections

a) The list of issues that must be studied:

1. General characteristics and classification of family picornavirus. The division of families.
2. General characteristics of enterovirusus. Classification: poliomyelitis, Coxsackie, ECHO.
3. The role of enteroviruses in human pathology. Epidemiology, pathogenesis of poliomyelitis and other enteroviral infections. Immunity.
4. esion of oral mucosa with angina caused by Coxsackie virus group A.

5. Methods of laboratory diagnostics of enteroviral infections.
6. Specific prophylaxis and treatment of enteroviral infections.
7. The genus of Aftovirusiv. FMD viruses. Biological properties. Pathogenesis of infection in humans.
8. Lesions of the oral mucosa under foot and mouth disease.
9. Laboratory diagnostics of foot and mouth disease. Specific prophylaxis.
10. General characteristics of coronavirusus. Role in human pathology. Laboratory diagnostics.

b) The list of practical skills that are necessary to master:

1. Microscope preparations in the light microscope with immersion lens
2. Ability to conduct consideration and evaluate the results of serological tests used in virology (hemagglutination inhibition test, neutralization reaction

Practical lesson's Protocol

Practical tasks should be done:

Task №1. To conduct consideration and estimate the results of neutralization reaction (NR) - the coloured test with examined serums and diagnosticum of poliomyelitis virus antigens of 1 type. To do a conclusion.

№ test tubes		1	2	3	4	5	6	7	Control	
Solubilization of serum (ml)		1:10 0,25	1:20 0,25	1:40 0,25	1:80 0,25	1:160 0,25	1:320 0,25	1:640 0,25	-	1:10 0.25
Nourishing media(ml)		-	-	-	-	-	-	-	0,25	0,25
Virus of 1th type 100 CPA $_{50}$		0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25	-
Cell culture 300000 – 4000000 / ml		0,25	0,25	0 , 2 5	0,25	0,25	0,25	0,25	0,25	0,25
Incubation at the temperature of 37°S during 4-7 days										
Consider	Serum № 1									
	Serum №2									

note: (+) – presence of cell culture (color of media is yellow);

(-) – absence of culture (color is raspberry).

Conclusion:

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Task №2. To conduct consideration and estimate the results of hemagglutination inhibition reaction (HAIR) with the serums and encephalitis diagnosticum. To do a conclusion.

№ test tubes / Ingredients		1	2	3	4	5	Control of serum	Control of diagnosticoumou
Solubilization of serum		1 :1 0	1:20	1:40	1:80	1:160	1:10	
Diagnosticum (+) -		+	+	+	+	+	-	+
Incubation at a room temperature during a 1 hour								
1% red corpuscles (+) -		+	+	+	+	+	+	+
Incubation at a room temperature during 45 minutes								
Consideration	Serum № 1							
	Serum № 2							

Conclusion:_____

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Task: №3. To describe immunobiological preparations for a specific prophylaxis and treatment of Enteroviral, Flaviviral and Coronaviral infections.

Preparations	Type	Purpose of application	Immunity
For active immunization			

For passive immunization			

Signature of teacher_____

Practical lesson № 5

Theme: Laboratory diagnostics of hepatitis A, B, C, D, E.

a) The list of issues that must be studied:

1. Hepatitis B virus . The structure of the virion. Sensitiveness to physical and chemical factors.
2. Antigens: NVs - surface antigen particles of Dane. Internal antigens: SiS, NVe, their characteristics.
3. Epidemiology and pathogenesis of hepatitis B. Persistence. Immunity.
4. Laboratory diagnostics of hepatitis B. Methods of detection and diagnostic value of markers of hepatitis B (antigens, antibodies, nucleic acids).
5. Specific prophylaxis and treatment of hepatitis B.
6. The virus of hepatitis A. The structure of the virion. Sensitiveness to physical and chemical factors.
7. Epidemiology and pathogenesis of hepatitis A. Immunity. Approaches to the specific prophylaxis.
8. Other causative agents of hepatitis (C, D, E, F, G), their taxonomic position, properties.
9. The role of viruses, hepatitis C, D, E, F, G in human pathology.
10. Possibility ofspread of hepatitis in the treatment of dental patients.
11. Methods of laboratory diagnostics of hepatitis caused by virus A, C, D, E, F, G.
12. The value of sterilization for the prophylaxis of hepatitis in dentistry.

b) The list of practical skills that are necessary to master:

1 .Ability to conduct consideration and evaluate the results of serological tests used in virology (ELISA).

Practical lesson's Protocol

Practical tasks should be done:

Task №1. To sketch the chart of hepatitis B structure.

Task №2. To do the analysis of different combinations of hepatitis B serological markers

Serologic markers	Hbs Ag	Hbe Ag	Anti HBc	Anti Hbe	Anti HBs	Analysis of results	Infectiousness of blood
1							
2							

Task №3. To estimate the results of the ELISA with the serums of patients

	1	2	3	4	5	6	7	8	9	10	11	12	
AND	0.005 NCl	-0.005 neg	0.0120 neg	0.002 neg	0.006 neg	0.006 neg	0.000 neg	****	****	****	****	****	AND
B	00.96 COl	0,002 neg	0,004 neg	0,003 neg	0,002 neg	0,004 neg	0,005 neg	****	****	****	****	****	B
C	0.266 CO2	0,003 neg	0,003 neg	0,004 neg	0,002 neg	0,005 neg	****	****	****	****	****	****	C
D	0.209 CO3	0,000 neg	0,016 neg	0,000 neg	0,270 POS	0,004 neg	0,004 neg	****	****	****	****	****	D
E	0,314 POS	0,002 neg	0,007 neg	0,003 neg	-0,001 neg	0,221 POS	0,002 neg	****	****	****	****	****	E
F	0.338 PC1	-0,005 neg	0,003 neg	0,005 neg	0,002 neg	0,005 neg	0,003 neg	****	****	****	****	****	F
G	0,002 neg	0,002 neg	0,015 neg	0,001 neg	0,004 neg	0,007 neg	0,005 neg	****	****	****	****	****	G
H	0,017 neg	0,003 neg	0,005 neg	-0,004 neg	0,003 neg	0,003 neg	0,004 neg	****	****	****	****	****	H
	1	2	3	4	5	6	7	8	9	10	11	12	

Conclusion:

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Task №4. To give comparative description of hepatitis, that are caused by the viruses of hepatitis A, B, C, D, E.

№	Viral hepatitis agents				
	Virus of hepatitis A	Virus of hepatitis B	The virus of hepatitis delta	Virus of hepatitis C	Virus of hepatitis E
1. Morphology					
2. Genome					
3. Source of infection					
4. Ways of transmission					
5. Receptive macroorganizm					
6. Entrance gates					
7. Pathogenesis					
8. Material for research					
9. Laboratory diagnostics					

Task №5. To describe immunobiological preparations for a specific prophylaxis and medical treatment of viral hepatitis.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Notes:

Analysis of different combinations of serologic markers during VHB (F.Deynhard, I.D.Gast, 1982)

HBsAg	HBeAg	Anti-HBc	Anti-HBe	Anti-HBs	Analysis of the results	Infectivity of blood
+	-	-	-	-	Acute stage VHB or chronic transmitter	++
+	+	_	-	-	Latent period and early acute stage	++
+	+	+	-	-	Chronic hepatitis or chronic transmitter	++
+	-	+	+	-	Late stage of acute hepatitis B or chronic hepatitis	+
-	-	+	+	+	Convalescence after acute hepatitis	-
-	-	+	-	+	Convalescence after carring one in past VHB	-
-	-	-	-	+	After immunization, after the contact (repeated) with HbsAg without development of infection, convalescence after carring one in past VHB	-

Signature of teacher_____

Practical lesson № 6

Theme: Laboratory diagnostics of the diseases caused by DNA–viruses

a) The list of issues that must be studied:

1. General characteristics and classification of families of DNA-containing viruses (poxviruses, herpesviruses, adenoviruses).
2. Structure of virions of poxviruses, herpesviruses, adenoviruses. Antigens, their localization and specificity.
3. Cultivation of DNA-containing viruses. Sensitiveness to physical and chemical factors.
4. Epidemiology and pathogenesis of diseases caused pox-, herpes-and adenoviruses. Immunity.
5. Lesions of the oral mucosa under herpes infections: acute herpetic stomatitis, chronic recediv herpes.
6. Persistence of herpes viruses and adenoviruses.
7. Methods of laboratory diagnostics of diseases caused by pox-, herpes-and adenoviruses.
8. Specific prophylaxis and treatment of diseases caused by DNA-containing viruses.

b) The list of practical skills that are necessary to master:

1. Microscope preparations in the light microscope with immersion lens
2. Ability to conduct consideration and evaluate the results of serological tests used in virology (reaction of complement fixation).
3. Reading and evaluation forms with the results of virological researches.

Practical lesson's Protocol

Practical tasks should be done:

Task **№1.Drow the herpes virus structure and mark its antigens.**

Task №2. To estimate the results of CBR results with the examined patients sera and diagnosticum with adenoviruses antigens.

		1	2	3	4	5	Control of serum	Control to the antigen
Solubilisation of serum Quantity of serum (ml)		1: 16 0,25	1:32 0,25	1: 64 0,25	1:128 0,25	1: 256 0,25	0,25	0,25
Diagnosticum (ml) («+» – bringing)		+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5	-
Complement (ml) («+» – bringing)		+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5
Solution (ml) («+» – bringing)		-	-	-	-	-	0,5	0,5
Incubation at the temperature of 4°C 30 minutes								
Gemolitic system (ml) («+» – bringing)		+ 1,0	+ 1,0	+ 1,0	+ 1,0	+ 1,0	+ 1,0	+ 1,0
Incubation at the temperature of 37°S during 18-20 hours								
Consideration	Serum №1							
	Serum №2							

Conclusion: _____

Task №3. To describe immunobiologic preparations for a specific prophylaxis and medical treatment of the DNA- viral infections.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher_____

Practical lesson №7 is control

Practical lesson №8

Theme: Sanitary-microbiological research of water, air, soil and food products

Practical lesson's Protocol

Practical tasks should be done:

Task №1. To define drinking-water microbe number.

Conclusion:_____
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Task №2. To define drinking-water coli-index and coli-titer. To do a conclusion.

Quantity of positive results of water analysis in 5 small bottles with 100 ml	Coli-index	Scopes of index (reliable scopes)		Coli-titr
		lower	overhead	
0	Less than 2	0	6,0	More Than 455
1	2	0,1	12,6	455
2	5	0,5	19,2	196
3	9	1,6	29,4	109
4	16	3,3	52,9	62
5	More than 16	8,0	-	Less Than 62

Conclusion:_____
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Task №3. To define drinking-water coli-index and coli-titer by the membrane filters method. To do a conclusion.

Conclusion:_____
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Task №4. To define the soil microbe number.

Conclusion:_____
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Task №5. To define the common microbe number of classroom air by sedimentation method.
Conclusion:_____
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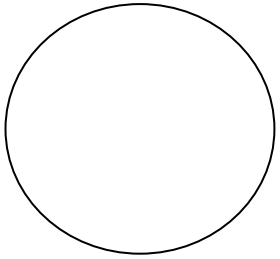
Task №6. Microscope the preparation made from yogurt. Stain by Gram



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To mark morphological and tinctorial properties of the microorganisms
Conclusion:_____
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Signature of teacher_____

Practical lesson № 9

Theme: Oral cavity micro flora

a) The list of issues that must be studied:

1. The main representatives of the permanent flora of the oral cavity: aerobic, anaerobic and optional obligate anaerobic microorganisms.
2. Representativesof interim oral microflora.
3. Factors that influence the formation of oral microflora.
4. Mechanisms of formation of the normal microflora. Adhesion and colonization. Koahrehation.
5. Microbial colonization of different parts of the mouth.
6. Changes in microflora depending on age, health and other factors.
7. Methods for microbiological researches used in dentistry.
8. Violation of microflora in the oral cavity. Dysbacteriosis.

b) The list of practical skills that are necessary to master:

1. Make preparations for microscopic research.
2. Stain preparations by simple and sophisticated methods .
3. Microscope preparations in the light microscope with immersion lens.
4. Differentiation of microorganisms by morphological and tinctorial characteristics .

Practical lesson's Protocol

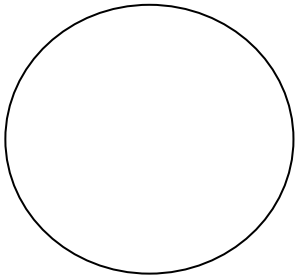
Practical tasks should be done:

Task № 1. To estimate the common state of oral cavity micro flora

Groups of microorganisms	Quantity
1. Grampozitive cocci	
2. Grampozitive bacilli	
3. The Gramnegative cocci	
4. The Gramnegative bacilli	
5. Candida	

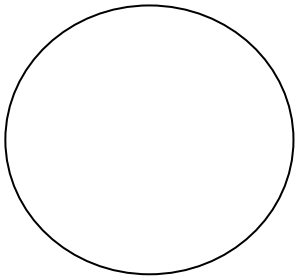
Conclusion:_____
_____

Task № 2. To prepare preparation from a dental raid, to stain by Gram, to microscope, to sketch.



To mark morphological and tinctorial properties of microorganisms

Task № 3. To prepare preparation by Storms from a dental raid. Microscope, sketch.



To mark morphological properties of microorganisms

Appendix: Composition of the oral microflora

In order to understand the aetiology of many oral and dental diseases and to interpret the results of microbiological analyses of clinical specimens, knowledge of the micro-organisms which comprise the resident flora within different ecological niches of the oral cavity is important. However, there are technical problems at all stages of analysing the oral microflora, which potentially compromise the accuracy of studies. The various methods and associated problems involved in specimen collection and processing. The initial sample collection is often the most important step in a microbiological investigation and will significantly influence the results obtained.

Most bactetia found in the oral cavity can be classified as Gram-positive or Gram-negative. Streptococci comprise a major part of the oral flora and play an important role in dental caries, purulent oral infections and infective endocarditis. Modern taxonomic methods have permitted their division into several major groups.

Some species of spirochaetes, protozoa and mycoplasma are also members of the normal oral flora bur are very difficult to cultivate. Spirochaetes are best demonsttated by the technique of dark field microscopy. Other bacteria such as the coliform *Escherichia colt* are present only transiently in the healthy oral cavity but may become established in the elderly or Immunocom-promised. *Candida albicans* is rhe most common fungal isolate and an average carriage rate of 40% has been

observed in asymptomatic adults. Candidal counts increase in the immunocompromised, denture wearers and following some types of antibiotic treatment. Certain viruses, for example Epstein-Barr virus and human herpes virus type 6, are also shed frequently in saliva of healthy individuals.

The oral microflora, in common with many other ecosystems, contains a large proportion (approximately 50%) of currently uncultivable bacteria. Therefore our present knowledge of oral microbiology and oral diseases is only partially complete. More recently, molecular biology techniques have identified some of these uncultivable micro-organisms- However, to under-stand their role in oral health and disease will require new methods to facilitate their isolation, culture and study.

Acquisition of the oral microflora

The oral cavity of the foetus is sterile and although during bitch the neonate comes into contact with the microflora of the mother's vagina, these organisms do not usually become established. The mouth is a highly selective environment for bacteria and only a few species are able to colonize the mouth of the new-born. From the first feeding, micto-organisms are transferred from the surrounding environment such as maternal saliva or the skin flora of the mother and nursing staff.

By 24 hours after birth the first (pioneer) species have become established. Streptococci, particularly *S. salivarius,* which bind to epithelial cells are usually the first to colonize. The early colonizers develop into a pioneer microbial community and begin co modify their environment by producing extracellular products, which enhance conditions for growth of other species. For example, *S. sa/ivarius* produces extracellular polymers from sucrose to which other bacteria, for example *Actinomyces* spp., can attach. This process of microbial succession and increasing diversity will result in the eventual formation of a climax community.

By one year of age, when teeth have erupted, the predominant species isolated are *Streptococcus* spp., *Neisseria* spp., *Veilhnetta* spp. and *Staphylococcus* spp. Less frequently isolated species include *Lactobacilus, Actinomyces, Prevotella* and *Fusobacterium.*

Tooth surfaces and gingival rissues provide new habitats for colonization, with resultant formation of dental plaque. Other shifts in the microbial flora take place during the lifetime of an individual; for example only 18% of 5 year olds have spirochaetes and black pigmenting anaerobes, compared with 90% of 13-16 year olds. The flora of adults remains relatively stable but denture wearers have an increased carriage rate *of Candida albicans.* From approximately 70 years of age there is an increased proportion of *Lactobacillus* and *Staphylococcus* species in saliva of non denture wearers, whilst after 80 yeats of age the number of yeasts increases.

Dental plaque formation

Adherence to a surface in the mouth is essential for survival of oral bacteria. In the case of supragingival plaque formation, organisms do not colonize clean enamel but interact with a layer of material on the tooth surface called the pellicle. The pellicle comprises mucins, salivary glycoproteins, minerals and immunoglobulins. Pellicle formation occurs in seconds on cleaned enamel and reaches a maximum thickness in 90—120 minutes.

The attachment of bacteria ro surfaces is a complex process and can be divided into four main stages as follows:

Bacteria must first approach the surface to which they will later bind. They can do this in several ways including liquid flow, diffusion through Brownian motion, or bacterial movement (chemotactic activity).

Two types of forces are involved at this stage. At distances of 10-100 nm, weak forces such as van der Waal's and electrostatic forces come into effect. These forces are highly dynamic and are influenced by the ion content of surrounding saliva. As a result they are readily reversible.

As rhe bacterium approaches closer (2 nm), strong forces, such as hydrogen bonding between hydroxyl groups in the pellicle and phosphate groups in the bacterial cell wall, come into play (Fig. 21.7).

Following initial adhesion, a more permanent attachmenr can occur by covalenr, ionic or electrostatic bonding.These bonds form between specific receptors on the host surface,
termed ligands, and components on the bacterium called adhesins. The latter ate often situated on bacterial appendages such as firnbriae. Oral bactetial attachment, and therefore plaque formation, is affected by a number of hosr and microbial factors and by saliva as summarized in Fig. 2..

Colonization and biofilm formation Once bound to a surface, the bacterium can divide and temain attached. Extracellular products are formed and daughter cells repeat the process so that microcolonies develop. Salivary glyco-proteins

and dietary sugars such as glucose and sucrose can be metabolized leading to the formation of bacterial cell wall, intra-cellular polysaccharides (IPS), and soluble and insoluble extracellular polysaccharides. The IPS serve as a nutrient store for the organism and are degraded to release energy and organic acids.

Gradually the microcolonies coalesce, producing a more complex three-dimensional arrangement. This is clue to co-aggregation between similar species (intrageneric) e.g. among streptococci, and aggregation between different bacterial species (intergeneric) e.g. between *S. sanguis* and *A. naeslundii* or between *Streptococcus* spp. and *Porphyromonas* spp.. The resultant biofilrn is called dental plaque which is a general term for the complex microbial community found on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin. Plaque matu-ration is characterized by increasing quantity and diversity of micro-organisms on the tooth surface. After 7 days streptococci ate still the main organisms present, but aftet 14 days there is a shift to anaerobic rods and filaments, with streptococci comprising only 15% of the cultivable flora. The whole process of plaque development is summarized diagrammatically in Fig. 21.11.

The situation on oral tnucosal cells is slightly different because of a modified pellicle that covers their surface. The number of bacteria initially adhering to mucosal cells is small and regular desquamation ensures a light microbial load.

There is increasing interest in the complex ecosystem of dental plaque, since this can be used to study the biofilm phenomenon.

A microbially derived sessile community characterized by cells that are attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with tespect to growth rate and gene transcription.

Biofilms are a major problem in many systems, for example contamination of dental unit water lines and infections associated with indwelling prosthetic devices such as hip joints. There is a growing body of evidence to support the fact that microbes behave differently when attached within a biofilm (sessile) as opposed to cells floating in a liquid (planktonic phase). These phenotypic changes may be expressed in a number of different ways. Certain genes may be switched on to alter the composition of the biofilm, for example production of more extracellular polysaccharide. One of the more important clinical effects of biofilms is their inherent resistance to physical or chemical removal. Thus, some micro-organisms can survive in biofilms at concentrations of antibiotics or disinfectants 1000 times greater than those required to kill their pianktonic counterparts. The resistance may be due to binding of the active ingredient to extracellular components of the biofilm or to the reduced growth rate *oi* bacteria. This phenomenon may be important in the generation of antibiotic resistant micro-organisms.

An equilibrium exists between the forces of retention and removal. Rough surfaces allow more plaque to adhere because of the increased surface area and protection from the forces of removal. In addition, surfaces with a low surface free energy (water-repel ling) such as Teflon® bind fewer micro-organisms than those with a high surface free energy (water-attracting) such as enamel. Therefore in order to minimize plaque accumulation, intra-oral devices should be designed with a smooth surface and possess a low surface free energy.

Calculus

Dental plaque can become calcined; saliva is super-saturated with calcium and phosphate ions which may be deposited within the deeper layers of plaque. These ions accumulare within the plaque matrix together with organic debris from dead micro-organisms, while plaque phosphatases and proteases degrade some of the calcification inhibitors in saliva (statherin and proline-rich proteins). These processes lead to the formation of insoluble calcium phosphate crystals, which coalesce ro form calculus. Many anti-calculus toothpastes contain pyrophosphate compounds designed to adsorb excess calcium rhus reducing intra-plaque mineral deposirion. Mature calculus consists of about 80% (dry weight) mineralized marerial (mostly hydroxyapatite) and 20% organic compounds, although the actual composition will vaty with the individual, age, site of the deposit and rhe location of the tooth.

The bacterial flora associated with calculus is relatively nonspecific and reflects the bacterial composition of the dental plaque with which it was associated. Thus, eatly supragingival calculus (2 days) contains primarily Grarn-positive cocci whilst older and gingival calculus will contain more Gram-negative rods. Calculus has a roughened surface and is potous, allowing bacteria and bacterial products such as toxins to be absorbed, thus providing an ideal reservoir for substances potentially harmful to the host. Calculus must therefore be removed from tooth surfaces to halt tissue damage and to promote healing from periodontal disease.

Factors affecting the growth of oral microorganisms

Maintenance of the mictobial community requires a degree of symbiosis between the micro-organisms and the host. This will reflect a balance between factors that encourage growth and those that tend to inhibit growth. The oral flora, specifically, is influenced by a wide range of factors which may be associated with the diet, saliva, gingival crevicular fluid, microbial products and host factors (Fig. 21.13).

Diet

The chemical composition of foods ingested will affect the availability of nutrients, since large molecular weight dietary poly-saccharides must first be broken down by salivary enzymes before they can be utilized. The presence of fermentable carbohydrates such as sucrose, maltose, lactose and glucose will lead to increased plaque formation and the accumulation of microbial products such as organic acids and dextrans. Thus, frequent eating of sucrose is associated with high caries activity. The physical consistency of large molecules such as starches and proteins restricts availability to bacteria, since they may be removed from the mouth before they have been degraded. However, some foods are retained between the teeth or stick to fissures more easily and the longer that bacteria have nutrients available, the more they can metabolise, grow and release by-products such as lactic acid.

Saliva

One of the essential properties of saliva is its action as an efficient buffer that regulates the pH of most sutfaces. The mean pH of unstimulated saliva is 6.75-7.25. Many of the predominant bacterial components of healthy plaque can tolerate brief exposure to low pH but are killed or inhibited by prolonged exposure. A regular decrease in pH by frequent sugar intakes will lead to the proliferation of *S, mutans* and *Lactobacillus* species and an increase in dental caries. Changes in the flow rate will affect the con-centrations of the ions bicatbonate, urea, ammonium, calcium and phosphate, which are of great importance to the balance of mineralization and deminetalization.

Saliva contains many important growth factors, such as glyco-proteins, proteins and minerals that may be utilized in bacterial adherence and metabolism. However, the microbial flora has to act synergistically to bring about the complete degradation of these compounds. Within the rnicrobial community there are a multitude of possible interactions between the products produced by different species, some beneficial and some harmful. Extracellular endoproducts produced by one species may be essential for the growth of another species; for example isobutyrate, a cell wall fatty acid produced by *Fusobacteria* may be used by *Treponema mlcrodentium.* Similarly, *Veillonella parvula* can produce vitamin K3 which, in turn, is essential for the growth of *Porphyromonas* spp.. Conversely, metabolic products such as hydrogen petoxide produced by the *Streptococcus oralh* group or butyrate produced by *Porphyromonas gingivalts* may inhibit othet bacteria.environment and favours the growth of anaerobes. Oxygen accepts electrons and raises the redox potential. Thus, a clean enamel surface has a tedox potential
of + 200mV whilst after 7 days of plaque accumulation the Eh has fallen to -141 mV.

Early colonisers of plaque utilise O_2 and produce **CO_2,** thus allowing bacteria that are capnophilic (CO_2 requiring), for example anginosus group streptococci, to become established. Late colonizers of plaque will produce H_2 and other reducing agents, such as sulphur-containing compounds, which will gradually lower the Eh, allowing mote anaerobic bacreria, such as *Prevotella* spp., to colonize. Thus, periodontal pockets are more reduced (-48 mV) than healthy gingivai crevices (+ 73 mV).

The host

In addition to host factors such as diet and salivary flow others, for example the presence of systemic disease, broad spectrum antibiotic usage, or chemotherapy for cancer, may disturb the host/microbial 6ora interactions. One of the easiest ways the host can influence the oral flora is by use of oral hygiene methods, such as tooth brushing. This produces a persistently young plaque con-taining many facultative anaerobic Gram-positive bacteria with limited numbers of obligate anaerobes, a flora which is compatible with oral health. However, in an effort to make effkienr plaque removal easier for patients, chemical agents have been introduced to reduce plaque build up.

Anti-plaque agents

Three main approaches have been utilized for chemically interfering with the formation of dental
These agents prevent bacterial proliferation on the tooth surface. Currently the most effective anti-plaque agent Is chlorhexidlne. The molecule has a positive charge at either end and binds readily to negatively charged sites on the enamel pellicle, mucosal cells and bacterial cell wall structures. Once bound, the chlorhexidine can exert its antimicrobial effect by damaging the rnicrobial cell membrane and precipitating the cell contents. Chlorhexidine also inhibits microbial adherence since it is able to adsorb onto a surface and is

slowly released, maintaining its antimicrobial activity, a property known as substantivity.

Sodium lauryl sulphare is used commonly in toothpastes and mouth rinses as an anionic detergent which solubilizes plaque to reduce its accumulation. In addition, it has a moderate degree of substantivity and antimicrobial activity, although much less than chlorhexidine.

Anti-adhesive compounds are designed to alter the surface binding within the oral cavity, thus preventing or blocking interactions between bacteria and the oral environment. Many are still at the experimental stage and are currently unavailable as commercial oral hygiene products. Fluoride-containing compounds such as sodium fluoride and stannous fluoride are reported to have anti-adherent properties, although the mechanism of action is uncertain.

Saliva also contains numerous antimictobial factors such as the salivary peroxidase systems, myeloperoxidase (peroxidases inhibit bacterial cell metabolism), lysozyme (lyses some bacterial cell walls), lactoferrin (binds iron which bacteria need for growth), histidine-rich peptides {inhibit *Candida albicam)* and immuno-globulins.

Gingival crevicular fluid (GCF)

This is a scrum transudate which contains proteins such, as albumin and immunoglobulins as well as amino acids, minerals, vitamins and glucose. GCF has protective functions for the host by virtue of its flushing effect and high numbets of viable poly-morphs, antibodies and complement proteins).

The gaseous environment

Most oral micro-organisms are facultative anaerobes, although if oxygen is present, aerobic respiration is preferred. However, some organisms are obligate anaerobes and are killed by oxygen.

The relative amounts of oxygen will help to determine the distribution of certain species within the oral cavity. Notmal air contains 20% **O_2,** the anteriot sutface of the tongue 16%, the posterior surface of the tongue 12% and the buccal folds 0.3%. This will be reflected in the number of anaerobic bacteria recovered from these sires. Sometimes the conditions available for the growth of bacteria are expressed as the oxidation-reduction level and are usually described as the redox potential (Eh), which is recorded in mV. A low Eh (negative value) is a highly reduced.

A. Questions for self-control

9. Basic representatives of oral cavity temporal and permanent microflora
10. Factors, that affect the oral cavity microflora forming.
11. Mechanisms of normal microflora forming. Adhesion and colonization. Coagregation.
12. Microbne colonization of oral cavity different areas.
13. Changes of microflora depending on age, the state of the human health and other factors.
14. Methods of microbiological research, that are used in stomatology.
15. Violation of oral cavity microflora. Disbacteriosis.

A. Tests:

1. A patient D appealed to the dentist with complaints on halitosis (unpleasant smell from a mouth). Which bacteria prevail among oral cavity microflora in this case?
A. Proteus
B. Bacteroides
C. Corynebacterium
D. Esherichia
E. Clostridia

2. Local immunity of oral cavity is provided by antibodies in saliva. The antibodies of which class are found in saliva in a maximal concentration?
A. Ig G
B. Ig A
C. IgM
D. Ig D
E. Ig E

3. 67 yeared patient C. after antibioticotherapy appealed to dentist concerning on edema, redness and irritation of oral cavity mucous. Which microorganism is the reason of such complications?
A. Veilonella
B. Staphylococcus
C. Streptococcus
D. Candida
E. Treponema

4. 59 yeared patient appealed to the dentist with concerning on an unpleasant smell from a mouth. After the examination a doctor set that the reason of unpleasant smell from a mouth (halitosis) was related with oral cavity

microflora. Which microorganism causes the development of halitosis?
A. Anaerobical bacteria (Prevotella, Bacteroides, Peptostreptococcus, Veilonella)
B. Bifidobacteria
C. Protozoa (Entamoeba gingivalis, Trichomonas elongate)
D. Facultative anaerobic coccus (Staphylococcus, Streptococcus)

5. Basing on the clinical displays the dentist suspected the chronic candidiasis and prescribed mycological research for confirmation the diagnosis. Which media should be used for inoculation?
A. MPA
B. Saboud`s media
C. Citt-Tarozzi`s media
D. Endo`s
E. Loeffler`s

6. The antibiotics treatment of 43 yeared patient C. caused oral cavity candidiasis. What species of Candida usually causes the oral cavity candidiasis?
A. C. albisans
B. C. tropicalis
C. C.parapsilosis
D. C. krusei
E. C. globrata
7. Name saliva enzyme wich killes bacteria.

A. Amilase
B. Phosphatase

C. Lysozime
D. Carbamilphosphatsintet
E.Glucose-6-phphatdehydrogenase

Signature of teacher: _____

Date: _____

Practical lesson № 10

Theme: Microbiological and immunological aspects of etiology and pathogenesis of caries

a) The list of issues that must be studied:

1. Features of oral diseases caused by resident micrflora.
2. Dental plaque, the composition and formation mechanism.
3. Caries - infection related to the resident flora of dental plaque.
4. Immunological basis of etiology and pathogenesis of dental caries.
5. Microbial flora under conditions of acute and chronic pulpitis.
6. Microbial flora under conditions of acute and chronic periodontitis.
7. Stomatogenic inflammation (periostitis, osteomyelitis, abscess, abscess), changes in symbiotic bacteria of the oral cavity under conditions of endogenous infections.
8. Features of collection of the examined material under caries, pulpitis and periodontitis.
9. Rules for collecting material for the allocation of anaerobic bacteria.
10. Anticaries vaccination.

b) The list of practical skills that are necessary to master:

1. Microscope preparations in the light microscope with immersion lens.
2. Differentiation of microorganisms by morphological and tinctorial characteristics .
3. To conduct consideration and estimate the results of titration of lysozyme.
4. Be able to determine the percentage of phagocytic neutrophils, phagocytic number

Practical lesson's Protocol

Practical tasks should be done:

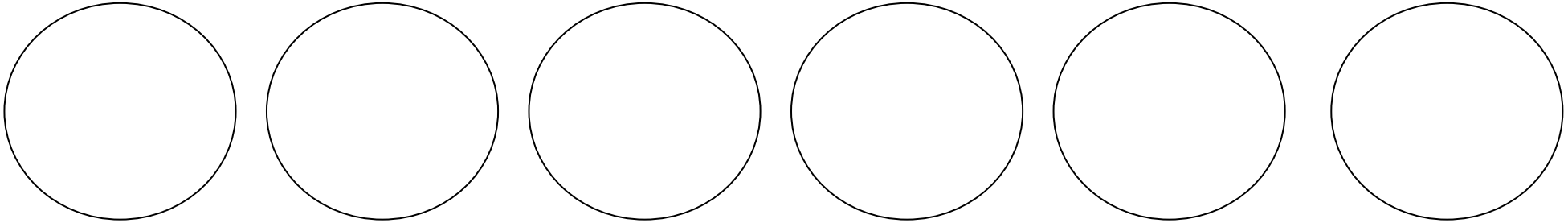
Task № 1. To bring to table the data about composition of micro flora of dental raid depending on the stage of his forming:

Stage of forming of dental raid	Composition of plaque
The stage I	_____ _____ _____ _____
The stage II	_____ _____ _____ _____
The stage III	_____

	_____ _____ _____

Task № 2. To sketch preparations of acid formation microorganisms:

a) S.mutans b) Lactobacillus c) Veillonella g) Leptotrichia d) Actinomyces e) Corynebacterium



_____

To mark morphological and tinctorial properties of microorganisms

Task № 3. To conduct consideration of activity of lysozyme of saliva of patients. To do a conclusion.

Concentration of lysozyme		1/8	1/16	1/32	1/64	1/128	1/256	C_s	K_c
Stock-taking	Patient with a single caries								
	Patient with a plural caries								

Conclusion:_____

_____

Task № 4. To conduct comparison of indexes of functional activity of neutrofillic leucocytes of peripheral blood of patients. To do a conclusion.

The evidence / The patients	Per cent of phagocyted neutrofillis	The phagocitosis number
Patient with the single uncomplicated caries	67	
Patient with plural one by a caries	18	

Appendix: Dental Caries (Decay)

Dental decay is due to the dissolution of tooth mineral (primarily hydroxyapatite, Ca10 (P04)6(0H)2) by acids derived from bacterial fermentation of sucrose and other dietary carbohydrates. These bacteria live in bacterial communities known as dental plaque which accumulates on the tooth surface. For almost a century it was believed that any bacterial community on the tooth surface could cause decay, and treatment was almost exclusively the mechanical cleaning of these surfaces by toothbrushing, using some type of mild abrasive. Such treatments based upon debridement and, in extreme cases, upon dietary carbohydrate restriction, were singularly unsuccessful in reducing dental decay. In fact, the prevalence of dental decay was so high among young men that it was the major cause of rejection from military service in World Wars I, II, and the Korean War. This staggering amount of dental morbidity led to the formation of dentistry as a separate health profession in the late 19th century; to the expectation that all people would, if they lived long enough, be edentulous (toothless); and to a dental health bill to the public of approximately 34 billion dollars per year in 1990.

Things have changed. Water fluoridation has proven to be a most cost-effective way of reducing decay; fluoride dentifrices were even more effective than initially projected; and research findings indicate that most carious lesions actually reflect a sucrose-dependent *Streptococcus mutans* infection. Individuals at risk for this infection can be diagnosed and treated by frequent mechanical intervention, by intensive application of prescription levels of fluorides or other antimicrobials (such as chlorhexidine), by restriction of ingestion of sucrose between meals, or by use of products that contain sucrose substitutes (such as xylitol). The net result is that dental decay in the late 20th century is a controllable infection and should be preventable in many individuals. Almost 50% of young children are caries-free, and the level of edentia among individuals over 65, has dropped from 50% to about 20%.

Etiology

Dental decay has been known since recorded history, but was not an important health problem until sucrose became a major component of the human diet. When sucrose is consumed frequently, an organism known as *Streptococcus mutans* emerges as the predominant organism, and it is this organism that has been uniquely associated with dental decay.

In 1924 *S mutans* was isolated from human carious lesions, but subsequently was not thoroughly studied until the 1960s when it was re-identified as the etiologic agent of a transmissible caries infection in rodent models. In these studies, all of Koch's postulates for infectivity were fulfilled in animal models. However, it proved difficult to show that *S mutans* was a human dental pathogen, because *S mutans* appears to be a member of the normal flora on the teeth, and it was difficult to show that an increase in *S mutans* actually preceded and/or coincided with the earliest clinical lesion.

Dental decay is measured clinically as a cavitation on the tooth surface. However, cavitation is a late event in the pathogenesis of decay, being preceded by a clinically detectable subsurface lesion known as a white spot (Figure 99-1), and prior to that by subsurface demineralization that can only be detected microscopically. From a diagnostic and treatment perspective, the lesion should be detected at the white spot stage. This usually cannot be done without rigorous descriptive criteria (not all white spots are due to the decay process) and because the white spot stage in the caries-prone fissures and approximal surfaces of the tooth cannot be directly visualized during a dental examination.

The prevalence of dental morbidity is documented in terms of the number of teeth (T) or tooth surfaces (S) that have obvious decay (D), contain a dental restoration or filling (F), or are missing (M). These DMF teeth (DMFT) and DMF surface (DMFS) scores do not discriminate as to the relative proportions of the score due to decay, versus fillings and extractions. This insensitivity of the DMFT and DMFS scores in quantitating the actual decay, independent of

morbidity led in early clinical studies to unimpressive associations between *S mutans* and DMFT or DMFS scores. However, when the comparison was limited to individuals with decayed teeth or when the plaque samples were taken from a decayed tooth site, a significant association between *S mutans* and decay was evident.

This association is clearly seen in individuals who developed xerostomia secondary to radiation treatment of head and neck cancer. *S mutans* and lactobacilli are normally present in low numbers in the plaque of these individuals. When the salivary flow decreases, the pH in the plaque drops, leading to a selection for aciduric (acid-tolerant) bacteria, such as *S mutans* and lactobacilli. New decayed lesions become obvious within 3 months after radiotherapy and the patient may average one or more new decayed surfaces per post-radiation month. During the development of decay, the proportions of, first, *S mutans* and then lactobacilli increased significantly. This sequence of events indicated that *S mutans* was involved with the initiation of decay, whereas the lactobacilli were associated with the progression of the lesion.

This bacterial succession is illustrated in Figure 99-2, which shows the sequence of events occurring on the surface of a caries-free tooth that either becomes carious or remains caries free. In either case, the tooth surface initially represents a carrier state relative to harboring a primary cariogen, such as *S mutans*, in the plaque on a smooth surface. The proportion of the cariogen in the flora is similar in both cases, but the location of *S mutans* differs within the plaques. In the tooth destined to develop decay, *S mutans* is located on the enamel surface, whereas in the tooth destined to remain caries free, *S mutans* is confined to the saliva-plaque interface. Debriding procedures, such as toothbrushing and flossing, might remove most plaque organisms, but could leave untouched those bacteria either firmly attached to the enamel surface or sequestered in defects in the enamel surface. In surfaces destined to become carious, the residual organisms would include *S mutans*, whereas in surfaces destined to remain caries free, *S mutans* would be absent. Over time these caries-free surfaces might alternately acquire and lose *S mutans*, thereby having an intermittent carrier-state status. However, in those surfaces in which caries will eventually develop, *S mutans* becomes a dominant member of the flora, undoubtedly secondary to frequent sucrose ingestion.

The incipient or white spot lesion occurs when the acidogenic activity of the cariogen causes tooth mineral to be mobilized from the subsurface enamel to buffer the pH at the plaque-enamel interface. Bacteriologic sampling at this stage should reveal both a proportional and an absolute increase in the levels of *S mutans*. When the lesion progresses to the stage of cavitation, the organisms penetrate into the enamel crystals (Fig. 99-2). Also, secondary cariogens, such as the lactobacilli, appear as a result of the selection for aciduric organisms in the plaque. When the lesion reaches the advanced clinical stage, conditions may be such that *S mutans* can no longer survive, and only secondary cariogens like the lactobacilli and opportunistic organisms can be found.

This model predicts that a bacterial succession occurs during the progression of a carious lesion and that the flora of the advanced lesion may bear little resemblance to the flora of the incipient lesion. Thus it was necessary to sample the plaque during the initial lesion or white spot stage to find the etiologic agents of decay. When this was done, *S mutans* dominated in the flora. However, for the lesion to progress to the stage of cavitation, lactobacilli seem necessary. Thus while *S mutans* could be isolated from both progressive and nonprogressive lesions, *L casei* could only be isolated from progressive lesions.

Pathogenesis

These clinical studies indicated that of the 200 to 300 species which can be isolated from plaque, only *S mutans*, and to a lesser extent the lactobacilli, can be consistently associated with dental decay. What makes these organisms cariogenic relative to all other bacterial types found in the plaque?

In the 19th century, microbial acid production from dietary substrates was linked to the etiology of dental decay in what was called the chemoparasitic theory of decay. But researchers were not able to associate any single acidogenic species with decay, and concluded that decay was bacteriologically nonspecific and due to the increased amounts of acid formed when bacteria accumulated in plaque on the tooth surfaces. It was noted that decay occurred at retentive sites on the teeth and recommended mechanical debridement of these sites as the best method of reducing decay. While the clinical observations were correct, there was no way of determining that the retentive sites were caries prone because they provide the micro-environment which selects for *S mutans*

and lactobacilli. In this section we shall examine those attributes of *S mutans* and the lactobacilli that enable them to be successful on retentive sites and show that these attributes constitute, in effect, the virulence factors which make these organisms specific odontopathogens.

Sucrose in the diet

Considerable evidence from epidemiologic observations and animal experiments indicates that, shortly after sucrose is introduced into the diet, a notably higher incidence of decay occurs. The relationship between sucrose ingestion and dental caries is reasonably well understood. The supragingival plaque flora derives its nutrients from various sources that include diet, saliva, sloughed epithelial cells, dead microbes, and gingival crevice fluid or exudate. All sources, except the foods in the diet provide only small amounts of nutrients. Dietary components are normally high-molecular-weight polymers (such as starch and proteins) that are in the mouth for short periods. They have a minimal effect on plaque growth except in those instances when food is retained between and on the teeth. Sucrose, however, changes this pattern because it is a low-molecular-weight disaccharide that can be rapidly sequestered and utilized by the plaque flora. Plaque organisms capable of fermenting sucrose have a decided advantage over the non-sucrose fermenters in that they can proliferate during periods of sucrose ingestion and thereby become the dominant plaque organisms.

Sucrose fermentation produces a rapid drop in the pH, to 5.0 or lower, at the point of interface between plaque and enamel. When sucrose is ingested during meals, sufficient saliva is secreted to buffer the plaque pH and decay does not occur. In fact, studies show that as much as one-half of a pound of sucrose consumed daily at meals for two years was not associated with an increase in dental decay; however, when the same or lesser amounts of sucrose were ingested between meals, subjects developed new decay at the rate of about three to four tooth surfaces per year. The frequent ingestion of sucrose has been shown to increase the lengths of time that sucrose could be detected in the saliva. This means that if this sucrose were available for microbial fermentation in the plaque, low plaque pHs would be present for long periods each day. When the plaque pH value falls below 5.0-5.2, the salivary buffers are overwhelmed and as lactic acid diffuses into the tooth, enamel begins to dissolve, releasing Ca and PO4 ions from sites beneath the surface enamel. Normally, the bathing saliva replenishes these minerals, but if the length of the flux from the enamel is great, repair does not occur and cavitation results. Thus, sucrose consumption per se does not cause decay, but the frequent ingestion of sucrose by prolonging the time period by which the plaque is acidic, is cariogenic.

Plaque bacteria that ferment sucrose produce acids, which *in vitro* lower the pH value to below 5.0. However, only *S mutans* of all these species reliably caused decay in germ-free animals fed a high-sucrose diet. This suggested that microbial acid production was not the exclusive determinant of decay and that *S mutans* had to possess other attributes which were responsible for its virulence. *S mutans* was subsequently shown to metabolize sucrose in a remarkably diverse fashion that is not matched by any other known plaque organism. The major pathway is concerned with energy metabolism; in this process, the enzyme invertase splits sucrose into its component glucose and fructose molecules, which are then converted to lactic acid by the glycolytic pathway. Other enzymes, called glucosyltransferases, split sucrose but transfer the glucose moiety to a glucose polymer known as a glucan. *S mutans* forms several complex glucans that differ in their core linkage, amount of branching, and molecular weight. The first glucan identified had a core linkage consisting of an a1-6 bond that classified it as a dextran. Later, a unique glucan having an a1-3 core linkage was identified and given the name mutan. *S mutans* also has enzymes that split sucrose and transfer the fructose moiety to a fructose polymer known as a fructan. Other plaque bacteria can use sucrose to synthesize one or more of these polymers, with the exception of mutan. Only *S mutans* can form all of them, a fact that led to an inquiry into the relationship between polymer production and caries formation.

A series of *in vitro* experiments showed that the glucans enable *S mutans* to adhere to surfaces. This suggested that *in vivo* these adhesive polymers would enable *S mutans* to adhere tenaciously to the tooth surface and to accumulate on these surfaces, thereby causing decay in the underlying surface.

Animal experiments in which rodents were infected with mutants of *S mutans* that lacked the ability to form either dextran or mutan, indicated that the absence of mutan was associated with a greater reduction in smooth surface decay than was the absence of dextran. In each instance, the amount of pit and fissure decay was not significantly affected by these mutations. Decay on smooth surfaces seems to depend on the retentive polymers formed by *S mutans*,

whereas in sites where retention is provided by the anatomy of the teeth (pits, fissures, and contact points between teeth), these polymers are not as important. Accordingly, pit and fissure decay may be caused simply by any acidogenic organism that can survive in these retentive sites.
This nonspecific explanation does not seem completely satisfactory, because in animal models and in human caries, *S mutans*, again, is the dominant organism involved or associated with pit and fissure decay. A few other organisms, such as *Lactobacillus casei* and *Streptococcus faecalis*, can cause fissure decay in germ-free rats. These three organisms are all relatively aciduric compared to other plaque bacteria; that is, they not only produce acids, but they are relatively resistant to the resulting low pH caused by acid accumulation. Lactobacilli are the most aciduric of the plaque bacteria, but these organisms only predominate by the time the carious lesion has extended into the dentin. At the time the earliest carious lesion is detected, only *S mutans* has reached significant levels and proportions (Figure 99-2). When *S mutans*, lactobacilli, and other plaque species were compared *in vitro* for their ability to ferment sucrose at different pH values, *S mutans* was found to be more active than the other bacteria at pH 5.0, and thus, it is probably most active *in vivo* at the very pH at which the teeth begin to demineralize.
This aciduricity best explains the involvement of both *S mutans* and lactobacilli in human decay. A retentive site is colonized by those organisms present in saliva. *S mutans*, although scarce in the initial inoculum (fewer than 0.1% of the initial colonizers), is selected for if the average pH value in the site is not well buffered by saliva. Frequent ingestion of sucrose-containing products predisposes toward lower pH values and thus selects for *S mutans*. When the pH remains in the vicinity of 5.0-5.5, tooth mineral is solubilized, thereby buffering the plaque and maintaining an environment suitable for growth of *S mutans*. Eventually, enough mineral is lost so that a cavitation occurs in the enamel, and if this enlarges so that it extends into the dentin, a semiclosed system is formed in which the pH value drops below 5.0. Under these acidic conditions, growth of lactobacilli is favored, and these organisms succeed as the predominant flora in the carious lesion.

Clinical Manifestation

Dental decay occurs at discrete sites on the surface of the enamel. Progress through the enamel is usually slow because of the remineralizing action of the saliva, and is asymptomatic. When decay spreads into the dentin, the process accelerates, most likely because the very low pH that can arise in this semiclosed environment denatures the collagen scaffold that holds the hydroxyapatite salts in place and rapidly solubilizes them. When the dentinal decay approaches the innervated tooth pulp, the pain can be intermittent or continuous, and dull or excruciating. Pain is the chief complaint of the patient.

Microbiologic Diagnosis

A microbiologic diagnosis for a *S mutans*/lactobacilli infection is rarely sought, primarily because the acute pain that brings the patient to the dentist is almost always relieved by a dental restoration or extraction. Thus, the knowledge of an underlying *S mutans* infection would not change the treatment. However, microbiologic diagnosis would be advantageous in the management of the patient to prevent or minimize future decay. Such situations would occur whenever an expensive treatment is planned, such as orthodontic treatment, or the placement of dental crowns and bridges to replace missing teeth. Microbiologic examination would also be useful at the end of any restorative treatment to determine the residual level of *S mutans* and lactobacilli colonization on the teeth.
Scandinavian investigators have empirically determined that 106 CFU *S mutans* per milliliter of stimulated saliva can be associated with future caries activity. Accordingly, they have recommended active intervention with fluoride, dietary counseling, and antimicrobial agents in individuals so infected. They have designed simple chairside tests that can, in a semiquantitated manner provide information on the salivary levels of *S mutans*. All of these tests rely on the fact that *S mutans* is resistant to 5 ug/ml of bacitracin and that it will grow in the presence of 20% sucrose. In liquid media containing these additives, *S mutans* will form adherent colonies on the side of glass, plastic strips, or any other solid surfaces that are present.
In a practical application of these tests, the clinician would not place orthodontic bands on an individual with 106 CFU of *S mutans*, because this individual would be apt to develop decay around the margins of the bands. Likewise an individual who is having extensive bridgework (the placing of dental

restorations across an edentulous space) would be at risk of developing new decay around the margins of these restorations. In both instances, the patient needs to be treated for *S mutans* infection prior to the placement of the dental devices or restorations.

Prevention and Treatment

Conventional dental therapy has not yet incorporated any microbiologically-based strategy into its armamentarium. Instead, a treatment based on response to symptoms has prevailed. The bankruptcy of this approach, which depends on a turn-of-the-century biologic base, has been demonstrated in the Scandinavian countries, where a socialized dental delivery system has made quality dentistry available to everyone. Because of the emphasis on treatment rather than prevention, the results have only prolonged the life span of the tooth by about 10 years, a rather poor therapeutic result. In England, where the health care system also emphasized treatment rather than prevention, one-half of the people over 35 years of age were edentulous in the 1970s. The Scandinavians, especially in Sweden, have changed their approach and have instituted plaque prophylactic programs for children and adults. Thorough dental cleaning with a 5% fluoride paste given at 2-4 week intervals combined with oral hygiene education, has lowered dental decay in children by about 80%-90%, compared to youngsters receiving symptomatic treatment. (Symptomatic treatment involves placing dental restorations in an obviously carious tooth, and pulling teeth.) Similar success has been achieved in adults with and without periodontal disease.

Thorough cleaning with fluoride apparently selects for the more desirable bacterial types, such as *S sanguis* and *S mitis*, which are capable of rapidly colonizing the tooth surfaces. *S mutans* presumably does not have an opportunity to become dominant, because the frequent debridement neutralizes its ability to be selected for by the low pH values that characterize an undisturbed plaque. Also, the 5% fluoride paste has an immediate bacteriostatic effect on the plaque organisms.

Fluoride as an Antimicrobial Agent in Plaque

The mechanisms by which fluoride prevents decay are multiple, and the relative contributions of each mechanism are not fully understood. The 30%-50% reduction in decay that follows water fluoridation is generally attributed to the fluoride replacing hydroxyl groups in the tooth crystal, thereby forming fluorapatite (Fig. 99-4). Fluorapatite is less soluble in acid than hydroxyapatite, which means that a tooth containing fluorapatite dissolves slowly in the low pH value found in plaque, and accordingly, remineralizes faster in the intervals between sugar ingestion. These explanations do not completely account for the proved efficacy of topically-applied fluorides and raises questions about other modes of fluoride action.

A. Questions for self-control

1. Features of oral cavity deseases.
2. Dental plaque, composition and formation mechanism.
3. Caries is the infection related with the resident flora of dental plaque.
4. Immunological bases of caries etiology and pathogenesis.
5. Microflora at acute and chronic pulpitis.
6. Microflora subject at acute and chronic periodontitis.
7. The dental inflammatory processes (periostitis, osteomyelitis abscess, phlegmona), changes of
8. oral cavity microorganisms at endogenous infections.
9. 8. Particular collections of specimen at caries, pulpitis and periodontitis
10. Rules of specimen collections for anaerobic bacteria pure culture isolation.
11. Anticariosis vaccinations

Signature of teacher_____

Date: _____

Practical lesson № 11

Theme: Microbiological and immunological aspects of ethyology and pathogenesis of parodontal diseases.

a) List of questions, that are subject to the study:

1. Microflora is the factor of parodontal diseases.
2. The Supragingival and subgingival dental name-plates, their composition and value in development of pathological process.
3. Participation of microorganisms of cavity of mouth in pathogenesis of parodontitis
4. The Parodontogenus species of microorganisms
5. Participation of the immune system in development of parodontitis
6. Unspecific resistance of organism subject at parodontal diseases

б) List of practical skills and abilities by which it is necessary to seize:

1. *Microscopy of preparations in a light microscope with immersion objective.*
2. *Differentiation of microorganisms after morphological and tinctorial signs.*

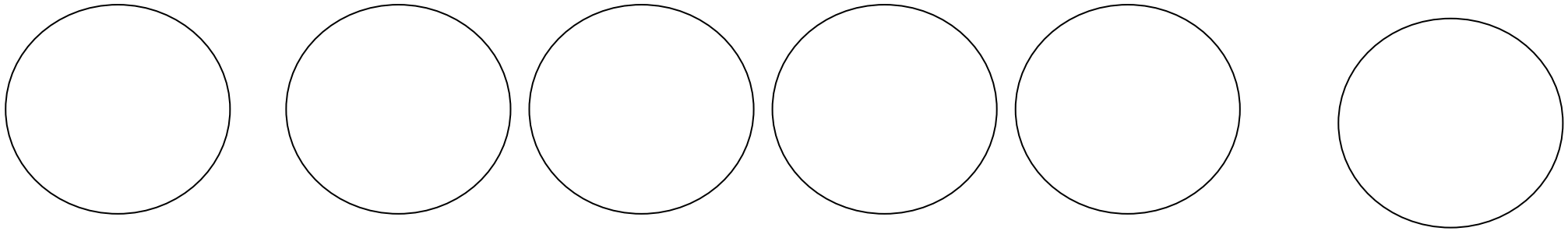
Practical lesson's Protocol

Practical tasks should be done:

Task №1. To bring in the Latin names of microorganisms, association of which co-stars in etiology of parodontitis, to table. To sketch them.

№ p/p	The Name
1	
2	
3	
4	
5	
6	

1) 2) 3) 4) 5) 6)



To mark morphological and tinctorial properties of microorganisms

Task №2. To bring to table the list of factors of pathogenesis of parodontogenus bacteria, that cause the following action:

Factors pathological	Character of wounded
	1. vazomotoricus violations 2. violation of cellular exchange 3the gemorogical necrosis 4. strengthening of transudation 5. activating of the plazmin-kinini system 6. secretion by macrophages and polimophonuclears leucocytes of collagen sis and and lysosomis sour gidrodasis 7. the inflammation
	defeat of the nervous ending violation of nervous–tropical processes at parodontal
	«Factors of distribution» and other enzymes pathogens.

Signature of teacher:_____

Periodontal Disease

Periodontal disease is the general description applied to the inflammatory response of the gingiva and surrounding connective tissue to the bacterial or plaque accumulations on the teeth. These inflammatory responses are divided into two general groupings: *gingivitis* or *periodontitis*. Gingivitis is extremely common, and is manifested clinically as bleeding of the gingival or gum tissues without evidence of bone loss or deep periodontal pockets. Pocketing is the term given to the pathologic loss of tissue between the tooth and the gingiva, creating spaces that are filled by dental plaque (Figure 99-1). Periodontitis occurs when the plaque-induced inflammatory response in the tissue results in actual loss of collagen attachment of the tooth to the bone, to loss of bone, and to deep periodontal pockets which, in some cases, can extend the entire length of the tooth root (15 to 20 mm).

Periodontitis is usually graded according to the severity of the tissue loss and the number of teeth involved. Periodontitis is not as prevalent as once thought: a recent survey of American adults revealed that only 8% of the population surveyed had one tooth site with attachment loss measuring 6 mm or more. This finding was surprising, given past surveys, which indicated that almost everyone would experience advanced forms of periodontal disease as they aged, but is in agreement with recent population surveys in other countries which show that from 5 to 15% of the population has periodontitis.

Etiology and Pathogenesis

The most important new finding concerning periodontal disease is the realization that these clinical entities are really specific infections. These infections are unusual in that massive or even obvious bacterial invasion of the tissues is rarely encountered. Rather, bacteria in the plaque touching the tissue elaborate various compounds, such as H_2S, NH_3, amines, endotoxins, enzymes (such as collagenases) and antigens, all of which penetrate the gingiva and elicit an inflammatory response. This inflammatory response, although overwhelmingly protective, appears to be responsible for a net loss of periodontal supporting tissue, and leads to periodontal pocket formation, loosening of the teeth, and eventual tooth loss. As will be described subsequently, neutrophils are extremely important in this inflammatory response and, if they are absent, as in various neutropenias, or compromised as a result of chemotherapy, an aggressive form of periodontitis is encountered. T4 helper cells play a role in this defense, as witnessed by the periodontitis encountered in patients with acquired immune deficiency syndrome.

Gingivitis

The simplest form of gingivitis is associated with the accumulation of supragingival plaque along the gingival margins of the teeth. This form of gingivitis has been extensively studied in human volunteers, and the sequence of events is well described. In these studies, individuals are brought to a state of health and then refrain from all forms of oral hygiene for a 3- to 4-week period. The initial colonizers of the teeth are streptococci, which proliferate and in turn become colonized by other bacteria present in saliva, such as various *Actinomyces* species and *Veillonella*. The greatest growth of the plaque occurs at the gingival margin, where plaque accumulations usually are visible after several days. This plaque may, in some instances, provoke a bleeding gingivitis in which spirochetes and *Actinomyces viscosus* are prominent members of the plaque flora. If this plaque remains undisturbed, the flora gradually shifts toward an anaerobic, Gram-negative flora that includes black pigmented bacteroides and several types of spirochetes. The increase in these anaerobic organisms can be explained by the low oxidation-reduction potential of the aged plaque and by nutrients derived from the inflammatory exudate at the site.

The gingivitis may resolve itself or fester subclinically for an indeterminate period; however, the potential for the formation of a periodontal pocket (periodontitis) exists at any time. When pockets are detected clinically, they usually are associated with calcified plaque deposits, called calculus, present on the tooth surfaces. For many years, calculus was thought to be the etiologic agent of periodontitis, because inflammation usually subsided when it was removed and the tooth surfaces were mechanically cleaned. However, calculus is always covered by plaque, and removal of calculus would be synonymous with debridement of plaque. The subgingival plaque flora associated with periodontitis is dominated by an anaerobic, Gram-negative flora in all cases but one, and that is a unique clinical entity formerly known as periodontosis, and now as localized juvenile periodontitis (LJP). LJP is an important clinical entity because of the understanding it has provided of the complex and dynamic interactions between the host and the flora in the pocket ecosystem.

Localized Juvenile Periodontitis (LJP)

LJP is different from all other periodontal infections, as it is not associated with plaque accumulations or calculus (in fact the absence of such led early investigators to consider it as a degenerative condition), is localized to certain anterior or front teeth and first molars, and is seen following puberty. It is a rather rare entity, occurring in about 0.1 to 0.5% of teenagers, but when found, is often clustered within families. This familial background suggested a genetic predisposition, which subsequently has been identified as a neutrophil defect associated with reduced chemotaxis. Bacterial examinations of subgingival plaque from affected teeth and adjacent healthy teeth, revealed that the diseased teeth were colonized by an essentially Gram-negative flora dominated by organisms subsequently identified as various *Capnocytophaga* and *Wolinella* species and *Actinobacillus actinomycetemcomitans*. It is *A actinomycetemcomitans* that appears to be the etiologic agent of LJP, and the arguments for its involvement are illustrative of the arguments made to implicate other species in other forms of periodontitis.

Once LJP has been recognized clinically, most of the tissue damage has already occurred, thereby permitting only a retrospective diagnosis of an *A actinomycetemcomitans* infection. *A actinomycetemcomitans* is found at a higher prevalence in tooth sites associated with LJP and at a lower prevalence in healthy sites in the same mouth, or at sites in periodontally healthy individuals. It is often found among other family members in a household with an LJP individual, and indeed among siblings at risk to LJP, there is suggestive data that colonization by *A actinomycetemcomitans* precedes the development of a pocket and subsequent bone loss. But what has been the most important reason for implicating
A actinomycetemcomitans as a periodontopathogen, is its killing effect on neutrophils.
A actinomycetemcomitans produces a leukotoxin that kills neutrophils *in vitro*. It is clear that this leukotoxin is expressed *in vivo*, because patients with LJP have developed circulating antibodies which can neutralize this toxin *in vitro*. From this finding, a scenario can be developed that explains the localized nature of LJP. Children with a neutrophil chemotactic defect become colonized by *A actinomycetemcomitans* in early life, presumably by contact with infected household members. The colonization spreads to those permanent teeth that erupted at ages 5 to 7, but remains quiescent as an infection during the time that the primary or baby teeth are lost, and new permanent teeth appear at about ages 11 to 13. The individual entering puberty, has a dentition com posed of first molars and incisors that are colonized by *A actinomycetemcomitans* and newly erupted teeth that either are not colonized or only minimally colonized.
Something then triggers the relative overgrowth of *A actinomycetemcomitans* in the subgingival plaque, and some of these organisms invade the gingival tissue and cause attachment and bone loss in the absence of an obvious inflammatory response. The latter can be explained by both a sluggish neutrophil response to the bacteria and by the leukotoxin inhibiting the neutrophils, and thereby preventing a protective host response in the pocket microenvironment. The leukotoxin is antigenic and elicits an antibody response which may neutralize the leukotoxin at other tooth sites, thereby limiting the infection to the originally colonized molars and incisors.
This scenario, while incomplete, does explain the localized nature of LJP, partially explains the absence of an inflammatory response in the tissue, and demonstrates the dynamic role of neutrophils and circulating antibodies in defending the periodontium. Presumably, this theme is operating in the more commonly found cases of adult periodontitis. Certainly, the central role of the neutrophils in host defense is unquestioned, as individuals with neutropenias, chronic granulomatous disease and various leukemias often present with advanced forms of periodontal disease.

Early-Onset Periodontitis (EOP) and Adult Periodontitis (AP)

The more common forms of periodontitis comprise at least two clinical entities, an early onset form in mainly young individuals and a chronic form seen in older adults. The early-onset periodontitis (EOP) is more aggressive looking, while the adult periodontitis (AP) may reflect a stable, but tenuous, stand-off between the host's defensive systems and the plaque bacteria. It is not clear whether these entities represent multiple types of infections with two clinical manifestations, or a single mixed anaerobic infection with different levels of host containment. The inability to distinguish microbiologically between these two general patterns reflects methodologic procedures relating to the sampling of the subgingival plaque and the inability of any one culture medium and/or technique to give the total picture of the 200 to 300 bacterial species found in the plaque flora. For example, the spirochetes cannot be quantitatively cultured and may account for more than 40% of the flora in EOP and AP. They can be enumerated by microscopic examination of the plaque but would be ignored in cultural studies. These cultural studies, in turn, reveal a bewildering array of species, many of them either newly-described or as yet unspeciated. None of these cultivable species predominates in all disease-associated plaques. For example, *Bacteroides forsythus*, a nonpigmenting fusiform organism has been associated with the active periodontal lesion. *B forsythus* is present in 13% of the active sites and at 8% of the inactive sites, a difference which is hardly indicative of etiologic association. Yet the authors concluded that *B forsythus* is a probable periodontal pathogen because its levels, when present, were on the average 4 times higher in the active sites than in the inactive sites, i.e., 2.5% *vs* 0.6%. This difference is well within the error of the methods used to isolate the organisms.
Despite these problems in assigning virulence to any one species, it is clear that the bacterial communities at disease sites are different from the communities at healthy and successfully-treated periodontal sites (Table 99-2). The diseased sites are dominated by anaerobes, and in particular, by spirochetes and black-pigmented bacteroides species, such as *Porphyromonas gingivalis* and *Prevotella intermedia*. Among the latter, *P gingivalis* most often is associated with EOP, whereas *P intermedia* is found in both EOP and AP. No species, except the ubiquitous spirochetes, are consistently found in all lesions. Among the spirochetes, *Treponema denticola* is the only species that can be reliably cultured. It has been shown to possess a wide array of enzymes, such as a collagenase, peptidases, hyaluronidase, and a keratinolytic enzyme, and to produce noxious end products, such as butyrate, NH3, H2S, and endotoxin, that could cause, if they entered the periodontal tissue, an inflammatory response. However, comparable enzymes occur in *P gingivalis* and other anaerobic species found in the plaque, so that it would be difficult to assign etiologic significance to any one of these organisms based on the production of these enzymes. This being the case, it may be best to consider that the collective overgrowth of all these anaerobic species in the plaque causes a mixed infection that is responsible for tissue loss in EOP and AP.

Clinical Manifestations

Periodontal disease is usually painless until late in the disease process, when the teeth are so loose that some discomfort may appear upon chewing. Retention of food in a pocket site may provoke a sudden burst of microbial growth which could result in a painful abscess. At other times, the anterior teeth may become so loose that they separate and the patient visits a dentist

because of the resulting poor aesthetics. However, under ordinary circumstances, it is bleeding upon brushing and/or concern over halitosis that brings the patient to the dentist. A thorough dental examination should find any pockets which may exist. If these pockets bleed upon probing, such bleeding is synonymous with tissue inflammation and warrants therapeutic intervention.

Microbiological Diagnosis

Microbiologic diagnosis is not commonly used in the management of periodontal disease. Several methodologies are, or soon will be, available that permit identification and quantification of the periodontopathogens listed in Table 99-2. The oldest method is the use of darkfield and phase contrast microscopy to identify spirochetes and other motile organisms in plaque samples. However, as spirochetes are detectable in most plaques, it is necessary to establish some critical value above which a spirochetal infection can be diagnosed. Our experience suggests that $\geq$ 20% spirochetes in any plaque sample permits the diagnosis of an anaerobic infection.

A microscopic examination cannot distinguish the species of bacteria present unless one uses an immunologic staining reagent specific for the organism in question. Such immunodiagnostic reagents have been used to detect and quantitate the levels of *P gingivalis*, *P intermedia*, *T denticola*, and *A actinomycetemcomitans* in the plaque. Cultural methods can, if the appropriate nonselective and selective media are used, provide information on the levels of *A actinomycetemcomitans*, black pigmented species, *Campylobacter* species, and other periodontopathogens. Also, because viable organisms are available, antibiotic sensitivities of the isolated organisms can be determined, which may be useful in certain instances.

Other diagnostic reagents are being developed to detect, in plaque, specific microbes or metabolites or enzymes unique to inflammation or infection. For example, specific microbes can be demonstrated in plaque by the use of DNA probes. Probes for *A actinomycetemcomitans*, *P intermedia* and *P gingivalis* are commercially available for testing via a reference laboratory. Future diagnostic procedures may rely on the detection of hydroxyproline, a collagen degradation product; prostaglandin, an inflammatory mediator; and enzymes, derived from either the host or the microbes. A trypsin-like enzyme is present in *T denticola*, *P gingivalis*, and *B forsythus* and is absent from at least 60 other subgingival plaque organisms. This enzyme can be detected by the hydrolysis of the trypsin substrate benzoyl-DL-arginine naphthylamide (BANA). The ability of subgingival plaque to hydrolyze BANA was associated with elevated levels and proportions of spirochetes and with probing depths greater than 6 mm. Subsequently, BANA hydrolysis was shown to be related to the *T denticola* and *P gingivalis* content of the plaque and to the clinical diagnosis of health or disease. As *T denticola*, *P gingivalis* and *B forsythus* are anaerobes, a positive BANA test may be useful in the diagnosis of an anaerobic plaque infection.

When these identification procedures were performed on the same plaque samples, the DNA probes and immunological reagents were significantly more likely to detect *P gingivalis, B forsythus, A actinomycetemcomitans* and *T denticola* than was the traditional cultural approach. In fact, this study suggested that culturing may be the least accurate detection procedure for these plaque species. When the probes and immunological reagents were compared to the BANA test, the probes and antibodies were slightly more accurate, i.e., 88% vs. 83%. All three approaches were essentially comparable indicating that reliable non-cultural methods are available to aid in the microbiological diagnosis of periodontal infections. Because the BANA test detects an enzyme(s) found in three anaerobic species, it may be used to detect an anaerobic periodontal infection.

Prevention and Treatment

Gingivitis can be prevented by good oral hygiene and professional surveillance. Gingivitis can be effectively treated by debridement of the teeth, and, if needed, by short-term use of products containing chlorhexidine, stannous fluoride, or other antimicrobial agents. Mouth rinses, gels, and toothpastes, when used in conjunction with toothbrushing and flossing, are probably adequate to deliver any antimicrobial agents to subgingival sites that are 1 to 3 mm in depth. At probing depths greater than 3 mm, there may not be sufficient penetration of the agent to the bottom of the pocket, and infection may persist. Subgingival scaling (debridement) by a professional is indicated, and additional benefits can usually be obtained by the use of irrigating devices containing an antimicrobial agent.

There is rarely any need to use systemic antimicrobial agents to treat gingivitis associated with pocket depths of 1 to 4 mm, with the exception of an increasingly rare and painful condition known today as acute necrotizing ulcerative gingivitis (ANUG) and formerly as trench mouth. Cases of ANUG that are refractory to mechanical debridement and topical antimicrobial agents respond, quickly and dramatically, to systemic metronidazole. The recognition of metronidazole's efficacy in ANUG led to the discovery that metronidazole has bactericidal activity against anaerobes. ANUG is characterized by tissue invasion by spirochetes and possibly other anaerobes and by elevated plaque levels of spirochetes and *P intermedia* (Table 99-2). ANUG thus resembles periodontitis in being an anaerobic infection.

Clinical dentistry has been about 80 to 85% successful in treating periodontitis by debridement and surgical procedures. However, surgery is labor intensive, and therefore costly. This limits the number of individuals who can be treated in a cost-efficient manner. However, if the majority of clinical cases of periodontitis represent specific bacterial infections, then an alternate treatment strategy would be to diagnose and treat the infection. It would seem that the crucial determination for the clinician in his treatment plan will be the diagnosis of either a micro-aerophilic infection, due to *A actinomycetemcomitans*, or an anaerobic infection characterized by the overgrowth of spirochetes and other anaerobic species.

A actinomycetemcomitans is sensitive to tetracycline, and early uncontrolled studies showed that tetracycline, scaling and root planing, periodontal flap surgery, and topical treatment with chlorhexidine were able, to save hopeless teeth in LJP patients. Additional studies, but none of a double-blind nature, confirm the usefulness of tetracycline in the treatment of LJP.

Subsequently it was shown that tetracycline is concentrated in the fluid that seeps out of the periodontium into the pocket micro-environment. This fact, combined with the demonstration that *A actinomycetemcomitans* can be found in some plaques associated with EOP and AP (Table 99-2), has led to the use of tetracycline in those clinical entities. Results have been equivocal, but this has not detracted from the popularity of tetracycline as a treatment for periodontitis.

Most bacteriologic studies implicate anaerobes as the etiologic agent(s) of EOP and AP, and this would point to the use of a drug such as metronidazole. However, early animal studies that employed lifetime feeding of extremely high dosages of metronidazole suggested that the drug might be tumorigenic. These studies have not been confirmed and, indeed, in 1981 the FDA approved metronidazole for treatment of anaerobic infections. In dentistry, this concern has caused a reluctance to use metronidazole, but has also allowed time for well controlled clinical trials of metronidazole.

Six double-blind studies have demonstrated that metronidazole, given for periods of time as short as 1 week, can significantly improve periodontal health. In all cases, the metronidazole was given in conjunction with professional debriding of the teeth. Maximal benefits were obtained when the metronidazole was given after the debridement. The best clinical response was often noted in patients with more advanced disease, in which the pocket depths were $\geq$ 6 mm, whereas there was only a moderate benefit when the pocket depths were from 4 to 6 mm. In these advanced cases some teeth, that were initially scheduled for extraction upon reexamination, were found no longer to need extraction and thus, in a sense, were saved.

Metronidazole has subsequently been evaluated to determine whether it can reduce the need for periodontal surgery. In three double-blind studies, the unsupervised usage of metronidazole for one week, when combined with the standard debridement procedures, was able to significantly reduce the number of teeth needing surgery when compared to the debridement procedures plus placebo treatment. This sparing effect on surgery has lasted for several years after the one-week period of systemic treatment.

The localized nature of the periodontal infection and the easy access of the teeth has prompted the development of delivery systems which release the antimicrobial agent directly into the periodontal pocket. The first of these delivery systems that is commercially available is a tetracycline impregnated cord which can be wrapped around the tooth below the gingival margin. This cord releases over 100μg of tetracycline per ml of gingival crevicular fluid during the entire period that it is *in situ*. In this manner, patient compliance is assured and the plaque microbes are constantly exposed to therapeutic levels of the agent.

These data from the double-blind metronidazole studies indicate that EOP and AP respond to treatment as if they were anaerobic infections and would seem to presage the more frequent usage of anti-anaerobic agents, such as metronidazole, in the future treatment of periodontal disease. Further developments of delivery systems which release antimicrobials directly into the periodontal pocket should assure that in the future, most periodontal infections will be medically managed.

Date: _____

Practical lesson № 12

Theme: Microbiological and immunological aspects of etiology and pathogenesis of infectious defeats of mucus shell of cavity of mouth

List of questions, that are subject to the study:

1. Stomatitis, glositis, heylitis. Role of resident flora in the origin of unspecific inflammatory defeats of mucus shell of cavity of mouth.
2. Immunological bases of etiology and pathogenesis of unspecific inflammatory defeats.
3. Fuzospirohitos (ulcer-uncritical stomatitis of Vensana).
4. Bacterial stomatitis (gonococcus, scarlatinus, diphtherias).
5. Defeat of mucus shell of cavity of mouth subject to the condition tuberculosis, lepris, Syphilis, actinomycosis.
6. Virose stomatitis (influenzus gerpetichnus).
7. Defeat of slizovoi shell of cavity of mouth subject to the condition Vil-infecti, measles, to the foot-and-mouth disease.
8. Candidoz of mucus shell of cavity of mouth.
9. The Yatrogenna infection in stomatological practice (viral hepatitis, AIDS).

List of practical skills and abilities by which it is necessary to seize:

1. *Microscopy of preparations in a light microscope with immersion objective*
2. *Differentiation of microorganisms after morphological and tinctorial signs.*

Practical lesson's Protocol

Practical tasks should be done:

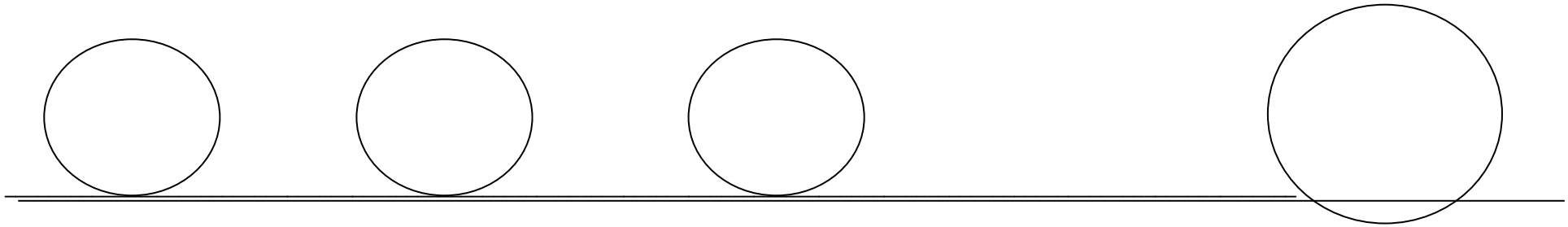
Task № 1. To sketch preparations of microorganisms, that cause the defeat of mucus shell of cavity of mouth (unspecific, specific or are mediated). Under pictures to mark the type of defeat, morphological and tinctorial characteristics.

a) Neisseri qonorrhoeae

b) Streptococcus

c) Shigella

g) Mycobacterium tuberculosis

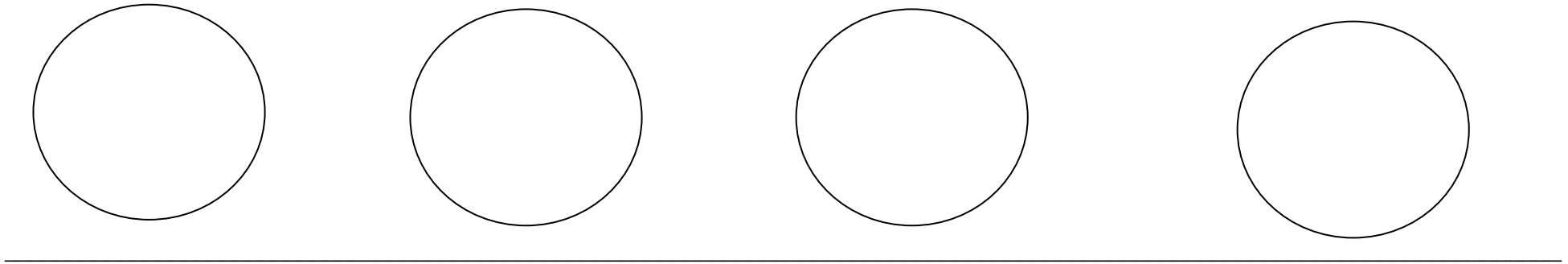


d) Treponema pallidum

e) Actinomyces

h) Candida

f) fuzobacteria and treponema (Vensan stomatitis)



Task № 2. Bring in to table the data about viruses, that cause the defeat of mucus shell of cavity of mouth.

№ p\	Virus	Family	Supercapsid (+/-)	Integration to genom (+/-)	Type of infection (sharp persistence)
RNA-genom:					
1.	Flu				
2.	Measles				
3.	Foot-and-mouth Disease				
4.	HIV				
DNA-genom:					
1.	Simple herpes				
2.	Windy pox				
3.	Cytomegalovirus				
4.	Epstain-Barr				

Signature of teacher:_____

Practical lesson № 13

Topic: Methods of sterilization and disinfection in dental practice

The list of issues that must be studied:

1. Microorganisms, as an object of disinfection and sterilization.
2. Connection with the structure of microbial cells operating factors. The main target of molecular structure of microbial cells exposed to certain factors.
3. The difference in terms of contamination and decontamination, disinfection and sterilization, asepsis and antisepsis.
4. Effects of physical factors.
5. Effects of chemical factors. Antiseptics and disinfectants.
6. The main methods of sterilization and disinfection used in dental practice.
7. Classification of tools, instruments and means of action (see appendix to practice).
8. Methods of monitoring the effectiveness of disinfection and sterilization.
9. The emergence of nosocomial infections (storey) in health care hospitals and dental clinics.

The list of practical skills that are necessary to master:

1. Compliance with the rules of anti-epidemic regime and safety in the bacteriological laboratory.
2. Disinfection of infected material, antiseptic treatment arms of these materials or contaminated culture of microbes.
3. Be able to prepare for sterilizing the dishes, nourishing environment.
4. Interpret biological properties of pathogenic and nonpathogenic microorganisms, viruses, and patterns of their interaction with microorganisms, with human population and the environment.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1. To conduct and bring to the table the molecular structure - the target microorganisms for such operating factors: high temperature, ionizing radiation, small doses of UV - radiation, light and infrared radiation, ultrasound, pressure, chemical factors (alcohols, aldehydes, dyes, acids, surfactants substances (SAS) and others.).

№	The current factor	The molecular structure - the target microorganisms
1	High temperature	
2	Ionizing radiation	
3	Small doses of UV - radiation	

4	Light and infrared radiation	
5	Ultrasound	
6	Pressure	
7	Chemical factors	

Task № 2. Bring to the table the terms corresponding to the sense of these antimicrobial methods.

№	Terms	Antimicrobial methods
1		Complete or partial removal of microorganisms from objects of the environment and human habitat
2		Physical and chemical methods of sterilization; chemical, physical, mechanical and biological methods of disinfection
3		The methods of antisepsis and chemotherapy

Task № 3. Demonstration of sterilization equipment.

Task № 4. Introduction to antiseptic and disinfectant agents with bactericidal, tuberculicidal, sporocidal, fungicidal and virulocidal action. Demonstration drugs.

Task № 5. To condact and to bring to the table the processing facilities and types of their impact on instruments used in dental practice

№	Name of instruments	The nature and types of treatment
1	Scalpels, needles of syringes, implants, burs, surgical instruments, which have contact with the tissues	
2	Mirrors, crowns, tips turbines, prints (casts) teeth, prosthesical material	
3	Thermometers for measuring temperature, D - baths, UV - lamps, physiotherapyinstruments, spoons for molds	
4	Table devices	

Systematics of devices, processes and processing equipment for disinfection and sterilization

Classification of tools	Main types of tools	The nature of processing and types of actions
Critical - penetrate in sterile tissue or blood vessels	All invasive surgical instruments that have contact with bloodprovided tissues, scalpels, needles of syringes, implants, and other	Sterilization - virulitsydni, sporotsydni, tuberkulotsydni, bactericidal action. Prolonged exposure: gamma rays, plasma, long gas and chemical sterilization, autoclaving (2 atm. 15 min.), Dry heat (max, mode, 2 hours)
Halfcritical — faced with mucous membranes (except for some dental instruments listed above)	Flexible endoscopes, catheters, instruments similar to flexible endoscopes, mirrors, crowns, lugs turbines, as well as prints (casts) teeth.	Sterilization of high level — bactericidal, tuberculicidal, sporocidal, fungicidal and virulocidal action Prolonged exposure: gamma rays, plasma, long gas and chemical sterilization, autoclaving (2 atm. 15 min.), Dry heat (max, mode, 2 hours)
	Thermometers for measuring the temperature of the mucous membrane, hydrotherapy baths, ultrasonic baths and UV lamp, physiotherapy instruments, spoons for molds.	Disinfection of average level: virulocidal, tuberculicidal, bactericidal action. Means for chemical disinfection, indicating at marked tuberculicidal activity.
Uncritical - by contact with intact skin	Thermometers for measuring skin temperature, stethoscope, cuff devices for measuring pressure, table devices, etc.	Disinfection of low level: bactericidal action. Means for chemical disinfection without indication of the presence marked tuberculicidal activity.

Signature of teacher:_____

Practical lesson № 14

Topic: Computerized testing of students' knowledge.

Held in the form of testing.

Contence

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