

Topic P07: Diagnostics anaerobic bacteria

Table for major results of Task 1 to Task 4 (to be filled step by step):

Strain		K	L	M	N
Gram stain of a strain – Task 1b (including eventual information concerning spore formation)					
Culture – task 3	Blood agar (“KA”) Growth Y/N				
	VL agar (“VLA”) Growth Y/N				
	VL broth Growth Y/N				
	Description of colonies on BA/VLA*				
FINAL CONCLUSION (result of Task 4 – ANAEROtest, or result of previous tasks for non-anaerobes)					

*Use VLA (VL agar) for bacteria not growing on BA (blood agar)

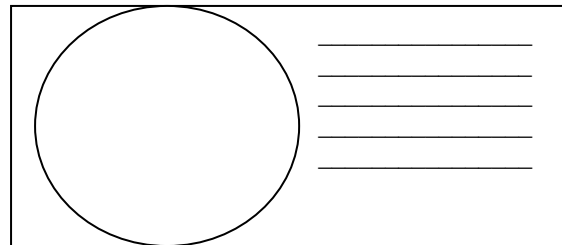
Task 1: Microscopy of a clinical specimen and microscopy of strain

a) Observation of a clinical specimen

Observe and Gram stained smear.

You will probably find a mixture of various bacteria, as it is typical for anaerobic infections, that usually not one microbe, but a mixture of them is responsible for an infection. Besides bacteria, you might see leucocytes (mostly polymorphonuclears), eventually epithelial cells, tissue detritus and so on.

Do not forget to **describe** your picture (use lines)!



b) Microscopy of suspicious strains

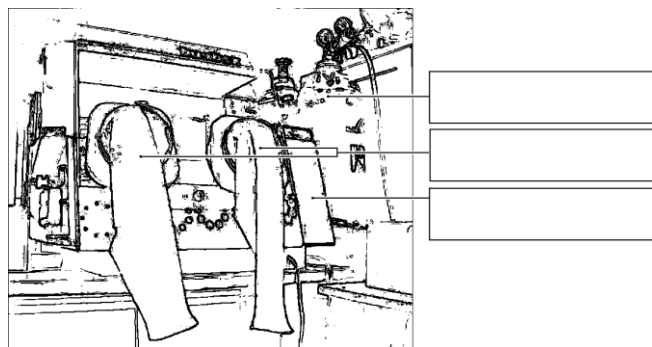
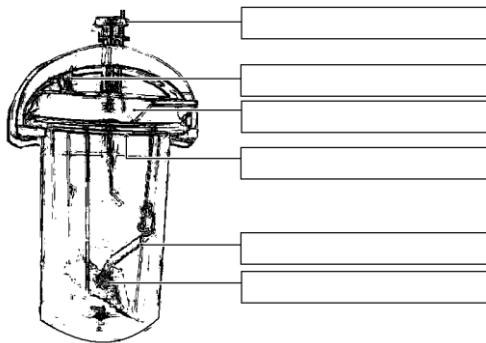
Anaerobic bacteria could be cocci or bacilli, Gram positive or Gram negative; so it is not different from other bacteria. On the other hand, anaerobes use to be much more pleomorphic. In genus *Clostridium*, the endospores are used as an important diagnostic sign. Try to find endospores in one of your strains (robust G+ rods).

Task 2: Anaerobic jar and anaerobic box

To obtain anaerobiose, three ways are used in the laboratory:

- a) for liquid media, **parafin oil** is used as a barrier between the medium and the atmosphere
- b) solid media are placed into an **anaerobic jar**, where oxygen is chemicaly replaced by a mixture of gases
- c) solid media might be also placed into an **anaerobic box**; anaerobic atmosphere comes from a cylinder

Add your description to the pictures of an anaerobic jar and an anaerobic box (you will see a real anaerobic jar and pictures of both anaerobic jar and anaerobic box in the slideshow)



Task 3: Cultivation on agar media

Describe cultivation results of the given strains on both aerobic and anaerobic media

a) Aerobic culture on blood agar (BA)

Write down, whether bacteria do grow or not, and eventually describe the colonies

b) Anaerobic culture on VL agar (VL blood agar)

VL (blood) agar is similar to blood agar, but it has decreased redox potential and it is cultured either in anaerobic jar or anaerobic box. Write down what strains do grow on it and describe those not growing on BA

c) Multiplication of anaerobic bacteria in VL broth

VL broth is used especially for multiplication of rare anaerobic bacteria. Check the presence of turbidity (= growth) in VL broth, write it to the table and compare with the results of part b)

Task 4: Species diagnostics of anaerobic bacteria using biochemical tests

In strains, found to be anaerobes, read the biochemical microtest (ANAEROTest 23 by Lachema) inoculated one day before. Read it according to the scheme. Attention! The codebook has four parts, so you have to find a proper part according to the microscopy. Results of „B“ and „A“ columns are NOT used for code counting. So, you obtain 6 position code: only for results of tests in columns H to C.

Strain:		H	G	F	E	D	C	B	A	Code:	
	1									Identification:	
	2									% of probability:	
	4									Typicity index:	
	Code										
Strain:		H	G	F	E	D	C	B	A	Code:	
	1									Identification:	
	2									% of probability:	
	4									Typicity index:	
	Code										

Notes:

Task 5: Susceptibility tests of anaerobic bacteria to antibiotics

Perform in vitro susceptibility testing of gram-negative cocci to suitable antibiotics.

Evaluate the diffusion disc susceptibility tests to antibiotics in strains found to be gram-negative cocci and that are pathogenous. Into the table, write the abbreviation of the antibiotics according to a card and for all tested strains measure the susceptibility zones. On your card, you have limit zones – according to them, interpret the zones as susceptible (S) resistant (R) and dubious (D).

Strain →				
Antibiotic (full name)	Zone Ø (mm)	Interpretation	Zone Ø (mm)	Interpretation

Task 6: Detection of toxins of clostridia

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c) Detection of A and B toxins of *Clostridium difficile*

Pseudomembranous colitis due to *Clostridium difficile* toxins is very dangerous, especially in hospitalized patients. The testing is performed using an immunochromatographic test, that was already performed in J09 practical. What is practically important: a bit of stool (NOT rectal swab) must be sent to the laboratory.

Observe result of A + B toxins of *Clostridium difficile* detection in stool specimens X and Y, and write down the results: **Specimen X** is *positive – negative* **Specimen Y** is *positive – negative*

Task 7: Demonstration of detection of anaerobic bacteria within facultative anaerobic flora

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