

## Topic J07: Introduction to serology: agglutination and precipitation, dilution

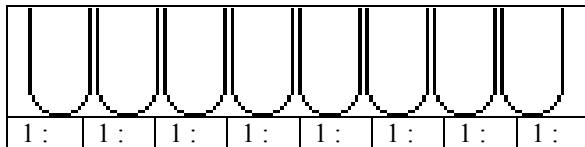
To study: Search WWW for terms: Antigen; Antibody; Serological reactions; Agglutination; Precipitation

### Task No. 1 Dilution in geometric series

In case of antibody detection, it is often good to know also quantity and not only presence/absence of antibodies. The quantity is not measured in units like mg/l or mmol/l, but it is defined as so named „titre“: the highest dilution that still gives a positive reaction. For this purpose, it is necessary first to prepare a dilution series of the serum to be examined. The most common way how to do it is to make a row of 2-fold serial dilutions.

So, try to make a row of 2-fold serial dilutions (1:2, one part serum + one part diluent, etc.).

- ❖ Prepare 8 test tubes and pipette 0.2 ml of saline into each test tube; pipette by means of a rubber suction bulb
- ❖ Add 0.2 ml of the serum (dyed blue for the purposes of this task) to the first tube
- ❖ Mix by aspirating and expelling from the pipette 3 times – now, the sample is diluted 1:2 in the first tube
- ❖ Transfer 0.2 ml of the 1:2 diluted serum to the second test tube and mix again, the sample in the second tube would be diluted 1:4
- ❖ Continue (always transfer 0.2 ml to adjacent test tube, mix and do the same with the next test tube)
- ❖ Discard 0.2 ml from the last test tube into the disinfectant solution; put the pipette into the disinfection, too
- ❖ Draw the colours and write down the dilutions



### Task No. 2 Antibody titres

Antibody titre is the highest dilution dilutin of sera in which was still apparently developed exact serological reaction.

Try to solve following problems:

a) In serological reaction a serum sample is diluted in 2-fold serial dilutions ; the titre of this serum is 1:128. In which well from the left is the reaction visible? Draw the result.

	Conclusion:								
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b) The same serological reaction was made twice in 2-fold serial dilutions with the same sample – for the first time from 1:40, repeatedly from 1: 60. Using the first dilution, we assessed the titre 1:320, using the second dilution we found 1: 480. Is it possible? Which test result would you choose as the best reflecting the real titre? Draw the result and write dilutions in the individual wells.

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### Task No. 3 Agglutination

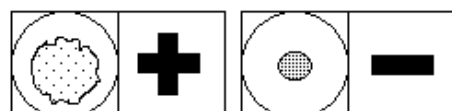
Agglutination is a reaction between antibodies and corpuscular antigen. That term „corpuscular“ means that instead of using molecule of antigen determinant only we use the whole microorganism. It is also possible to bind an originally colloid antigen to a corpuscle (agglutination on carriers, see Task No. 4)

#### 3a) Agglutination in service of antibody detection

Read a microtitration plate with serum samples, in which antibodies to *Yersinia enterocolitica* were determined. In the first well, serum is diluted 1:100 with 2-fold dilution. Antigens for the reaction are plain bacterial cells. Agglutination is a white map on the boottom of a positive well. Read and draw a result, write a titre.

	1:100	1:200	1:400	1:800	K-	NOT USED	ANTIBODIES Y/H	TITRE
K+								1 :
1								1 :
2								1 :
3								1 :
4								1 :

Interpretation:



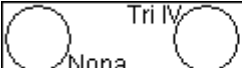
### 3b) Agglutination in service of antigen analysis

Using the agglutination reaction, determine whether the given strain of *E. coli* is enteropathogenic, i.e. it carries antigens connected with the ability to cause the specific type of diarrhoea. Use a nonavalent serum (containing antibodies against 9 antigenic types) and a trivalent serum IV (containing antibodies against three more antigenic types). In case of positivity of a nonavalent serum, we would continue with three trivalent sera (I, II, III) and monovalent sera; in case of positivity of trivalent serum IV we would continue with three monovalent sera.

**(In the practical session for technical reasons we do not continue, in case of positivity of one of polyvalent sera we just conclude „it is an EPEC“)**


- ❖ With a pasteur pipette, place a drop of saline on the slide, and beside it, drop a polyvalent serum containing antibodies against several enteropathogenic serotypes of *E.coli*
- ❖ Remove about half a loopful of the strain, place part of it on the slide closely next to the drop of the serum
- ❖ Mix the remainder in a drop of saline
- ❖ Without flaming the loop, suspend the strain placed on the slide in a drop of the serum
- ❖ Rock the slide with circling movements for about one minute, and follow the rise of agglutination

Draw the result and make a conclusion (strike off, what is not true).

	Conclusion: Strain is – is not an EPEC	Nonavalent serum (I, II, III)									Trivalent (IV)		
		Trivalent (I)			Trivalent (II)			Trivalent (III)			Trivalent (IV)		
		0111	055	026	086	0119	0127	0125	0126	0128	0114	0124	0142

### Task No. 4 Agglutination on carriers

Read a result of TPHA (*Treponema pallidum* haemagglutination test) in which treponemal antigen is coated on a surface of erythrocyte and reacts with antibodies. The test is used in diagnostics of syphilis. For this test serum is not titred and a result is red in a semiquantitative way „1 to 3 crosses“. Draw how are various results look like and write down numbers of eventual positive sera.

+++ ++ + +- - 	Positive are sera No
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### Task No. 5: Precipitation

Precipitation is a reaction between antibodies and the soluble antigen.

#### 5a) Precipitation in the proof of antibodies

Rapid reagin reaction (RRR) is a nice example of the reaction of that type. RRR is used in the screening syphilis. It is interesting, that we do not search for antibodies against *Treponema pallidum*, but antibodies against so named kardiolipin, that is present in body of patients infected with syphilis.

**The following guidelines are only an info, in practice, you have the test already prepared for reading.**

- ❖ Mark the location of the sera in the wells on a perspex panel
- ❖ Add 0.05 ml of negative serum to the first well, and 0.05 ml of positive control serum to the next one
- ❖ Then pipette 0.05 ml of the patient's sample into the next wells
- ❖ Between pipetting wash the pipette 3times with 0.05 ml of saline
- ❖ Finally, pipette 0.05 ml of kardiolipin antigen into each well containing serum and shake the panel carefully
- ❖ Stir about five minutes and then read the precipitation result against a dark background

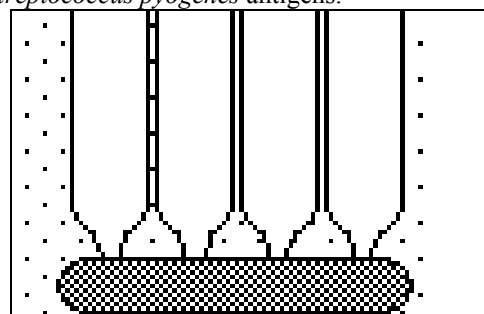
Draw your result and make a conclusion:

	Conclusion:
K-   K+   1   2   3	

#### 5b) Precipitation in the proof of antigen

Ring precipitation enables to find, which of given extracts contains *Streptococcus pyogenes* antigens.

- ❖ Take a short pasteur pipette and stick the capillary end in a piece of modeling clay
- ❖ Using another pipette, pour serum containing antibodies to a group A streptococci antigens into the stuck one; add the serum to a height of about 3–5 mm
- ❖ Using a clean pasteur pipette, carefully layer an extract from the examined strains of *Streptococcus* on the serum
- ❖ After five minutes see whether or not a precipitation ring has formed at the boundary of the two fluids (read the result in front of a dark background)



Draw the result and write the conclusion.

Conclusion: Positive is the extract No.

## Topic J07

### Check-up questions:

1. What type of specimen is needed for antibody detection?
2. What types of specimens can be used for antigen detection?
3. What does mean the term „antigen analysis“?
4. Does presence of antibodies allways mean an accute infection? Explain.
5. What does the term „titre“ mean? Is it assessed in antibody detection, antigen detection, or both? Explain.
6. What is the difference between precipitation and agglutination reaction?
7. Why it is necessary to perform confirmation in case of a positive RRR reaction?
8. Is it possible to examine antibodies angainst a coloid antigen using agglutination reaction?
9. Give examples of suitable disinfectants for surfaces contaminated by serum. (Serum migth contain viral agents, for example viruses of hepatitis.)