

## Topic J08: Complement fixation test (CFT) and a follow-up of titres

To study: Web-search: Complement, amboceptor, anticomplementarity

### Task No. 1 Examination of function of indicator system for the CFT

Complement causes hemolysis of sheep erythrocytes in complex with amboceptor (= rabbit antibodies against sheep erythrocytes). Absence of any of needed components leads to absence of haemolysis.

Pipette into tubes individual parts of reaction according to the table. Wash your pipette always carefully three times by saline to avoid contamination. Incubate 1 hour in a thermostat. Finally evaluate the results.

**Note: All amounts are in millilitres (ml)**

tube	saline	sheep ery	amboceptor	complement	rabbit ery	Haemolysis (encircle)	In case of no haemolysis, explain, why it is absent
1	0.2	0.2	0.2			yes – no	
2	0.2	0.2		0.2		yes – no	
3		0.2	0.2	0.2		yes – no	
4			0.2	0.2	0.2	yes – no	

### Task No. 2 Assessment of proper amount of complement to be used in CFT (titration)

CFT uses exactly measured amount of guinea pig complement, given in hemolytic units (patient's complement is inactivated by heating of serum). Hemolytic unit is the exact amount of complement which causes hemolysis of given volume of RBC (in complex with amboceptor).

In four tubes, guinea pig serum is diluted 1:32, 1:36, 1:40 and 1:44. Serum was incubated with working volume of sheep erythrocytes with amboceptor.

Write, in which dilution is 1 hemolytic unit of complement. It is 1 : \_\_\_\_\_

### Task No. 3 Schematic analyse of course of CFT, including anticomplementarity testing

In following schemes judge where free complement remains free after the first phase of reaction (encircle what is true, cross, what is wrong) and add verbal description of the result (haemolysis, sedimentation of RBCs).

**Note: all schemes describe use of CFT for antibody detection.**

LAB Ag = laboratory antigen

PATIENT Ig = patient antibody

C = complement

Ery = sheep red blood cell

Amb = amboceptor (rabbit antibody against sheep red blood cells)

ANTICOMPLEMENT = anticomplementarity causing component

Description of situation	Phase 1	Complement after Phase 1	Phase 2	Result	Result description
Course of a positive CFT		free – bound			
Course of a negative CFT		free – bound			
Anticomplementarity testing, serum is anticomplementary		free – bound* *or inactivated			
Anticomplementarity testing, serum is OK		free – bound* *or inactivated			

**Task No. 4 Assessment of complementfixation antibodies to the most frequent causative agents of respiratory diseases**

Read the titres of CFT in individual patients. Remark anticomplementarity controls in the wells of the first column. CFT starts from the second well with 1:5 dilution. Draw your result, write titre values (in positive patients) and try to interpret results.

Disease		↔	↔	↔	↔	↔	↔	↔	↔	increase/decrease	diagnostic conclusion	
	I	○	○	○	○	○	○	○	○	TITRE = 1:		
	II	○	○	○	○	○	○	○	○	TITRE = 1:		
	I	○	○	○	○	○	○	○	○	TITRE = 1:		
	II	○	○	○	○	○	○	○	○	TITRE = 1:		
	I	○	○	○	○	○	○	○	○	TITRE = 1:		
	II	○	○	○	○	○	○	○	○	TITRE = 1:		
	I	○	○	○	○	○	○	○	○	TITRE = 1:		
	II	○	○	○	○	○	○	○	○	TITRE = 1:		
	I	○	○	○	○	○	○	○	○	TITRE = 1:		
	II	○	○	○	○	○	○	○	○	TITRE = 1:		

**Task No. 5: Assessment of complementfixation antibodies to tick-born encephalitis**

Read CFT titres in pair samples of patients. The first dilution is 1:4 in geometric series. Carefully evaluate controls of anticomplementarity. Draw a result, write titer and try to interpret results.

Patient		↔	↔	↔	↔	↔	↔	↔	↔	increase/decrease	diagnostic conclusion	
Positive control		○	○	○	○	○	○	○	○	TITRE = 1:		
Khaled	I	○	○	○	○	○	○	○	○	TITRE = 1:		
	II	○	○	○	○	○	○	○	○	TITRE = 1:		
Laura	I	○	○	○	○	○	○	○	○	TITRE = 1:		
	II	○	○	○	○	○	○	○	○	TITRE = 1:		
Maria	I	○	○	○	○	○	○	○	○	TITRE = 1:		
	II	○	○	○	○	○	○	○	○	TITRE = 1:		

**Task No. 6 Assessment of complementfixation antibodies in toxoplasmosis**

Read CFT titres in sera of patiens with suspected infection caused by *Toxoplasma gondii*. The first dilution is 1:8 in geometric series. Carefully evaluate controls of anticomplementarity. Draw a result, write titer and evaluate only as „positive“ / „negative“ / „anticomplementary

		↔	↔	↔	↔	↔	↔	↔	↔		
K+		○	○	○	○	○	○	○	○	TITR = 1:	
1		○	○	○	○	○	○	○	○	TITR = 1:	
2		○	○	○	○	○	○	○	○	TITR = 1:	
3		○	○	○	○	○	○	○	○	TITR = 1:	
4		○	○	○	○	○	○	○	○	TITR = 1:	
5		○	○	○	○	○	○	○	○	TITR = 1:	
6		○	○	○	○	○	○	○	○	TITR = 1:	

## Topic J08

### Check-up questions:

1. Why patients own complement is not used for CFT and guinea pig complement is used instead?
  
2. What is the method used for patient serum inactivation?
  
3. Which type of errors is caused by
  - a) anticomplementarity of serum?
  - b) too big amounts of used complement?
  
4. Why 2-fold increase of titre cannot be considered significant? (Think about error of measuring.)
  
5. Why it is recommendable to use pair sera when using reactions like CFT?
  
6. What is meaning of the term „seroconversion“? What can be considered more significant, seroconversion of 4-fold increase of titre? Explain.
  
7. How would result of Task No. 4 influence decision about starting antibiotic therapy? (Answer during the practical session)