

Topic J09: Neutralisation (ASO, HIT, VNT)

To study: Search WWW for: ASO, rheumatoid fever, acute glomerulonephritis, viral haemagglutination
Neutralisation reactions are based on inhibition of specific influence of antigen by antibodies.

Task No. 1 ASO – a proof of antistreptolysin O

Principle: Antibodies prevents hemolysin (streptolysin O – i.e. antigen) to hemolyse rabbit RBC.
ASO levels increase after beta-hemolytic streptococci group A (less commonly also other groups) caused infections. In risk for late sequelae, ASO increase over 200 I. U. (international units) is seen.
On your serological panel, read ASO values of positive control and seven patients.

	100	120	150	180	225	270	337	405	506	607	759	911	ASO value (IU)	Interpretation
K+	○	○	○	○	○	○	○	○	○	○	○	○		
P1	○	○	○	○	○	○	○	○	○	○	○	○		
P2	○	○	○	○	○	○	○	○	○	○	○	○		
P3	○	○	○	○	○	○	○	○	○	○	○	○		
P4	○	○	○	○	○	○	○	○	○	○	○	○		
P5	○	○	○	○	○	○	○	○	○	○	○	○		
P6	○	○	○	○	○	○	○	○	○	○	○	○		
P7	○	○	○	○	○	○	○	○	○	○	○	○		

Task No. 2 HIT – hemagglutination inhibition test

Principle: Antibodies prevents virus to agglutinate erythrocytes. Serum samples taken from patients with suspect tick-born encephalitis are examined. Read, draw results and make a clinical conclusion.

Patient	Specim	:1:5	:1:10	:	:	:	:	TITRE (fill in for positive sera only)	Increase/decrease (how many times)	Diagnostic conclusion	1 – control of antigen (normal is haemagglutination)
Positive control		○	○	○	○	○	○	TITRE = 1:	Positive control is – is not positive		
Kebonye	I	○	○	○	○	○	○	TITRE = 1:			2 – control of RBC (normal is absence of haemagglutination)
	II	○	○	○	○	○	○	TITRE = 1:			
Luis	I	○	○	○	○	○	○	TITRE = 1:			Controls 1 and 2 OK? yes – not
	II	○	○	○	○	○	○	TITRE = 1:			
Mathew	I	○	○	○	○	○	○	TITRE = 1:			
	II	○	○	○	○	○	○	TITRE = 1:			
Natasha	I	○	○	○	○	○	○	TITRE = 1:			
	II	○	○	○	○	○	○	TITRE = 1:			

Task No. 3 VNT – virus neutralisation test

Principle: Antibodies present virus to kill cells of a cell culture growing in a microtitration plate.
A cell culture of monkey kidneys was cultivated in a microtitration plate and then various Coxsackievirus B serotypes and serum from a patient suffering from myocarditis were added. Medium get red in wells in which virus kill cells. On the contrary, if antibodies neutralise virus, cells stay alive and medium get yellow by products of cell metabolism. – Draw the result and try to interpret findings.

Coxsackievirus	Serum	:1:5	:1:10	:	:	:	:	TITRE (fill in for positive sera only)	Increase/decrease (how many times)	Diagnostic conclusion	Controls
B1	I	○	○	○	○	○	○	TITRE = 1:			A – control of cells B – control of serum I C – control of serum II
	II	○	○	○	○	○	○	TITRE = 1:			Controls A, B, C should be yellow
B2	I	○	○	○	○	○	○	TITRE = 1:			D, E, F, G, H, I – controls of coxsackieviruses B1 to B6
	II	○	○	○	○	○	○	TITRE = 1:			
B3	I	○	○	○	○	○	○	TITRE = 1:			Controls D to I should be red
	II	○	○	○	○	○	○	TITRE = 1:			
B4	I	○	○	○	○	○	○	TITRE = 1:			Controls OK? Yes – not
	II	○	○	○	○	○	○	TITRE = 1:			
B5	I	○	○	○	○	○	○	TITRE = 1:			
	II	○	○	○	○	○	○	TITRE = 1:			
B6	I	○	○	○	○	○	○	TITRE = 1:			
	II	○	○	○	○	○	○	TITRE = 1:			

Check-up questions:

1. In which clinical situations ASO diagnostics is rational?

2. Why it is not suitable to classify ASO as „indirect diagnostic reaction for microbial detection“, although it is a method of antibody detection?

3. Some viruses are unable to agglutinate RBC – how does the fact influence HIT diagnostic?

4. Which is a Czech abbreviation for ASO?

5. Why neutralisation reactions are rare in bacteriology?

6. A bonus question for clever students: Try to find an arrangement for HIT, if it should be used for antigen detection.