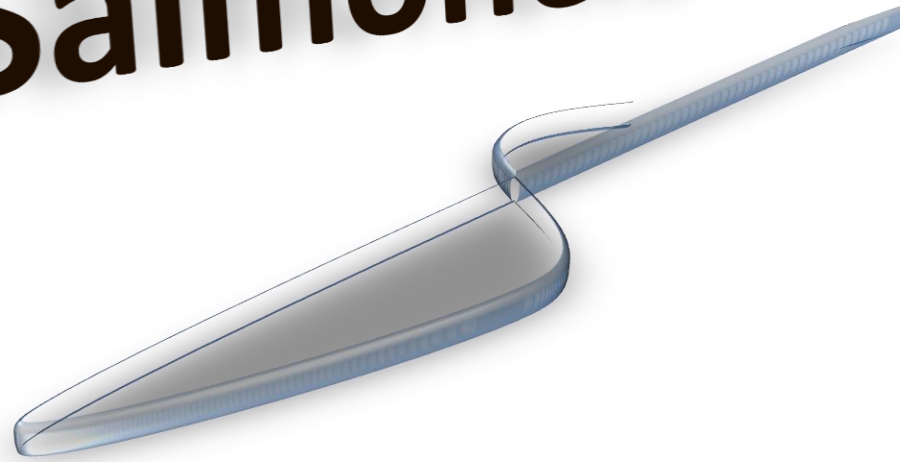
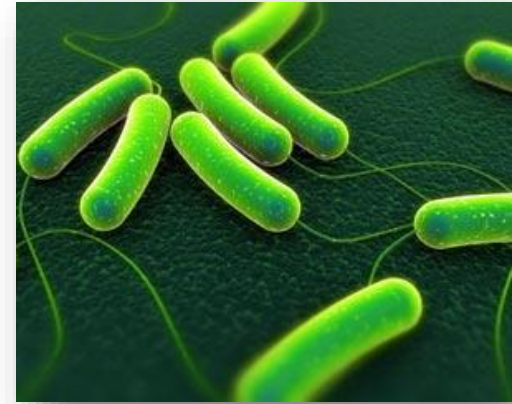


Salmonella test



Morphology

- 1- Salmonella is bacteria
- a- Short bacilli
- b- Gram -ve bacilli
- c- Non- capsulated



Salmonella has several species which can cause disease in human.



Pathogenicity:

The following diseases are caused by salmonella:

- **Enteric fever:** includes typhoid & paratyphoid
Caused by: S. Typhi & S. Paratyphi
- **Diarrheal disease:**
Caused by: S. Typhimurium (food poisoning),
S. Enteritidis (enterocolitis)
- **Septicaemia:**
Caused by: S. Choleraesuis



Symptoms of Salmonellosis

- ✓ Diarrhea
- ✓ Nausea
- ✓ Vomiting
- ✓ Stomach pain
- ✓ Headache
- ✓ Fever
- ✓ Onset 12-72 hours after infection



Enteric fever:

- Caused by:
- **S. Typhi** (most serious form)
- **S. Paratyphi** (milder form) contain 3 types
 - A
 - B
 - C (common in Europe)



Mood of infection

- The source of infection is ingestion of the organisms in contaminated food or water from contaminated hands [Feces, Flies, Fingers, Food].
- These salmonella penetrate the intestinal mucosa so, detected in blood, urine and stool



Laboratory diagnosis

Specimen	Time	
Blood	1st week	
Urine	2nd week	Culture
Stool	3rd week	



Diagnosis of Enteric fever serologically:

- Detection of a specific antibody by:
 - a) Widal test.
 - b) Ig M antibody immunoassay.



Serological characters

- The bacteria has γ antigen:
- Somatic antigen: O
- Flagellar antigen: H
- Widal test: detect the antibodies in serum against O Ag & H Ag.



A) investigation of typhoid:

S. Typhi O

S. Typhi H

B) investigation of paratyphoid

A&B:

S. Paratyphi A O

S. Paratyphi A H

S. Paratyphi B O

S. Paratyphi B H

- O Ag for S. Paratyphi A and S. Paratyphi B are not taken as they cross react with S. Typhi O Ag



Slide Widal Test

1. Qualitative test

2. Semi-Quantitative test



1. Qualitative test

1. Bring the test Rgs and sample to room temperature.
1. Resuspend the Ag vial gently.
2. Prepare white slide for using.
3. Put 0.0 μ (1 drop) of the Rg of S. Typhi O ,S. Typhi H ,S. Paratyphi AH and S. Paratyphi BH Separating each other by enough space



4. Qualitative test (cont.)

- o. Put 0.5μ serum at each reagent zone
6. Mix each Rg with its neighbor serum with a disposable stirrer, use a separate stirrer for each mixture.
- v. Rock the slide gently by hand or by shaker(1000 r.p.m).
8. Observe immediately under a suitable light source for any degree of agglutination



Readings:

-ve: non-agglutination Ag-
Ab Rx 1/ε.

+ve: agglutination Ag-Ab
Rx 1/λ.



Observations

- The agglutination has (occurred / not occurred) in H antigen circle.
- The agglutination has (occurred / not occurred) in O antigen circle.
- The agglutination has (occurred / not occurred) in A antigen circle.
- The agglutination has (occurred / not occurred) in B antigen circle.



2. Semi-Quantitative test

- Put 1 drop of the Ag as 0 times in white plate

1 drop + 100 μl ----- 1 in 100 dilution (1/100)

1 drop + 50 μl ----- 1 in 50 dilution (1/50)

1 drop + 20 μl ----- 1 in 20 dilution (1/20)

1 drop + 10 μl ----- 1 in 10 dilution (1/10)

1 drop + 0.5 μl ----- 1 in 200 dilution (1/200)



۲. Semi-Quantitative test (cont.)

- Rock the slide gently by hand or by shaker (100 r.p.m)
- Observe immediately under a suitable light source for any degree of agglutination.



Test tube agglutination

- Row with 6 test tubes
- 1,9 ml saline is transferred to first tube of each row.
- 1 ml saline is transferred to tubes 2-6 in each row.
- Put 0,1 ml (100 μ) serum to first tube and mix well. This is 1:20 dilution.



Tube agglutination test

- 1 ml of diluted serum taken from tube 1 is transferred to tube 2 and mixed well.
- This '**mix and transfer**' is repeated for each row till the last tube, from which 1 ml is discarded.
- Specific antigens are added.
- Finally, the tubes are incubated at 37°C for 2 hr.



Examination

- Examine under visible light
- Final serum dilution will be:
 - 1:2.
 - 1:4.
 - 1:8.
 - 1:16.
 - 1:32.



Interpretation of Widal test:

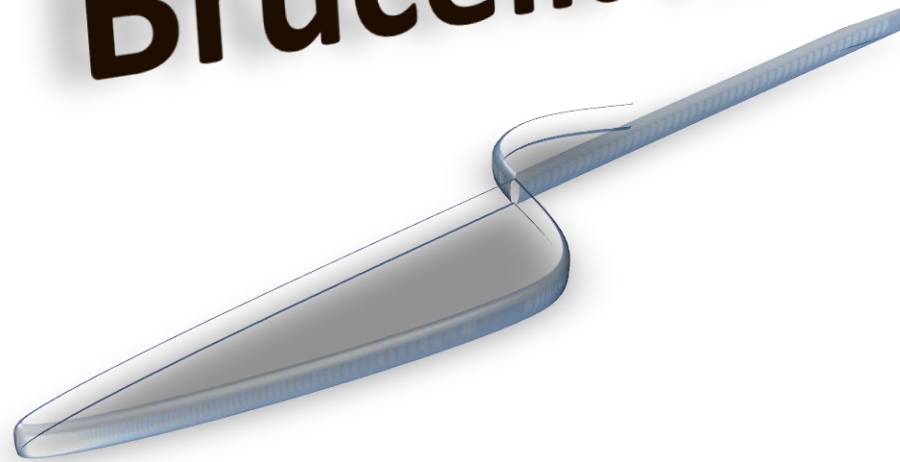
- Titre greater than 1:8 for O indicates recent infection.
- 1:16 for H indicates past infection or vaccination.



Timing of test is important, as antibodies begin to arise during end of first week. The titres increase during second, third and fourth week after which it gradually declines. The test may be negative in early part of first week



Brucella test



Nomenclature

Bang's disease


Gibraltar fever

Malta fever

Mediterranean fever

undulant fever



- 
- Malta fever, the disease now called brucellosis first came from Malta. The causal relationship between organism and disease was first established by Dr. David Bruce.
 - The popular name undulant fever originates from the characteristic undulance (or "wave-like" nature) of the fever, which rises and falls over weeks in untreated patients

Morphology

- Small
- Gram –ve
- Nonmotile
- Nonspore – forming
- coccobacilli



Types of Brucellosis

B. Melitensis:

pathogen of goats and sheep.

B. Abortus:

pathogen of Cows, Buffalo

B. Ovis:

pathogen of sheep

B. suis:

pathogen of Pigs

B. Canis:

pathogen of dogs (less common)



What causes brucellosis?


- Is a zoonosis.
- Brucellosis in humans is usually associated with the consumption of unpasteurized milk and soft cheeses made from the milk , undercooked meat of infected animals, or close contact with their secretions.
- Transmission from human to human, through sexual contact or from mother to child, is rare but possible



Signs and Symptoms

- Symptoms may show up anytime from a few days to a few months after infection.

Symptoms are similar to those of the flu and include:



fever	sweating	body aches	joint pain	fatigue
Dizziness	headache	loss of appetite	weight loss	cough
chest pain	abdominal pain	difficulty breathing	weakness	

- Brucellosis symptoms may disappear for weeks or months and then return.
- In some people, brucellosis becomes chronic, with symptoms persisting for years even after treatment



Serology for brucellosis

- Serology for brucellosis is a blood test to look for antibodies against brucella.



Slide brucella Test

١. Qualitative test

٢. Semi-Quantitative test



1. Qualitative test

1. Bring the test Rgs and sample to room temperature.
1. Resuspend the Ag vial gently.
2. Prepare white slide for using.
3. Put 0.05μ (1 drop) of the Rg of brucella melitensis, brucella abortus Separating each other by enough space



4. Qualitative test (cont.)

- o. Put 0.5μ serum at each reagent zone
6. Mix each Rg with its neighbor serum with a disposable stirrer, use a separate stirrer for each mixture.
- v. Rock the slide gently by hand or by shaker(1000 r.p.m).
8. Observe immediately under a suitable light source for any degree of agglutination



Readings:

-ve: non-agglutination Ag-
Ab Rx 1/ε.

+ve: agglutination Ag-Ab
Rx 1/∞.



Observations

- The agglutination has (occurred / not occurred) in H antigen circle.
- The agglutination has (occurred / not occurred) in O antigen circle.
- The agglutination has (occurred / not occurred) in A antigen circle.
- The agglutination has (occurred / not occurred) in B antigen circle.



2. Semi-Quantitative test

- Put 1 drop of the Ag as 0 times in white plate

1 drop + 1. μ ----- 1 in 2. dilution (1/2.)

1 drop + 2. μ ----- 1 in 4. dilution (1/4.)

1 drop + 4. μ ----- 1 in 16. dilution (1/16.)

1 drop + 8. μ ----- 1 in 64. dilution (1/64.)

1 drop + 16. μ ----- 1 in 256. dilution (1/256.)



۲. Semi-Quantitative test (cont.)

- Rock the slide gently by hand or by shaker (100 r.p.m)
- Observe immediately under a suitable light source for any degree of agglutination.



Tube agglutination test

- Row with 6 test tubes
- 1,9 ml saline is transferred to first tube of each row.
- 1 ml saline is transferred to tubes 2-6 in each row.
- Put 0,1 ml (100 μ) serum to first tube and mix well. This is 1:20 dilution.



Tube agglutination test

- 1 ml of diluted serum taken from tube 1 is transferred to tube 2 and mixed well.
- This '**mix and transfer**' is repeated for each row till the last tube, from which 1 ml is discarded.
- Specific antigens are added.
- Finally, the tubes are incubated at 37°C for 2 hr.



Examination

- Examine under visible light
- Final serum dilution will be:
 - 1:2.
 - 1:4.
 - 1:8.
 - 1:16.
 - 1:32.



Interpretation of Brucella test:

- **Normal results:**

Negative result usually means you have not come in contact with the bacteria that cause brucellosis.

However, this test may not detect the disease at the early stage. Your doctor may have you come back for another test in 10 days to 3 weeks.



Interpretation of Brucella test:

- **Abnormal results:**

Positive result usually means you have come in contact with the bacteria that cause brucellosis.

However, this does not mean that you have an active infection. Your doctor will repeat the test after a few weeks to see if the test result increases.

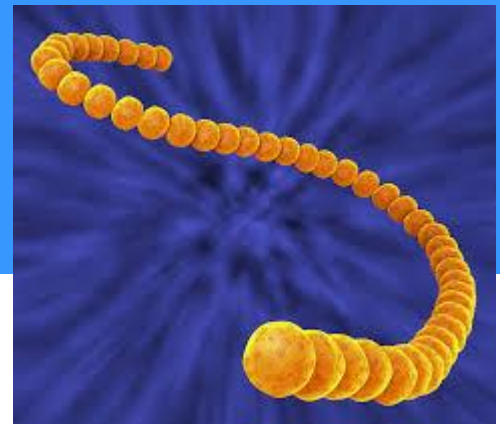
Dr A Khedr



Anti-streptolysin O



Streptococcus



- is a genus of spherical Gram-positive bacteria.
- Species of Streptococcus are classified based on their hemolytic properties

α hemolytic cause partial hemolysis

β hemolytic cause complete hemolysis

γ hemolytic cause no hemolysis

β Hemolytic Streptococcus


- Can be then classified according to specific carbohydrates present on the bacterial cell wall
- There are 20 described serotypes, named Lancefield groups A to V (excluding I and J).



Streptolysin

- Streptolysin is a streptococcal hemolytic exotoxin.
- It include γ types:
 - streptolysin O (SLO), which is oxygen-labile
 - streptolysin S (SLS), which is oxygen-stable



- 
- Streptolysin O is a hemolysin that is produced by β -hemolytic streptococci and is hemolytically active only in the reduced state
 - unlike streptolysin S, which is stable in the presence of oxygen.
 - Another difference is that that SLO is antigenic, while SLS is not antigenic due to its small size

Streptolysin O

- An oxygen -labile β -hemolytic enzyme belonging to a family of thiol - activated toxins.
- exists in two forms:
a reduced active state
oxidized inactive state
- Reversibly oxidized SO is activated by cleavage of disulfide bonds.



- streptolysin O produced by most strains of group A and many strains of groups C and G streptococci.

This cytolysin is capable of causing lysis in animal cells especially WBCs and RBCs by binding with the membrane cholesterol where it polymerizes to form a transmembrane channel (about 3. nm in diameter), which then causes cell lysis.



Antistreptolysin O

- When the body is infected with streptococci, it produces antibodies against the various antigens that the streptococci produce
- Anti streptolysin O is the antibody made against streptolysin O.
- These antibodies produced against the bacteria cross-react with human antigens (mainly collagen) and hence attack the cellular matrix of various organs, mainly the heart, joints, skin, brain.



What diseases does streptococcus cause?

- Direct invasion/toxin:
 - skin infections
 - strep throat
 - scarlet fever
- Antibody-mediated:
 - rheumatic fever
 - post-streptococcal glomerulonephritis



Rheumatic Fever

A severe infectious disease occurring chiefly in children, characterized by fever and painful inflammation of the joints and frequently resulting in permanent damage to the valves of the heart.

symptoms of rheumatic fever

- ١) Fever
- ٢) Myocarditis (inflammation of the heart muscle)
- ٣) Joint swelling (arthritis)
- ٤) Subcutaneous nodules

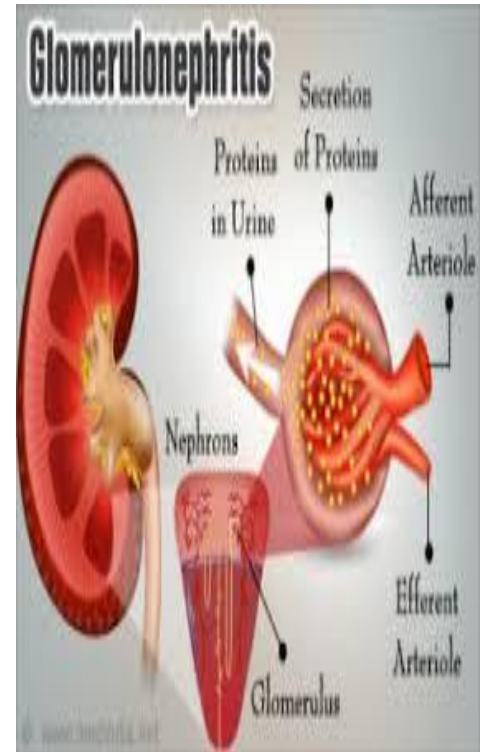


Glomerulonephritis

- A form of nephritis characterized by inflammation of the renal glomeruli.

after strep infection of pharynx or skin Antigens from this induce antibody response.

Resulting complexes travel to kidneys and get deposited in glomerular basement membrane, leading to local glomerular destruction in kidney



Symptoms of acute post-strep glomerulonephritis

Antibody-mediated inflammatory disease of kidney glomeruli.

Retention of water (puffy face),
dark urine (due to hematuria - blood in urine),

Oliguria

proteinuria



Clinical significance

- This test is a sensitive test for recent streptococcal infection.
- A rise in ASO begins about one week after infection and peaks two to four weeks later.
- In the absence of complications or reinfection, the ASO level will fall to preinfection levels within 7 to 12 months.
- Over 80% of patients with acute rheumatic fever and 90% of patients with acute glomerulonephritis due to *streptococci* have elevated levels of ASO.



Qualitative Test

1. Bring the test Rgs and sample to room temp.
2. Resuspend the Ag vial gently
3. Prepare black slide for using
4. Put 0.5μ (1 drop) of the Rg of Streptolysin o
5. Put 0.5μ serum at reagent zone
6. Mix each Rg with its neighbor serum with a disposable stirrer, use a separate stirrer for each mixture



- V. Rock the slide gently by hand or by shaker(1000 r.p.m).
- Λ. Observe immediately under a suitable light source for any degree of agglutination for 2 minutes



Reading

- If the precipitation occur the result is +ve
Give titre $\gamma \cdot \cdot$ IU/mL

If no ppt occurs the result is -ve



In +ve case we must repeat the following steps

1. Serum diluted by saline by the ratio 1:1
2. Take 10 μ from mix and put 10 μ from reagent on the black slide
3. Mix and rotate for 2 min.
4. If -ve so the titre 200 IU/ml
5. If +ve the titre 400 IU/ml
6. Repeat by dilution of last mix by the ratio 1:1
7. The titre in this test 200, 400, 800, 1600 IU/ml.


C - Reactive Protein




CRP

- CRP is synthesized by the liver.
- is a protein found in the blood, the levels of which rise in response to inflammation.
- C-reactive protein is an acute-phase protein.



- 
- Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (microbes & bacteria) in order to activate the complement system so enhances phagocytosis by macrophages.
 - Thus, CRP participates in the clearance of necrotic and apoptotic cells.

- 
- Measuring CRP level is a screen for infectious and inflammatory diseases, necrosis, malignancies, and autoimmune disorders.
 - Because there are a large number of disparate conditions that can increase CRP production, an elevated CRP level does not diagnose a specific disease.


- levels of CRP rapidly increase within γ hours of acute insult, reaching a peak at $\xi\Lambda$ hours.



Qualitative Test

١. Bring the test Rgs and sample to room temperature.
٢. Resuspend the Ag vial gently
٣. Prepare black slide for using
٤. Put 0.5μ (١ drop) of the Rg
٥. Put 0.5μ serum at each reagent zone



- 
7. Mix each Rg with its neighbor serum with a disposable stirrer, use a separate stirrer for each mixture. Rock the slide gently by hand or by shaker (1000 r.p.m).
 8. Observe immediately under a suitable light source for any degree of agglutination

Readings:

- ve: non-agglutination Ag-Ab Rx **less than 1 mg/dl**
- +ve: agglutination Ag-Ab Rx **(make titre)**

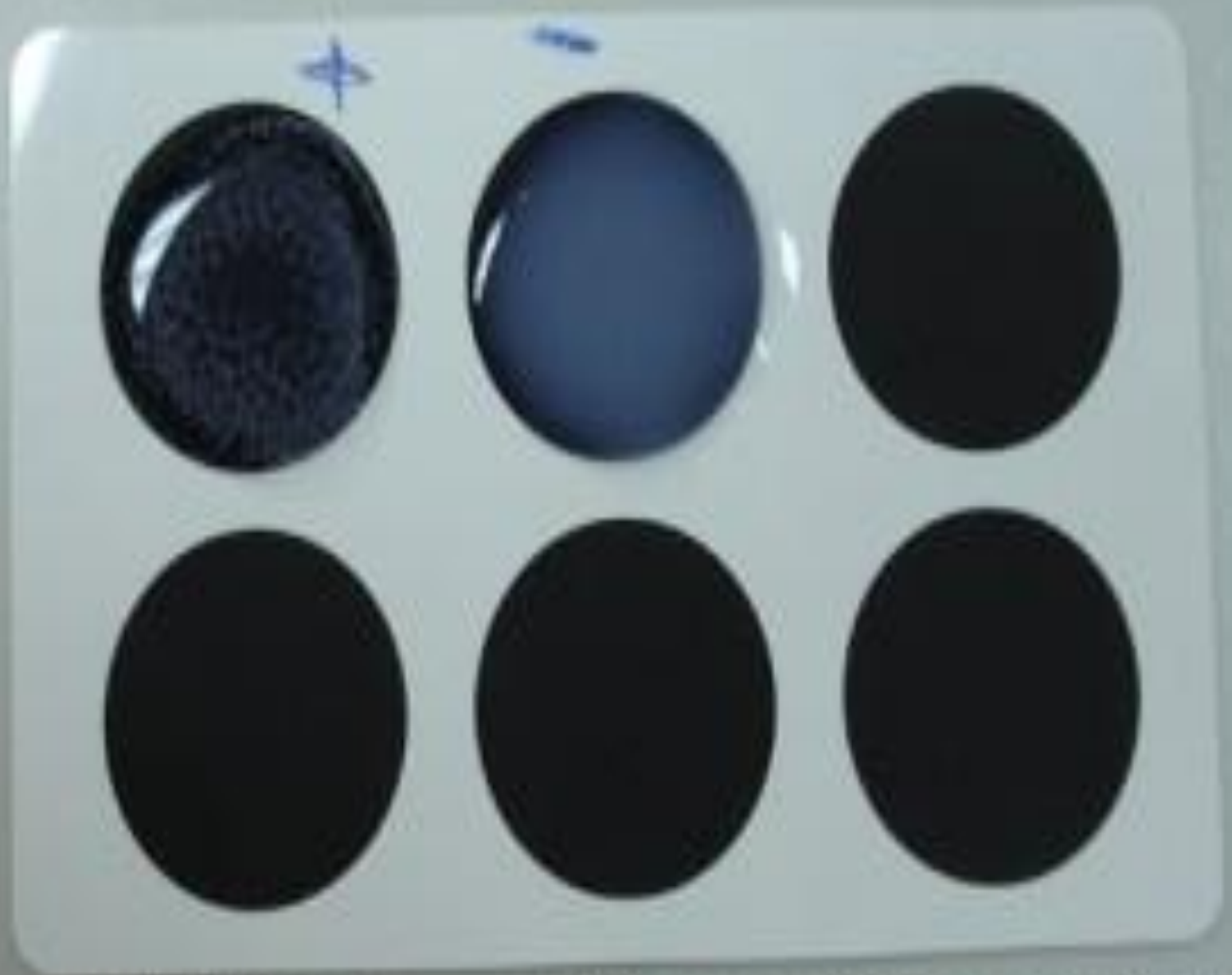
- DILUTION
(mg/L)

- 1:1
- 1:2
- 1:4
- 1:8

CONCENTRATION

7
12
24
48





(CRP test --- (C-reactive protien