ANTIGEN-ANTIBODY REACTIONS AND SELECTED TESTS

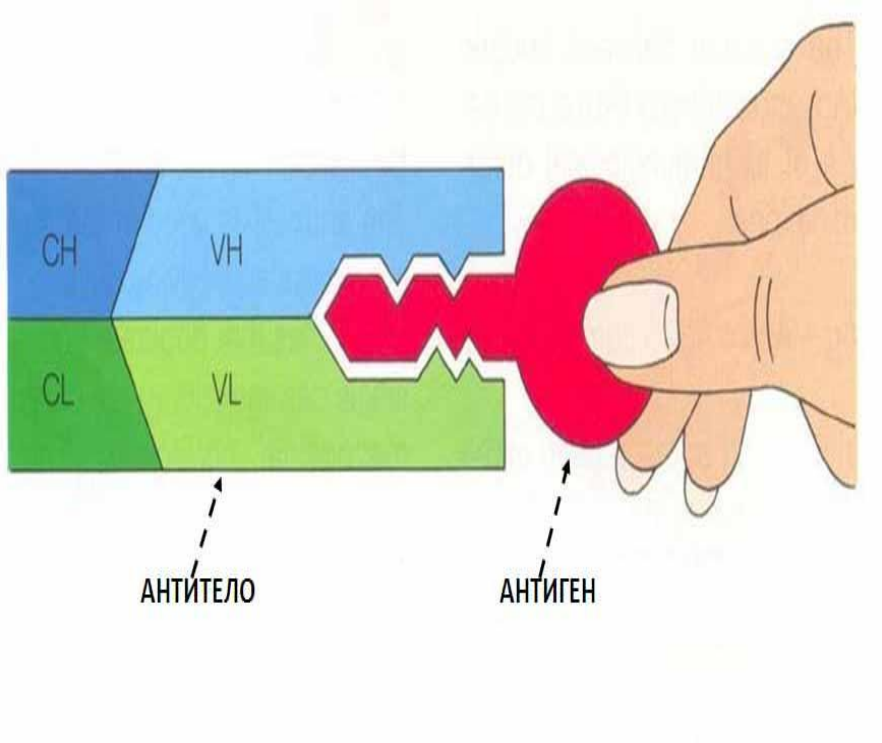
- **TEACHING OBJECTIVES**

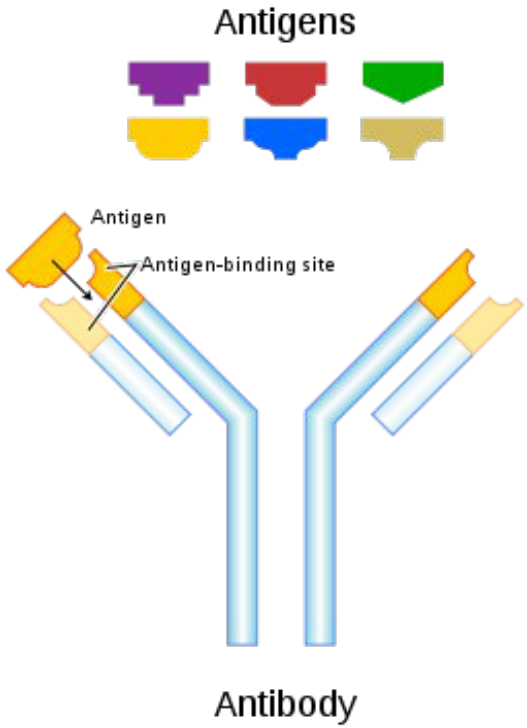
  1.To describe the nature of Ag-Ab reactions

  2.To compare and contrast antibody affinity and avidity

  3.To delineate the basis for antibody specificity and cross reactivity

  4. To discuss the principles of commonly used tests for antigen/antibody reactions

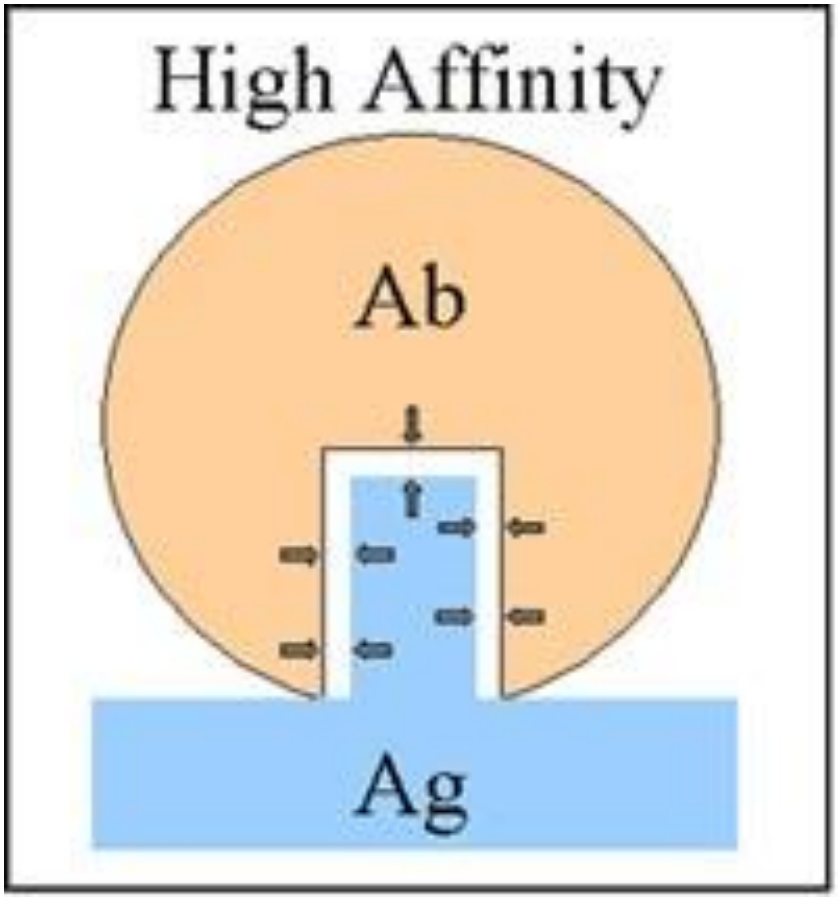


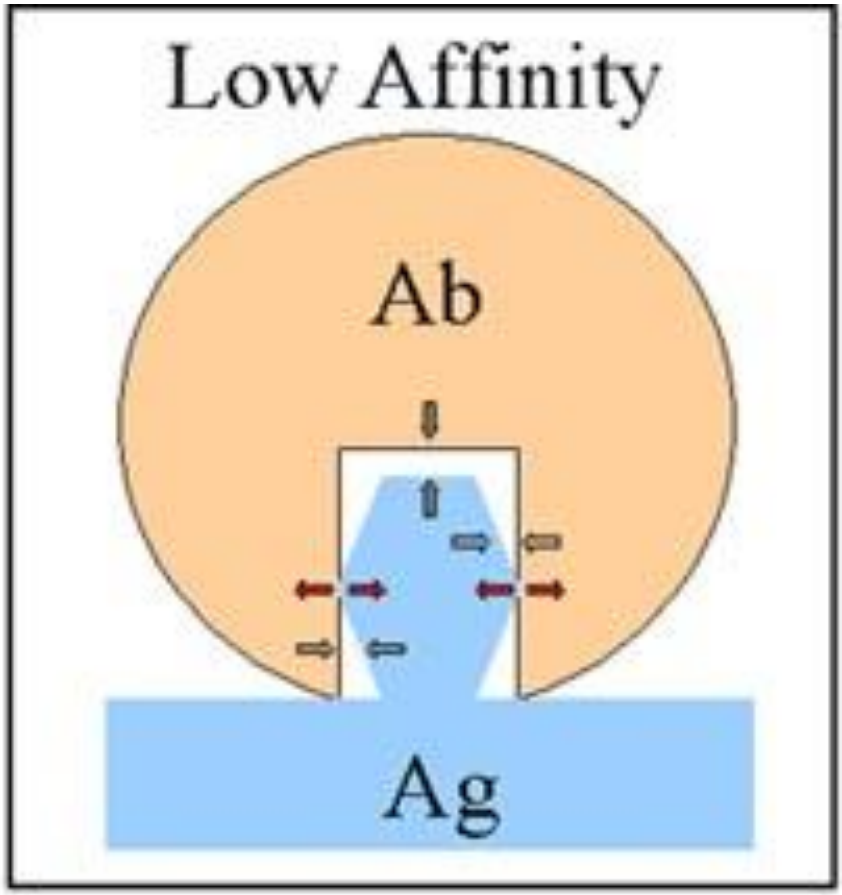


NATURE OF ANTIGEN-ANTIBODY REACTIONS

Lock and Key Concept

The combining site of an antibody is located in the Fab portion of the molecule and is constructed from the hypervariable regions of the heavy and light chains. Thus, the concept of antigen-antibody reactions is one of a key (*i.e.* the antigen) which fits into a lock (*i.e.*the antibody).





AFFINITY AND AVIDITY

Affinity

Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody. It is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site of the antibody as illustrated in Figure

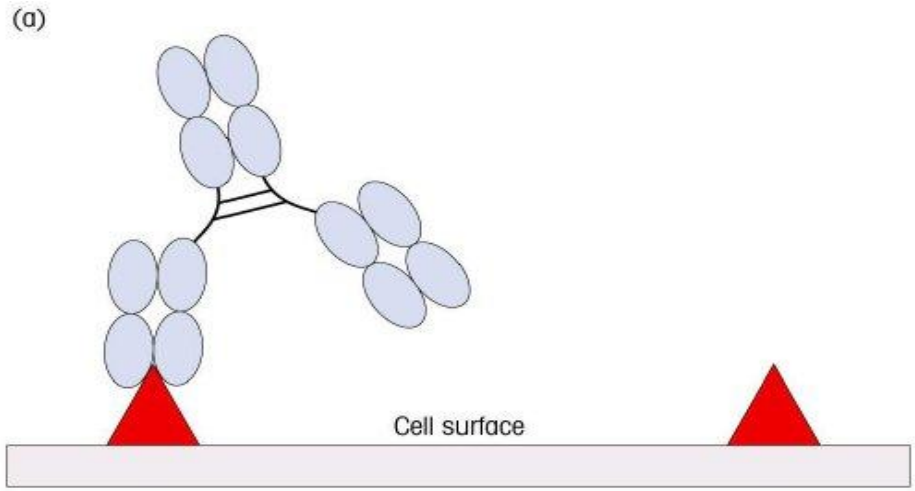
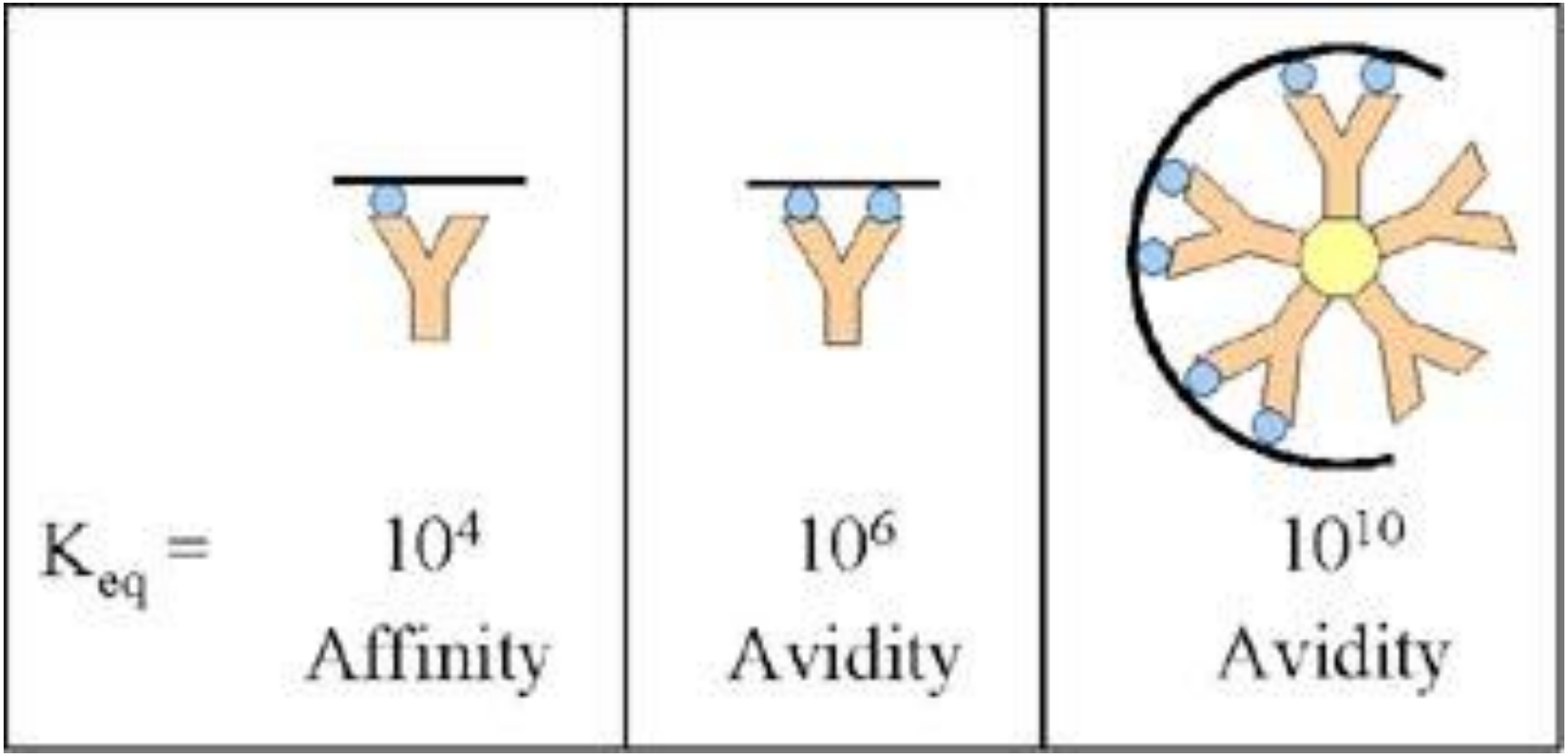


Figure 5.11. Divalent antibody binding to a cell surface.

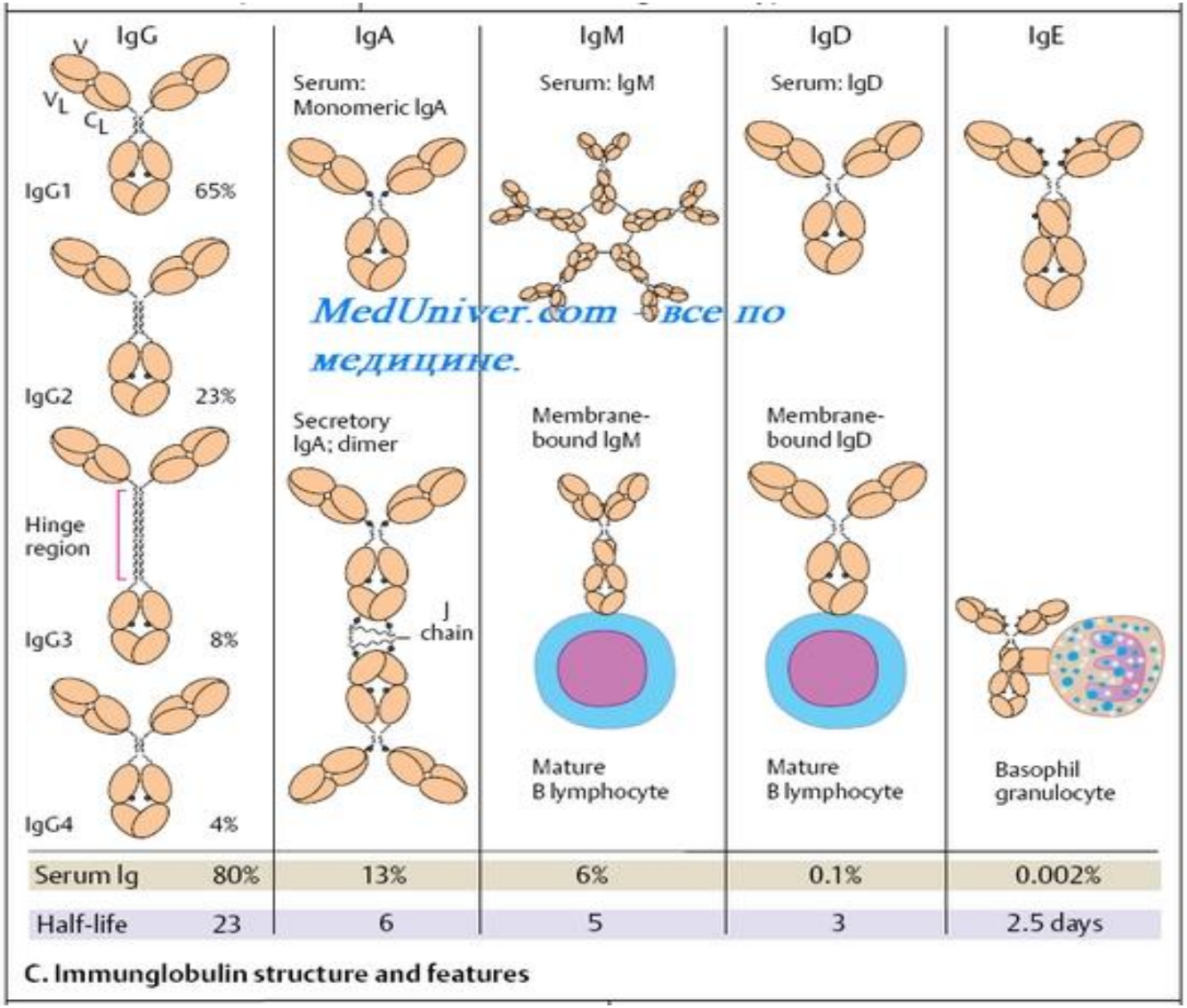
The affinity of an antibody that can bind divalently to a multivalent antigen (b), such as may be found on a cell surface, is enhanced relative to an antibody that can only bind monovalently (a).



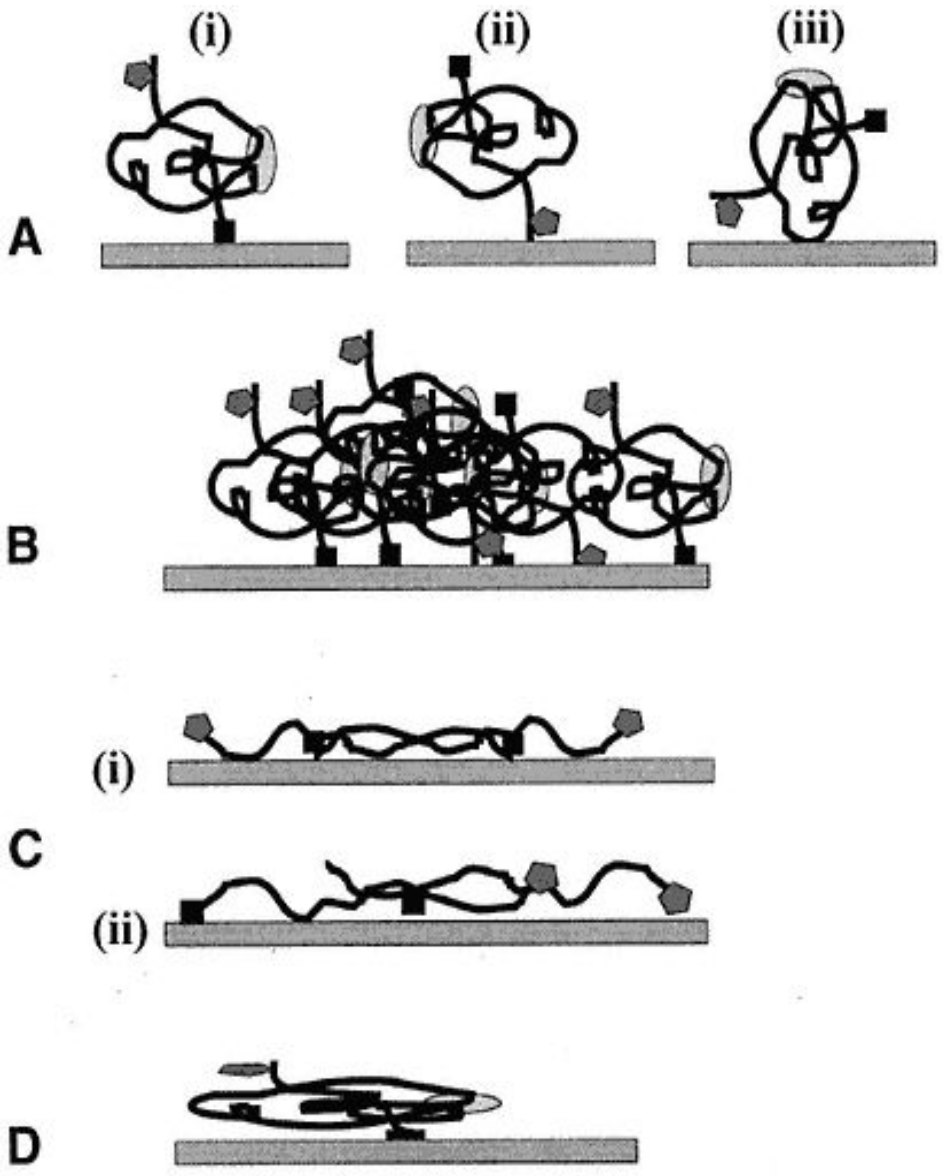
Avidity

Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies. Avidity is influenced by both the valence of the antibody and the valence of the antigen. Avidity is more than the sum of the individual affinities. This is illustrated in Figure.

To repeat, affinity refers to the strength of binding between a single antigenic determinant and an individual antibody combining site whereas avidity refers to the overall strength of binding between multivalent antigens and antibodies.



C. Immunglobulin structure and features



Possible effects on soluble protein of immobilization

Protein is shown as having three antigenic sites (epitopes). Two are linear (solid box and shaded pentagon), and one is conformational dependent (shaded oval).

(A) **(i) to (iii) The orientation of the molecule on the well affects the presentation of the individual epitopes**. This is true of passive and covalent binding to plastic.

(B) **Aggregation of the antigen can complicate presentation** and also lead to leaching following binding with detecting antibody.

(C) **The antigen may be altered through treatment before attachment**. In both (i) and (ii)the conformational epitope has been destroyed. Note also that the orientation of the molecules affects the presentation and spacing between individual epitopes.

(D) **Nondenatured protein can also alter its conformation by passive adsorption to plastic.**

SPECIFICITY AND CROSS REACTIVITY

Specificity

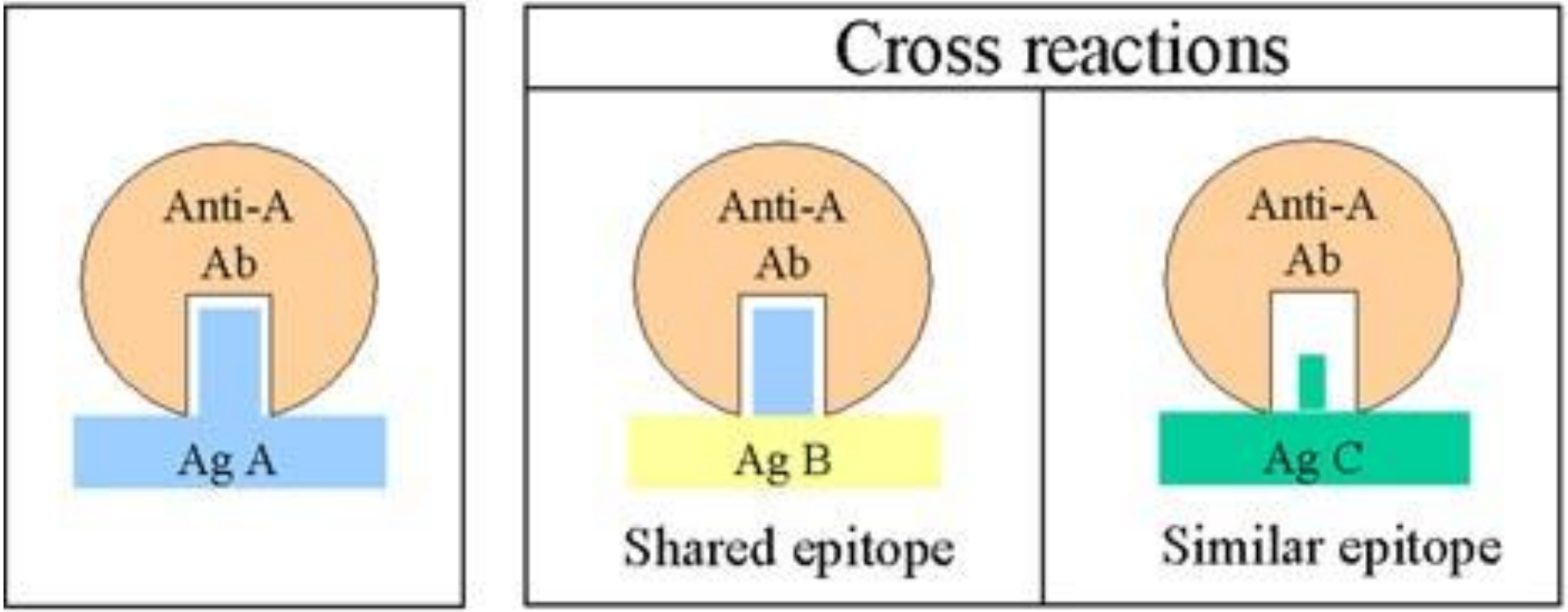
Specificity refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen.
In general, there is a high degree of specificity in antigen-antibody reactions.

Antibodies can distinguish differences in:

- **The primary structure of an antigen**
- **Isomeric forms of an antigen**
- **Secondary and tertiary structure of an antigen**

APLICATION OF ANTIGEN-ANTIBODY REACTIONS

1. Diagnosis of infectious and parasitic diseases and the establishment of detection antibody titers (serodiagnosis);
2. Diagnosis of diseases to identify antigens of pathogens in the body;
3. Identification of cultures of bacteria and viruses isolated from humans and animals;
4. Determination of the composition and characteristics of human tissue: blood group, Rh factor, transplantation antigens;
5. Identification of the human body and in the environment of any substances having antigenicity (hormones, enzymes, toxins, drugs, drugs, etc.).
6. Assessment of immune status to determine the quantitative and functional characteristics of immune system cells and their products.
7. Identification of immunopathological conditions, allergies, transplant and anti-tumor responses.

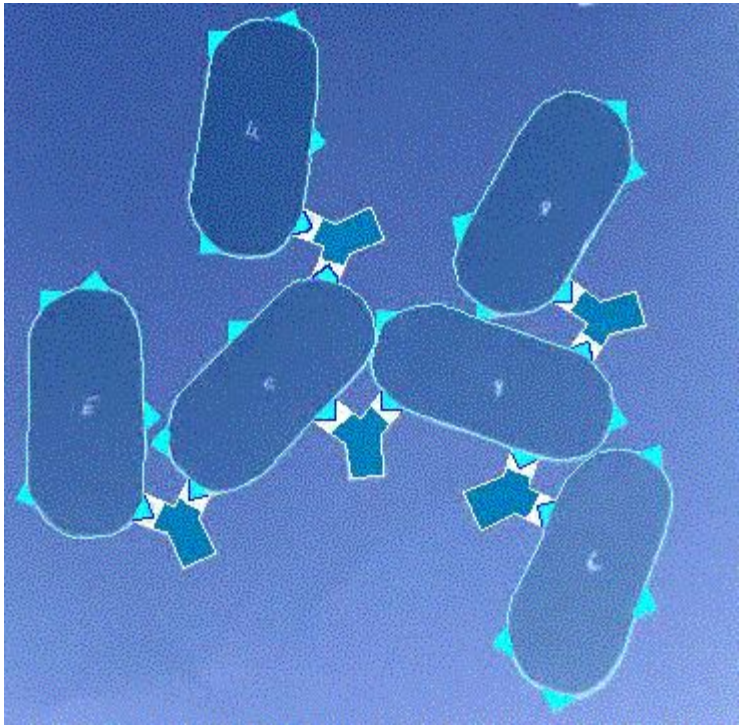


Cross reactivity

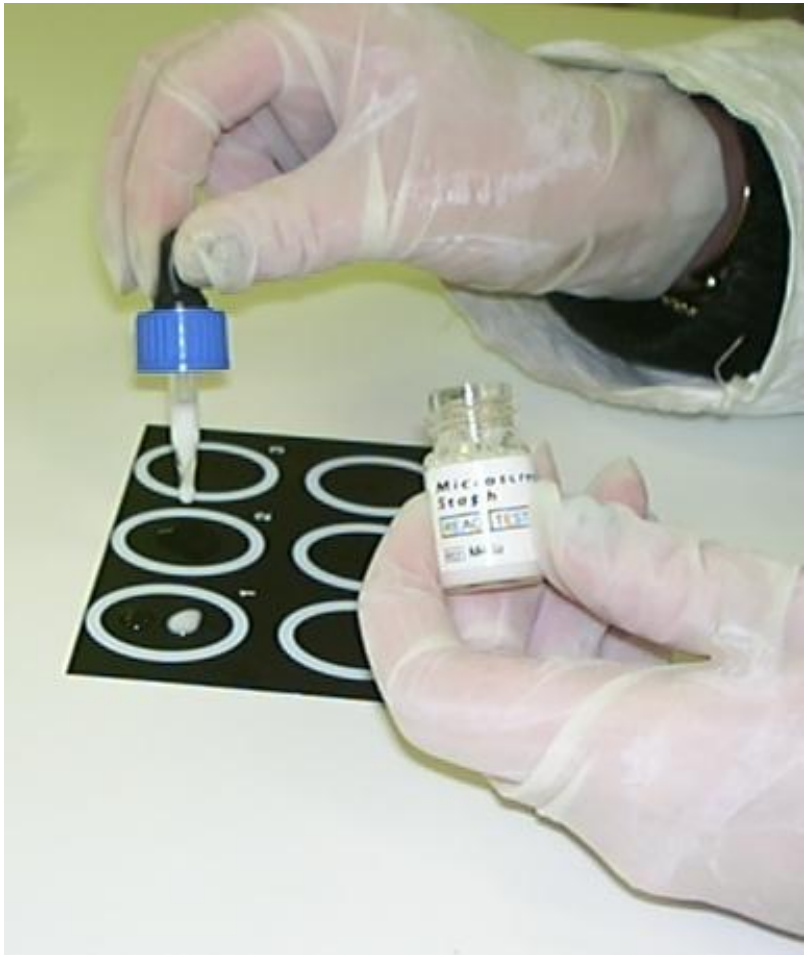
Cross reactivity refers to the ability of an individual antibody combining site to react with more than one antigenic determinant or the ability of a population of antibody molecules to react with more than one antigen. Figure illustrates how cross reactions can arise. Cross reactions arise because the cross reacting antigen shares an epitope in common with the immunizing antigen or because it has an epitope which is structurally similar to one on the immunizing antigen (multispecificity).

Agglutination test

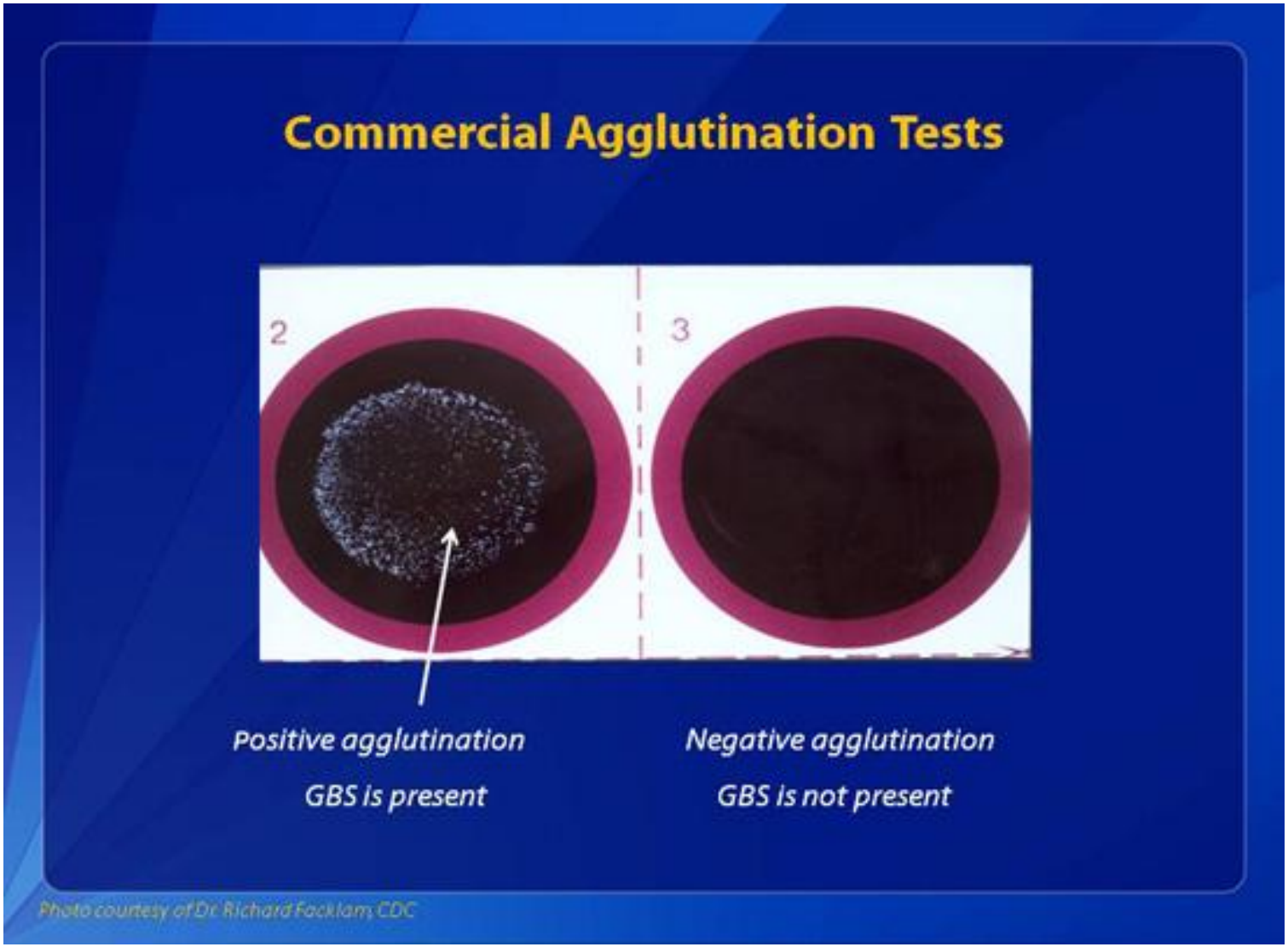
Agglutination test
(agglutinacio - склеивание)
- **gluing and precipitation of the bacteria under the influence of antibodies in an environment with the electrolyte.**



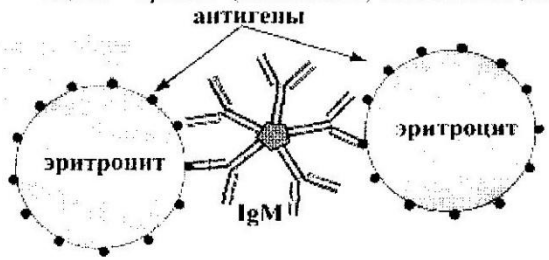
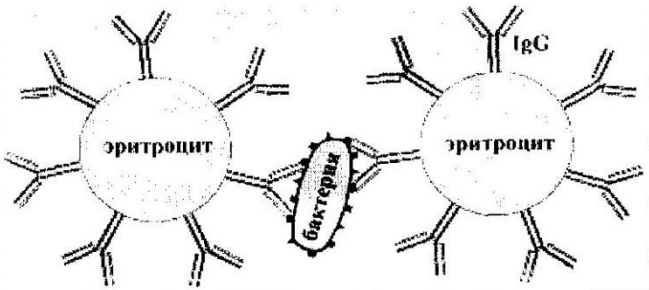
STATEMENT OF MICROAGGLUTINATION TEST



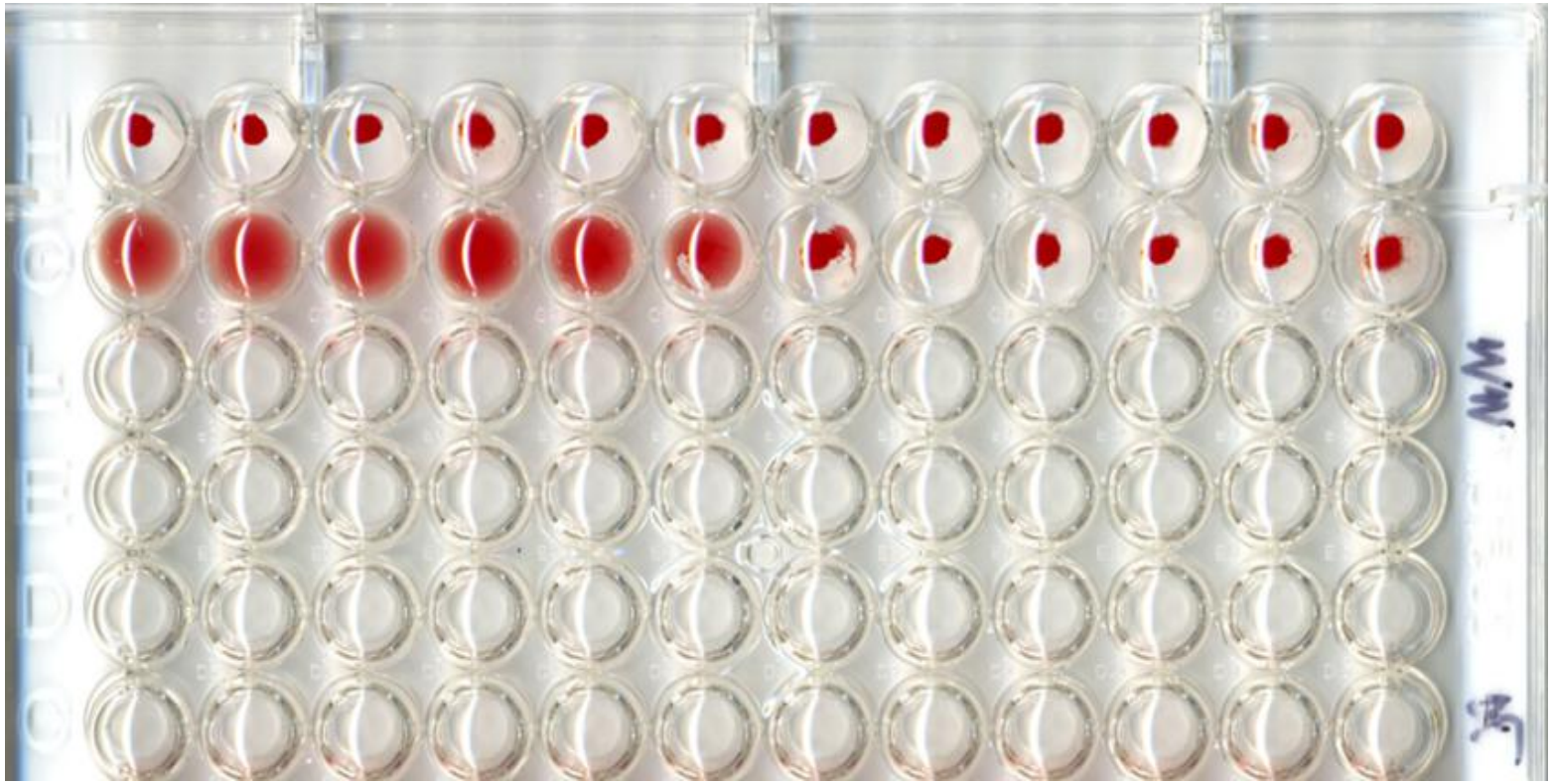
THE RESULTS OF MICROAGGLUTINATION TEST



Реакция непрямой (пассивной) гемагглютинации (РНГА, РПГА)

В **РНГА** выявляют антитела сыворотки крови с помощью **антигенного эритроцитарного диагностикума**, который представляет собой эритроциты с адсорбированными на них антигенами.	Антигенный эритроцитарный диагностикум 
Эритроциты (или частицы латекса) с адсорбированными на них антигенами взаимодействуют с соответствующими антителами сыворотки крови, что вызывает склеивание и выпадение эритроцитов на дно пробирки или ячейки в виде фестончатого осадка. При отрицательной реакции эритроциты оседают в виде пуговки.	Реакция непрямой (пассивной) агглютинации 
РПГА ставят в пластиковых планшетках или в пробирках с разведениями сыворотки крови больного, к которым добавляют эритроцитарный диагностикум.	
Иногда применяют **антительный эритроцитарный диагностикум** - эритроциты, на которых адсорбированы антитела. Например, можно обнаружить ботулинический токсин, добавляя к нему эритроцитарный антительный ботулинический диагностикум (такую реакцию называют реакцией обратной непрямой гемагглютинации (**РОНГА**).	Реакция обратной непрямой гемагглютинации 

Passive Hemagglutination



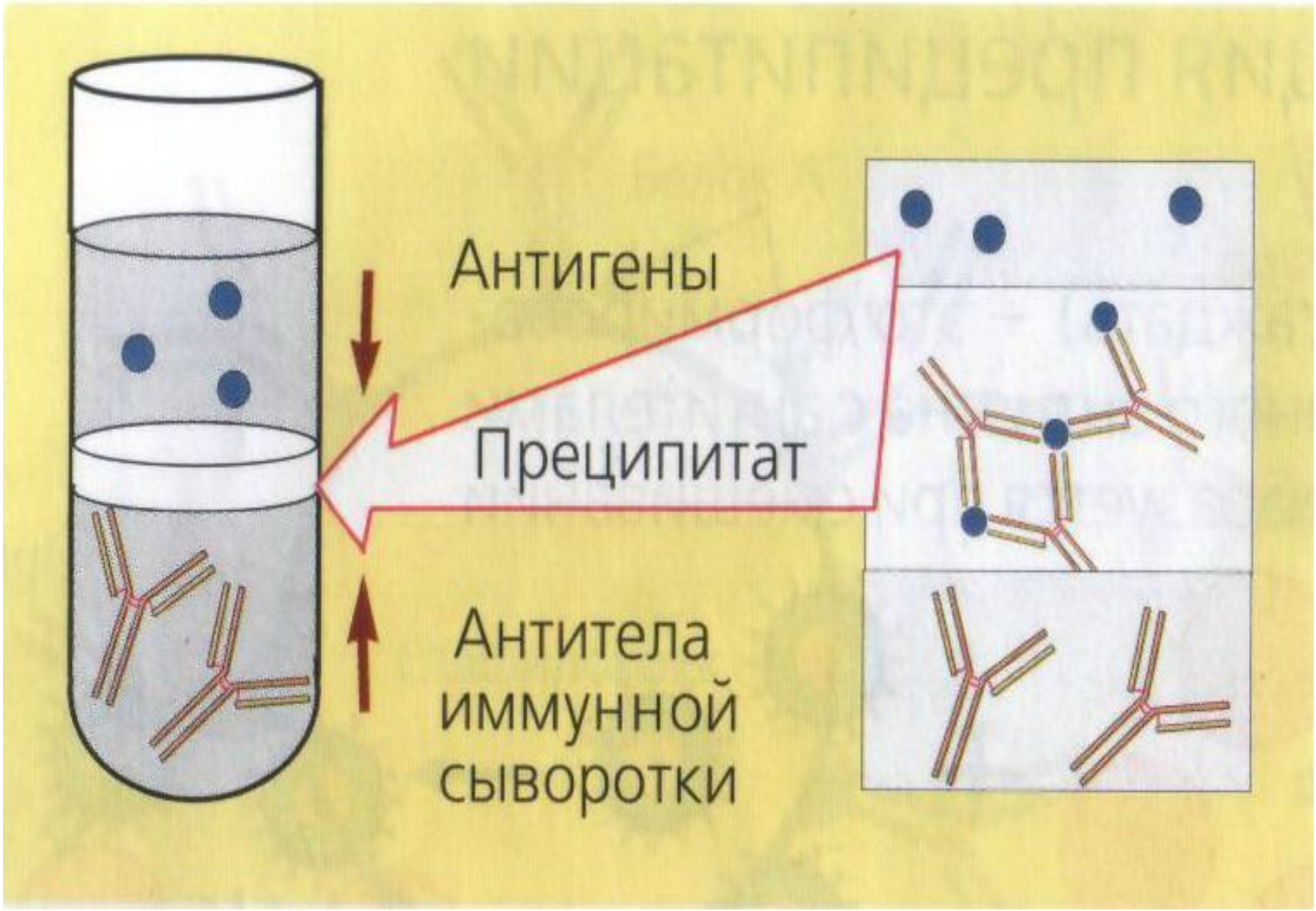
In positive cases precipitate has the form of a thin film of the red blood cells glued together (umbrella).

PRECIPITATION TEST

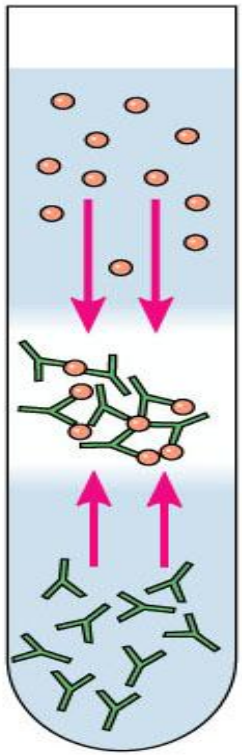
Principle: When interacting of soluble antigen with antibody in the presence of electrolyte (NaCl) complex Ag-Ab is formed as an insoluble precipitate.

- PT is used for two purposes: detection of antigens with the help of known antibody or antibodies using known antigens.
- With the help of PT falsification of fish and meat products is determined.

THE PRINCIPLE OF PRECIPITATION TEST

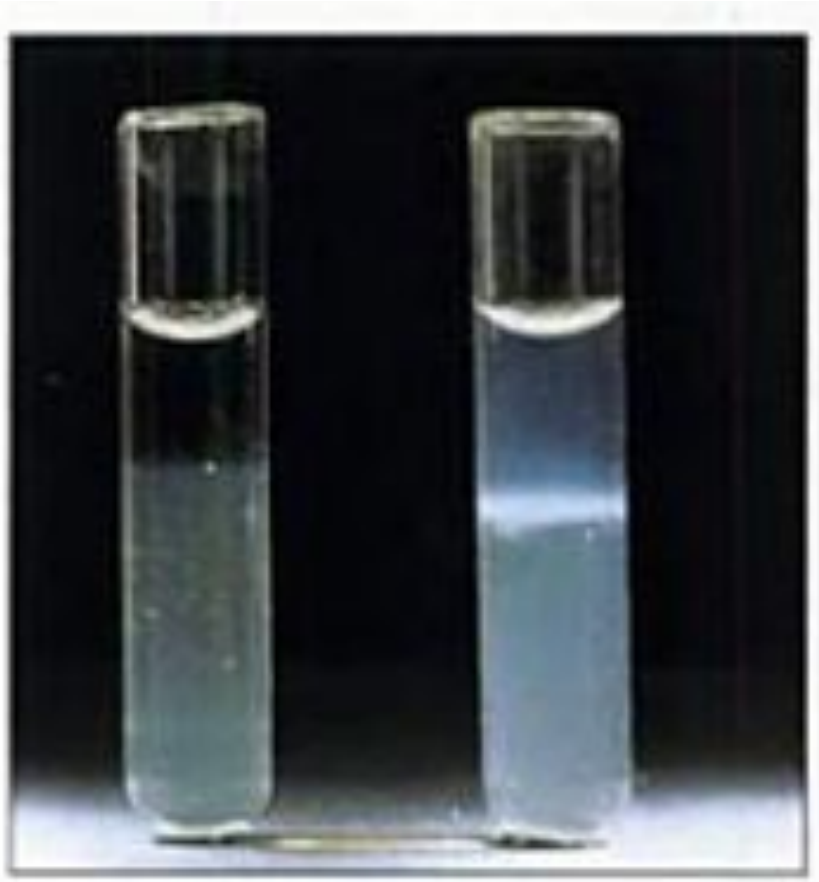


Ring-precipitation test



Formation of the Ag-Ab complex

The test is carried out by layering the antigen on the immune serum



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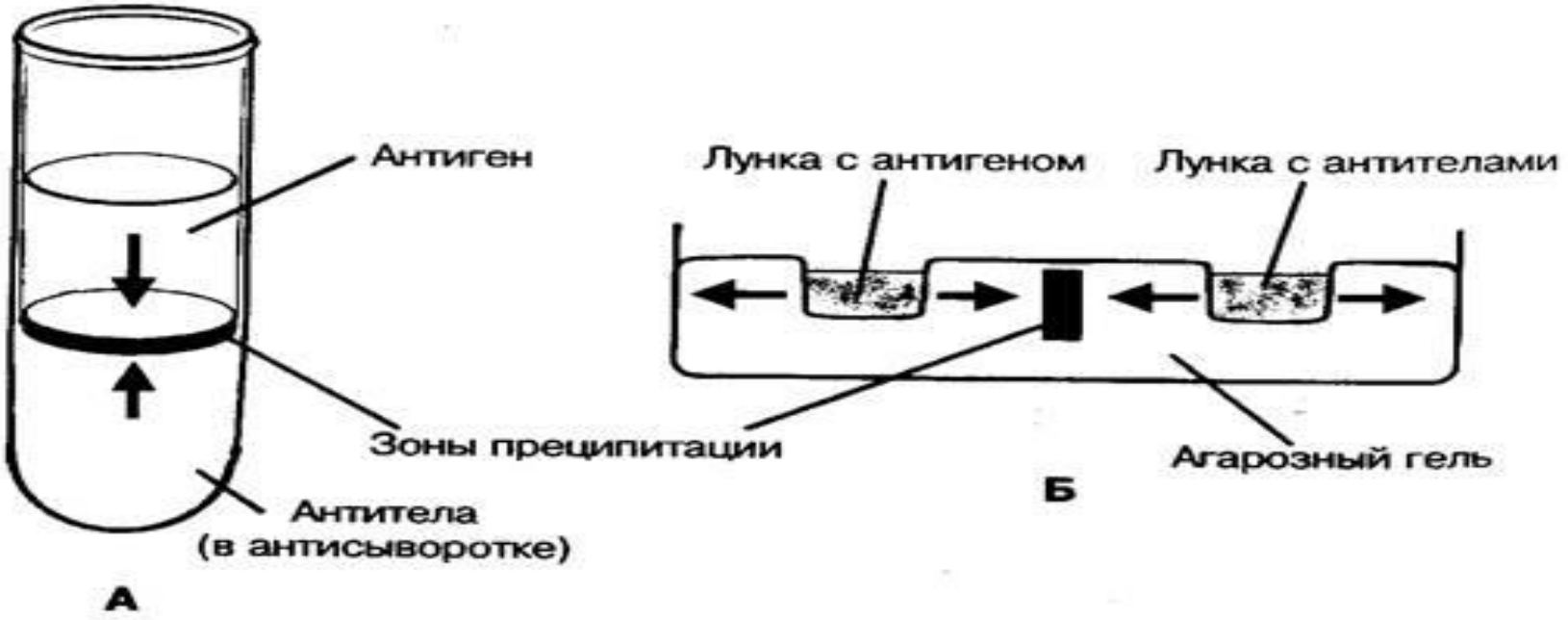
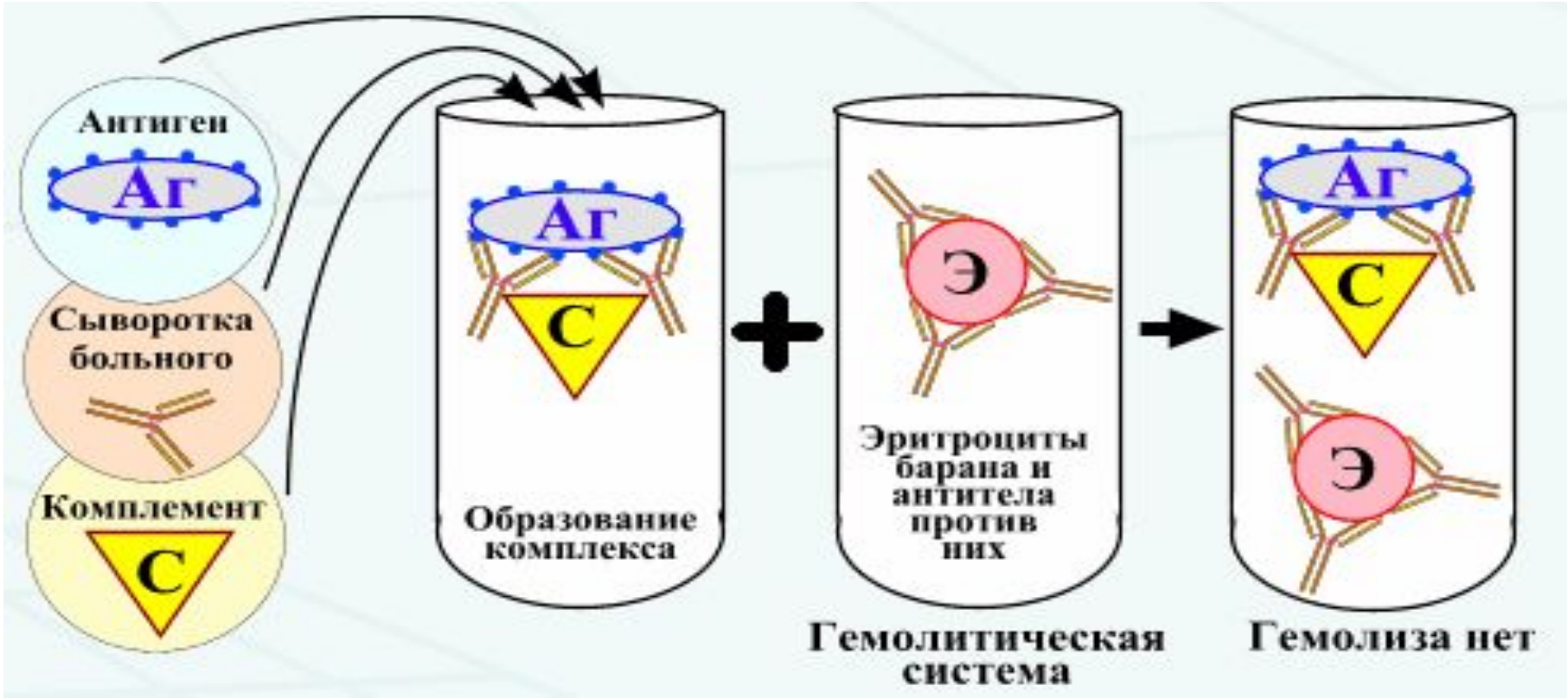
THE PRINCIPLE OF RADIAL IMMUNODIFFUSION TEST

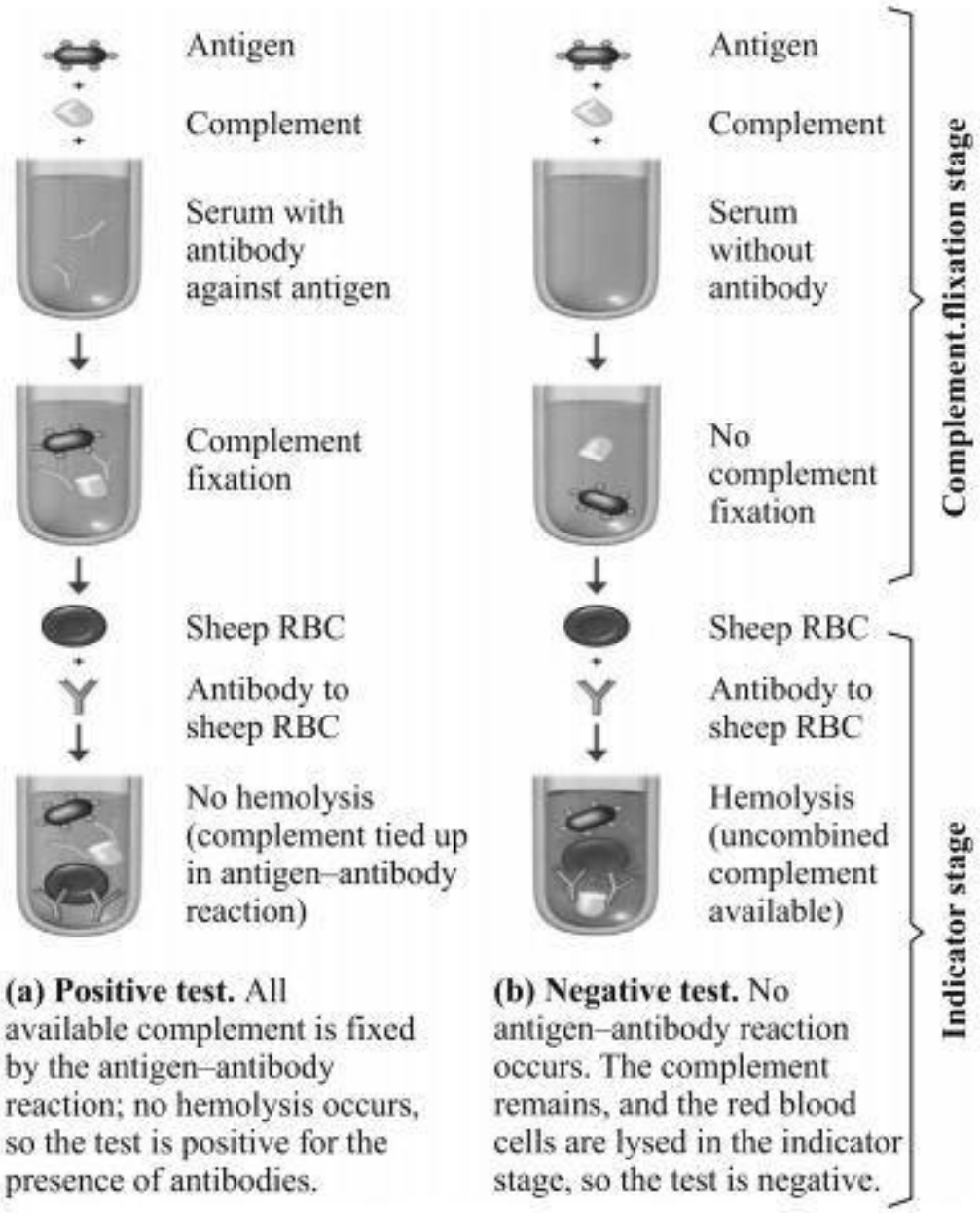


The test is carried out in agar gel plates or in Petri dishes. Holes are cut out In the frozen gel at some distance from each other, and filled with the solutions of antigen and antisera. In the case of optimally selected ratio of antigens to antibodies, precipitation bands are formed in the gel between the wells. Since the reagents diffuse from the wells concentrically, the method allows several simultaneous reactions to be carried out by placing several wells with antigens around the antiserum well.

Radial Immunodiffusion (RID)

Complement fixation test





(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

Materials and Reagents

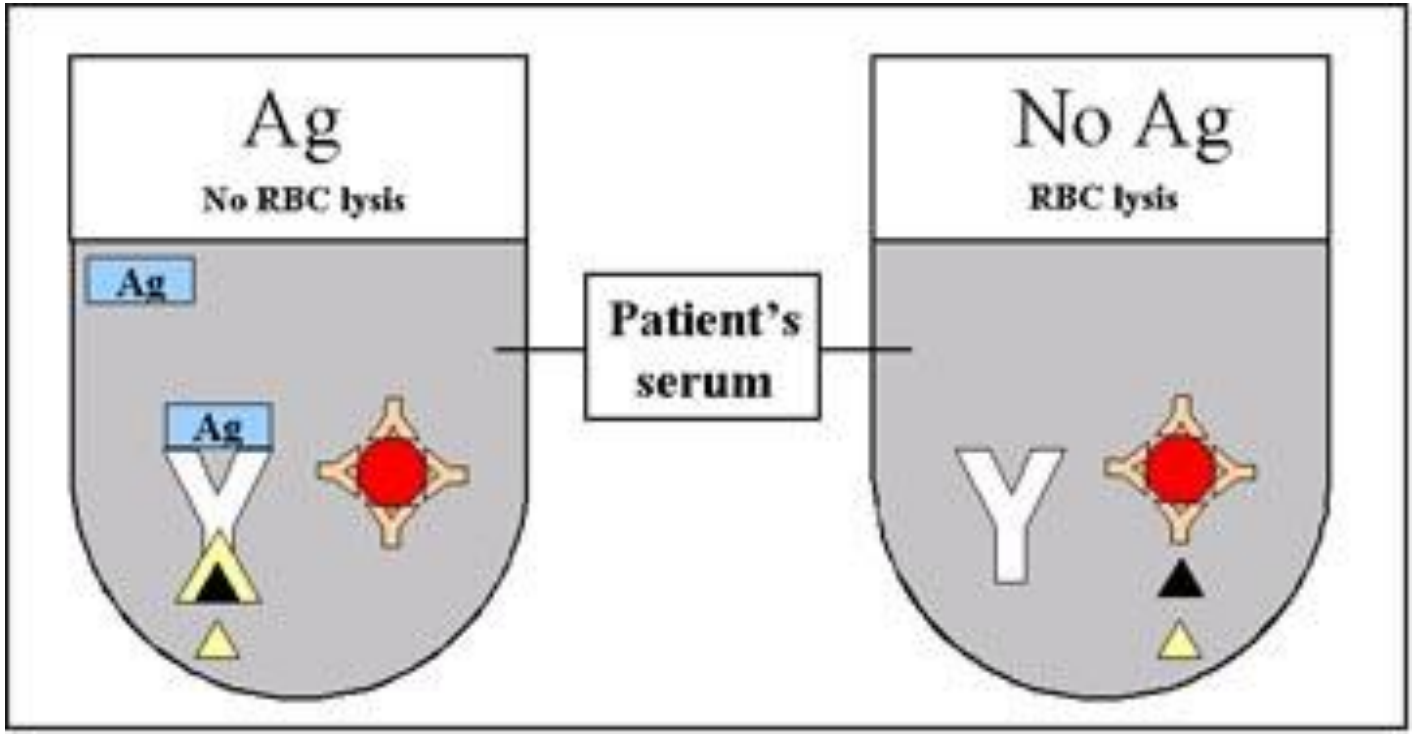
1. **Sheep erythrocytes suspension** (5% suspension of washed sheep RBCs)

2. **Hemolysin** (rabbit anti-sheep red-cell antibody)

3. **Guinea pig complement**, free of antibodies to the agent of interest

4. **Test serum**

5. **Antigen**



The principle of the complement fixation test is illustrated in Figure. Antigen is mixed with the test serum to be assayed for antibody and antigen/antibody complexes are allowed to form. A control tube in which no antigen is added is also prepared. If no antigen/antibody complexes are present in the tube, none of the complement will be fixed. However, if antigen/antibody complexes are present, they will fix complement and thereby reduce the amount of complement in the tube. After allowing complement fixation by any antigen/antibody complexes, a standard amount of red blood cells, which have been pre-coated with anti-erythrocyte antibodies is added. The amount of antibody-coated red blood cells is predetermined to be just enough to completely use up all the complement initially added, if it were still there.

Procedure of Complement Fixation Test

Complement Fixation Test (CFT) consists of two stage:

First step (Complement fixation stage): a known antigen and inactivated patient's serum are incubated with a standardized, limited amount of complement. **If the serum contains specific, complement activating antibody the complement will be activated or fixed** by the antigen-antibody complex. However, if there is **no antibody** in the patient's serum, there will be no formation of antigen-antibody complex, and therefore complement will not be fixed. But **will remain free**.

Second step (Indicator Stage): The second step detects whether complement has been utilized in the first step or not. This is done by adding the indicator system.
If the complement is fixed in the first step owing to the presence of antibody there will be no complement left to fix to the indicator system. There won't be any lysis of RBCs. However**, if there is no antibody** in the patient's serum, **there will be no antigen-antibody complex**, and therefore, complement will be present free or unfixed in the mixture. This unfixed complement will now react with the antibody- coated sheep red blood cells to bring about their lysis.

Results and Interpretation

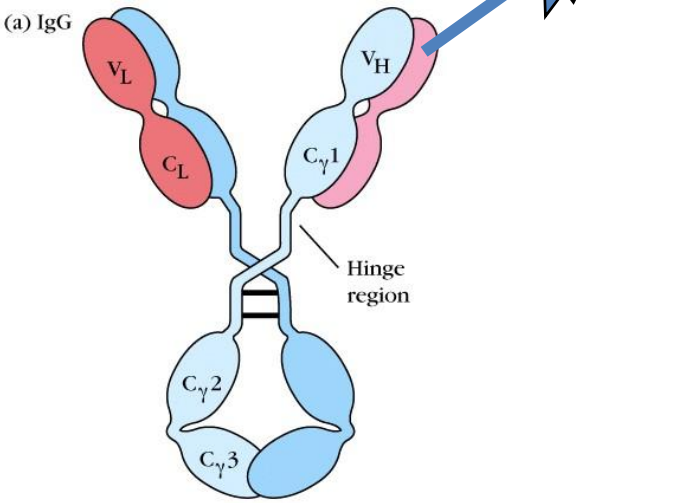
Thus, no lysis of sheep red blood cells (positive CFT) indicates the presence of antibody in the presence of antibody in the test serum, while lysis of sheep red blood cells (Negative CFT) indicates the absence of antibody in the serum.

Enzyme linked immunosorbent assay

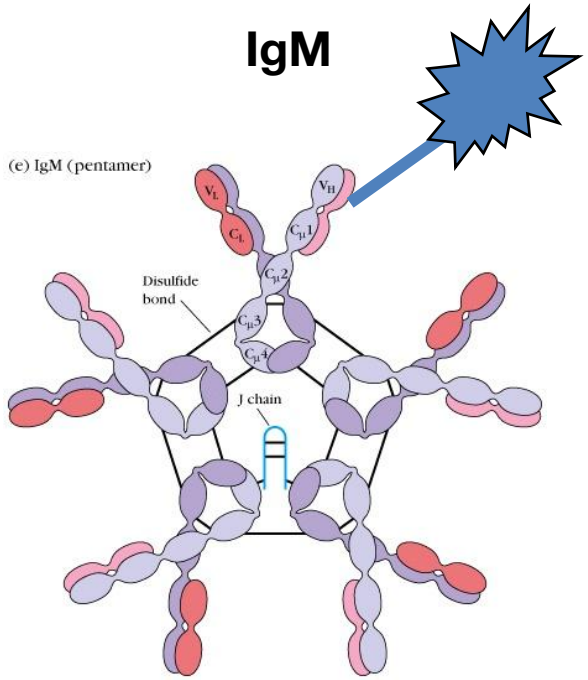
ELISA - it is serological test in which for the visualization of the formed antigen-antibody complex enzyme labels (horseradish peroxidase or alkaline phosphatase) are used . These marker (indicator) enzymes are able to cleave the substrate and cause a color change.

Серологические реакции, основанные на использовании меток

IgG



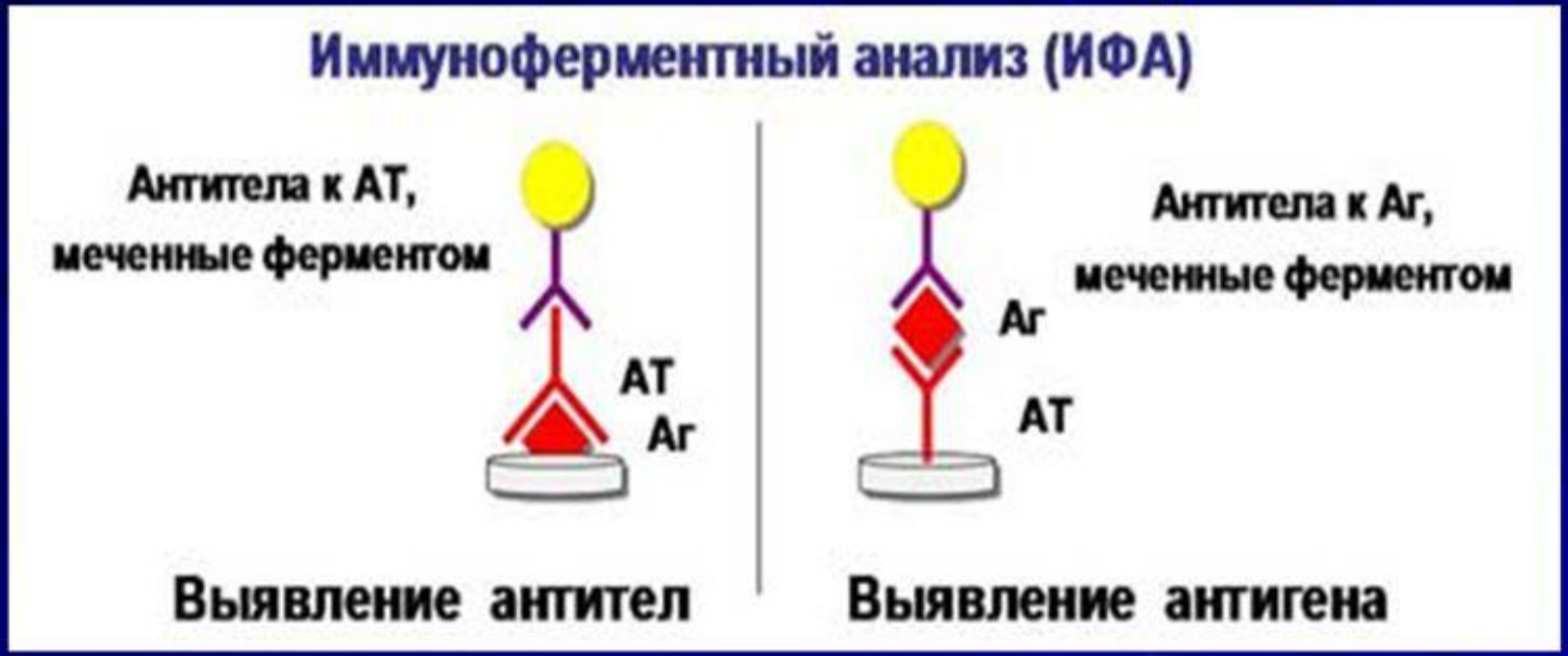
IgM



МЕТКИ

- **Ферментные**
- **Флюоресцирующие**
- **радиоизотопные**

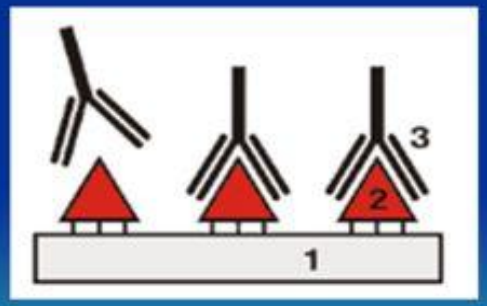
ИММУНОФЕРМЕНТНЫЙ АНАЛИЗ (ИФА)



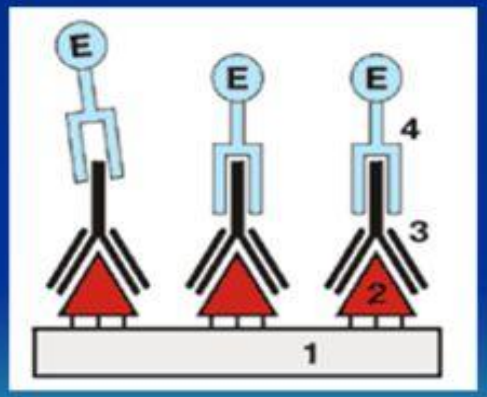
Метод основан на специфическом связывании АТс АГ, при этом один из компонентов конъюгирован с ферментом, в результате реакции с соответствующим хромогенным субстратом образовывается окрашенный продукт, количество которого можно определить спектрофотометрически.

Иммуноферментный анализ

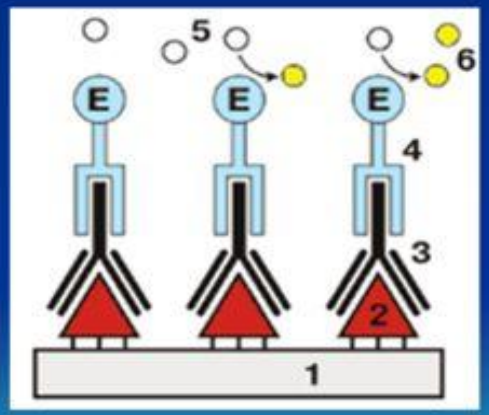
Этапы ИФА:



Взаимодействие аналита с лигандом

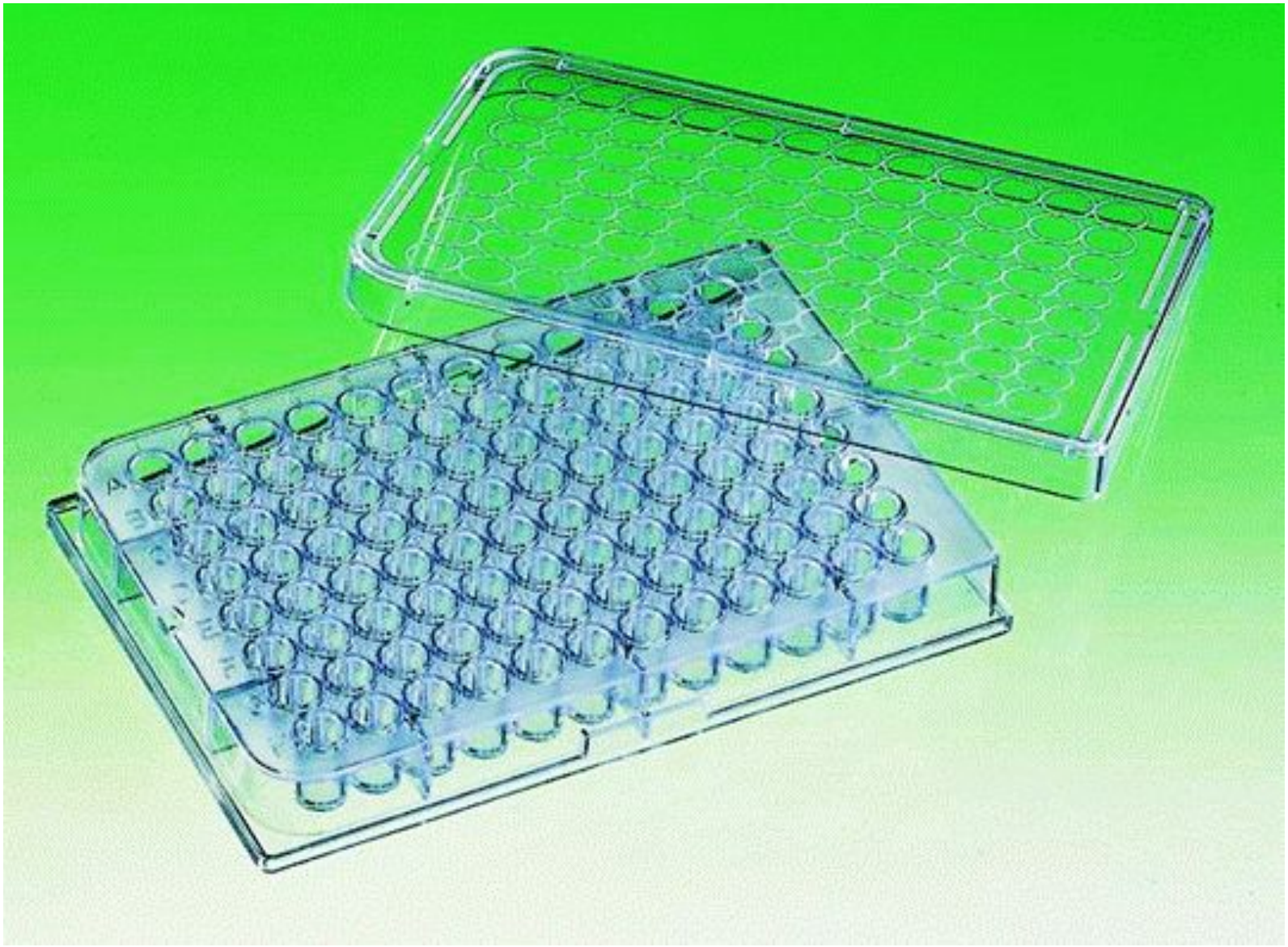


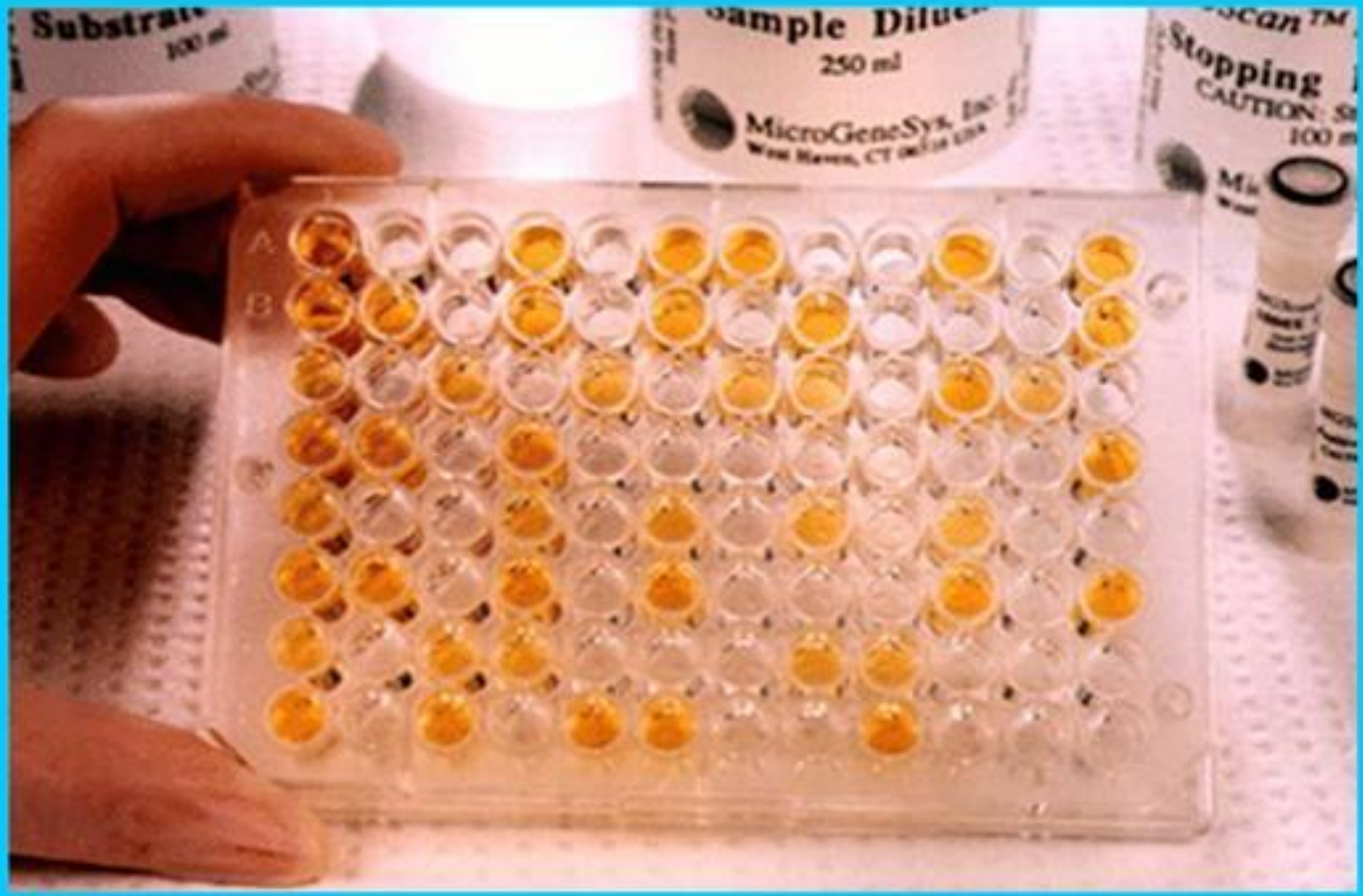
Формирование меченного комплекса



Измерение сигнала

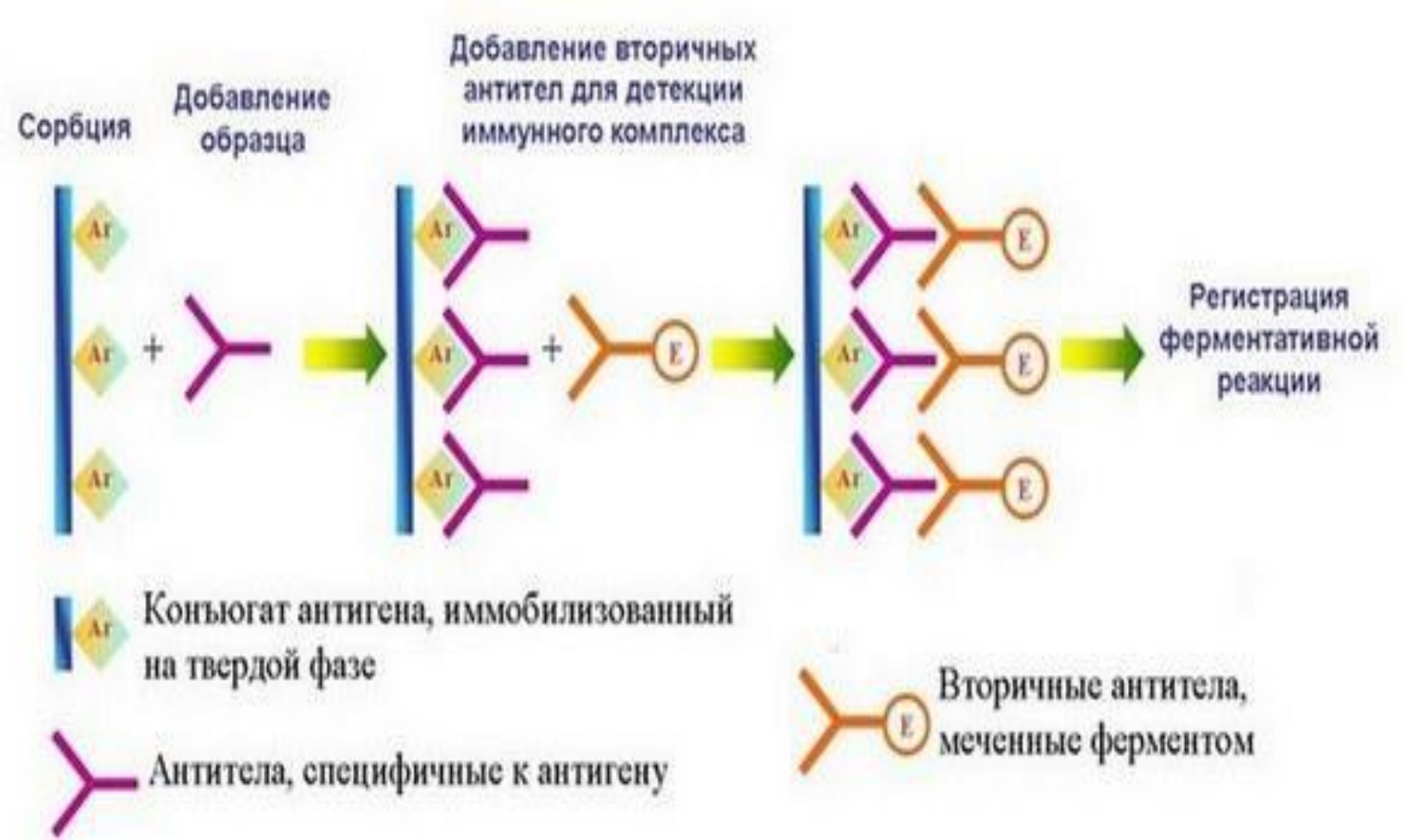
96-луночный планшет для ИФА



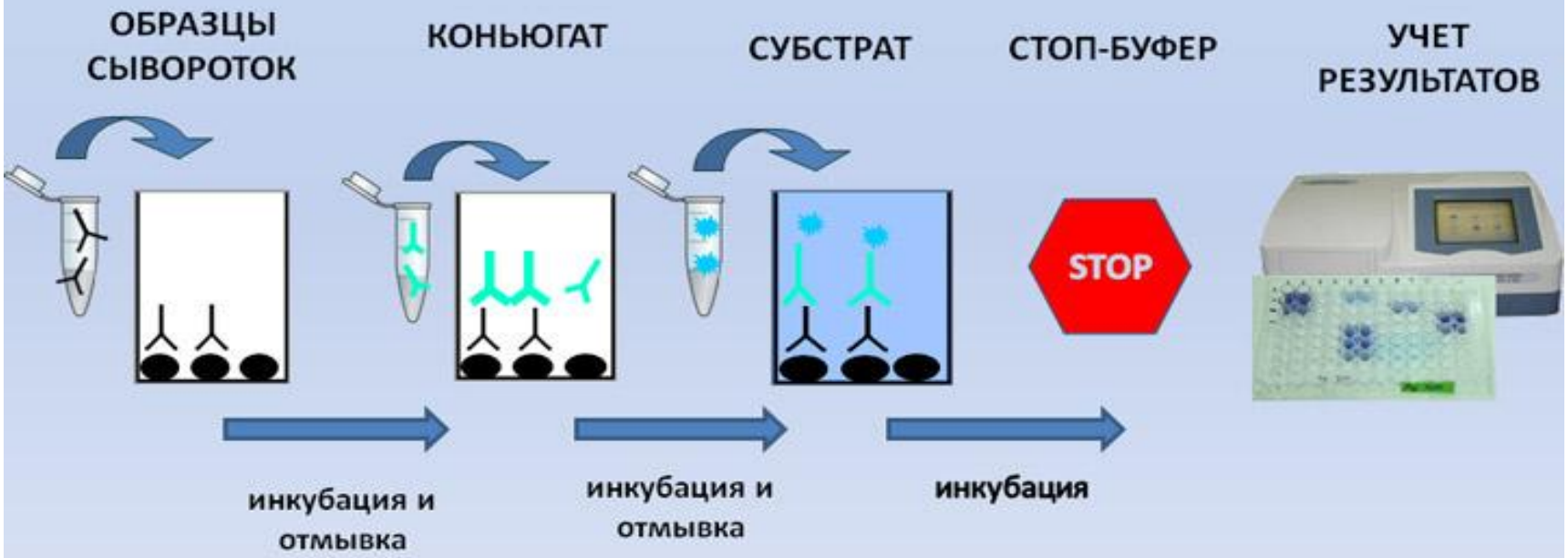

Substrate
100 ml
Sample Dilue
250 ml
MicroGeneSys, Inc.
Stopping
CAUTION:
100 m

Спектрофотометр для ИФА

ПРИНЦИП НЕПРЯМОГО ИФА



Непрямой вариант ИФА

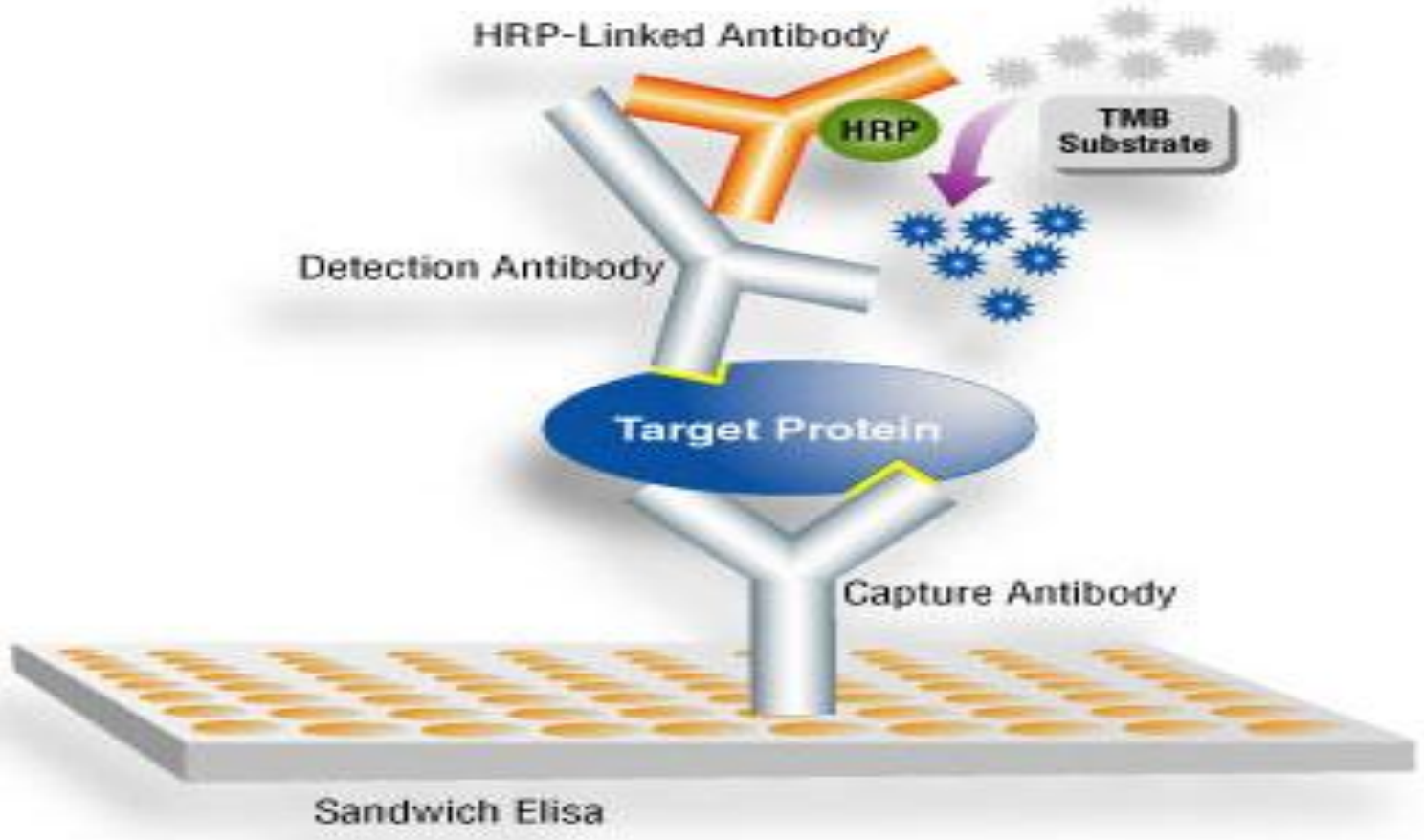


Sandwich ELISA

- **This variant of ELISA is extremely common for the determination of antigens possessing more than one determinant.**

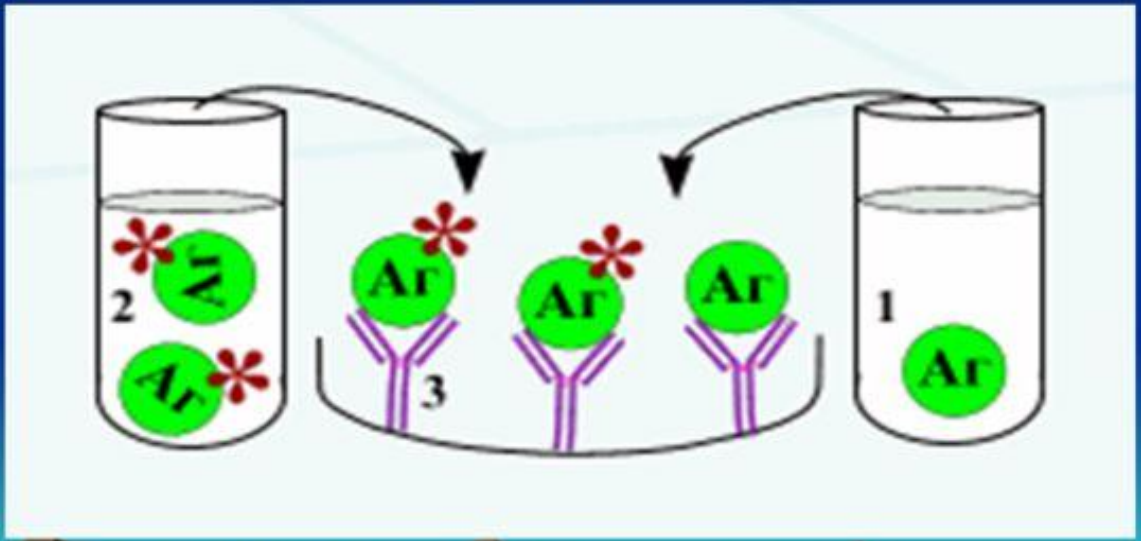
- **In the process of analysis, the antigen is "squeezed" between antibody molecules, which led to the name of the "sandwich" method. This name is now used practically in all literature as an official term.**

Принцип сэндвич-ИФА

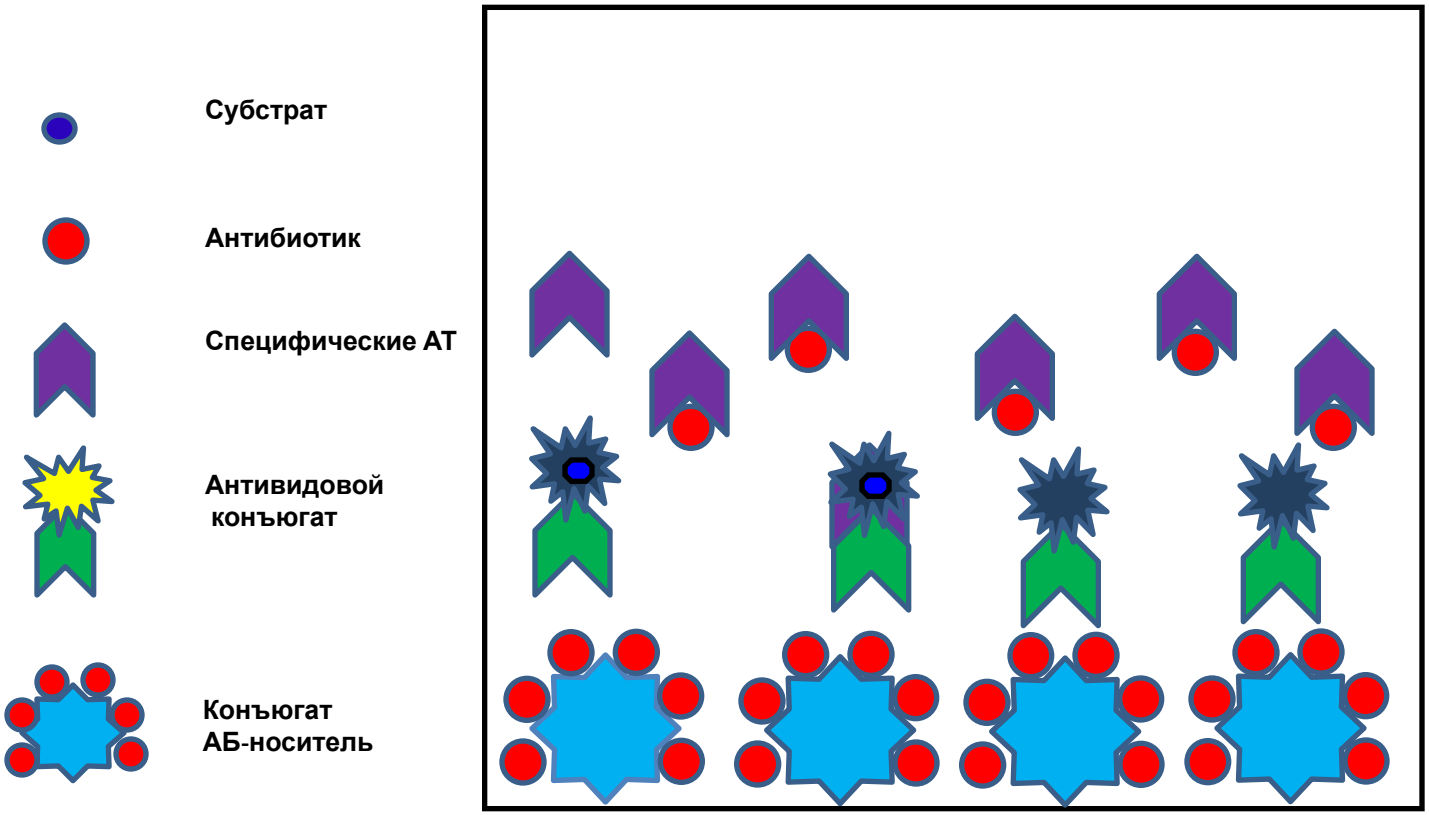


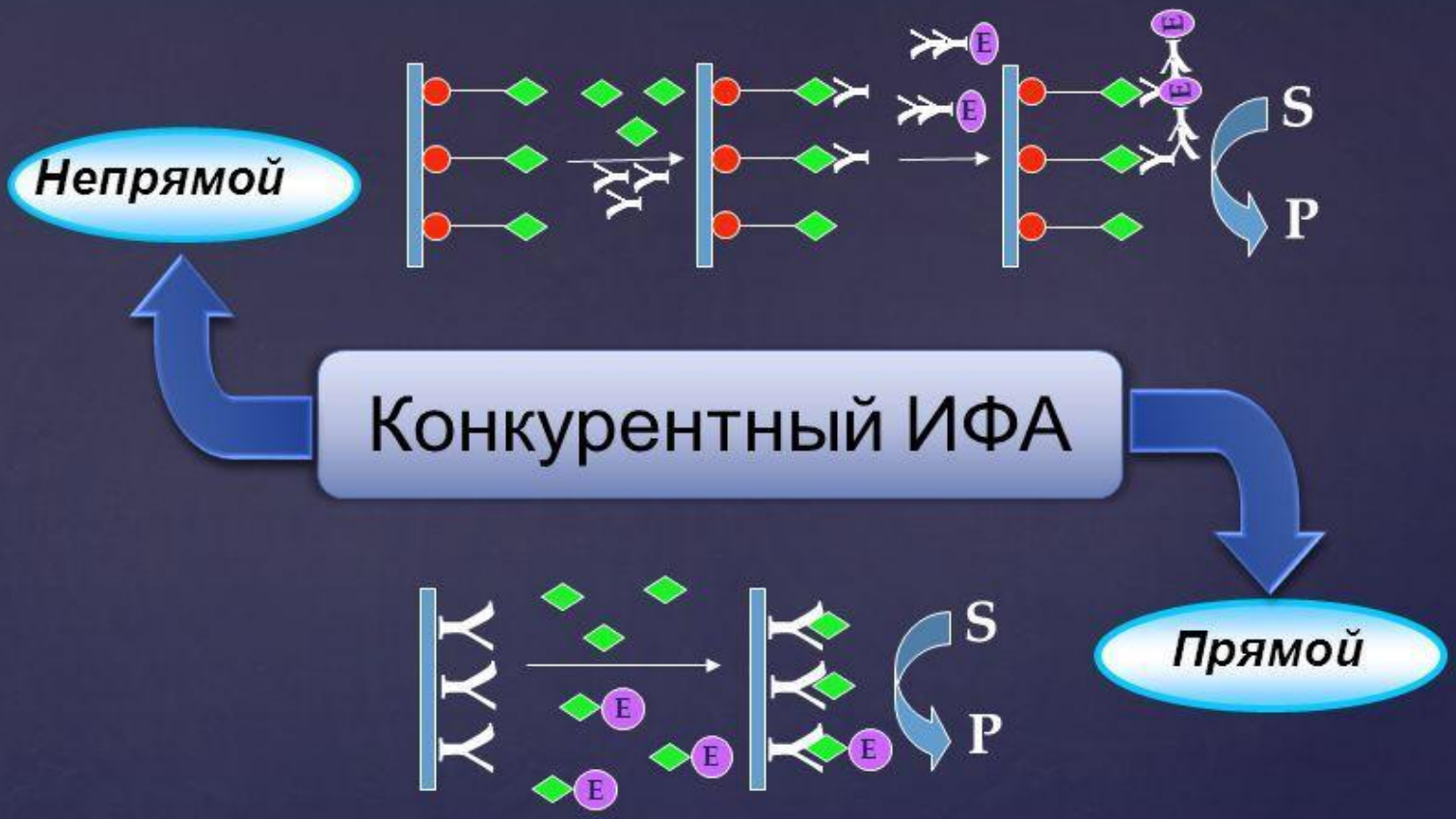
Конкурентный ИФА для определения антигенов

Искомый антиген(1) и меченый ферментом антиген(2) конкурируют друг с другом за антитела (3), сорбированные на твердой фазе.



Конкурентный ИФА для определения антигена (антибиотиков)





Иммуноферментный анализ

Конкурентный ИФА для определения специфических антител

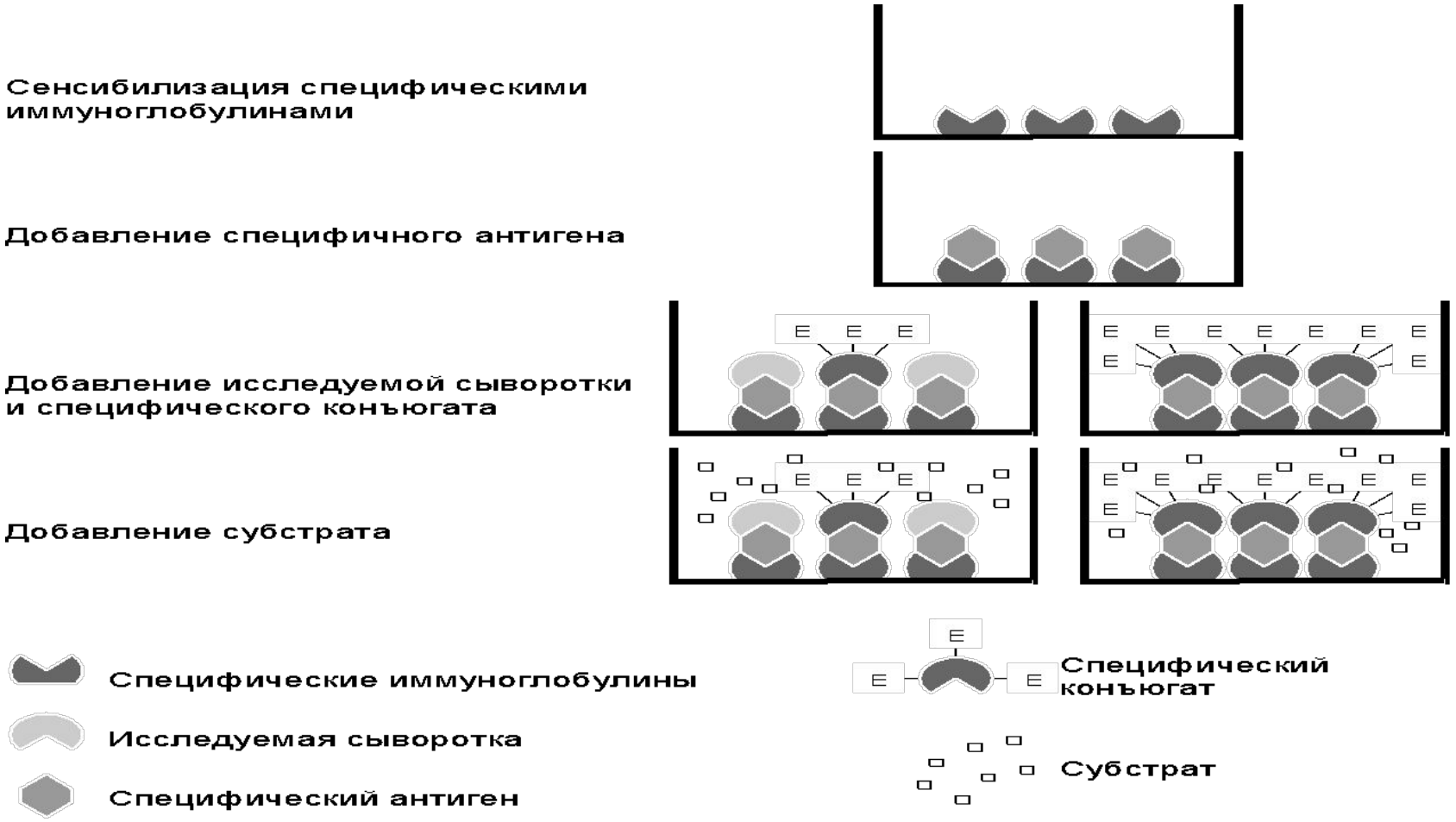
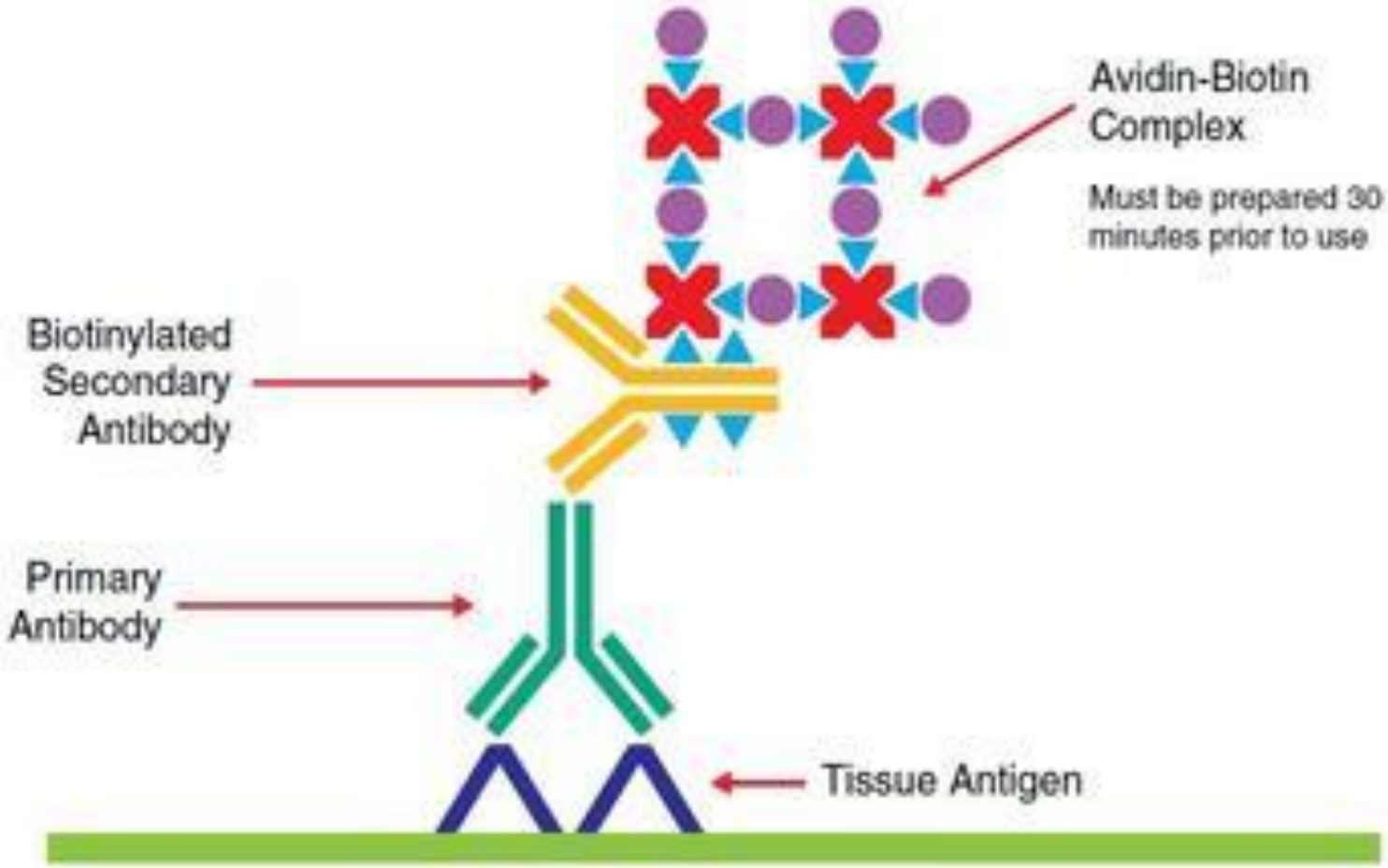


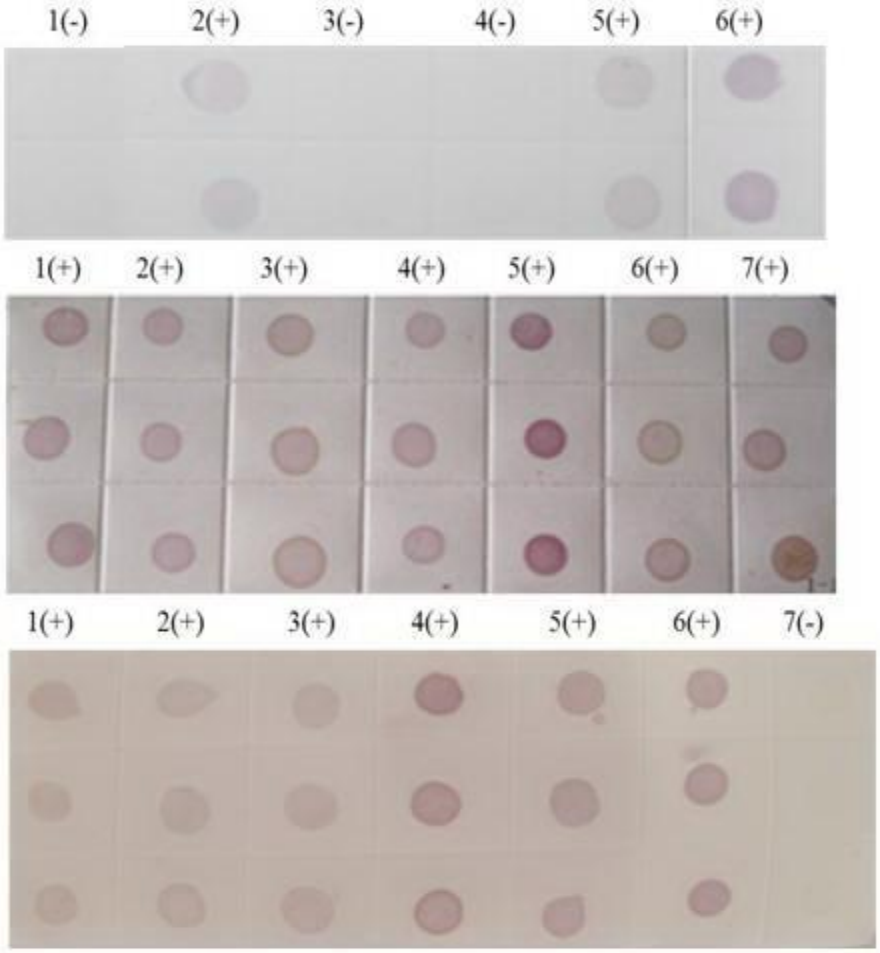
Рис. 3.

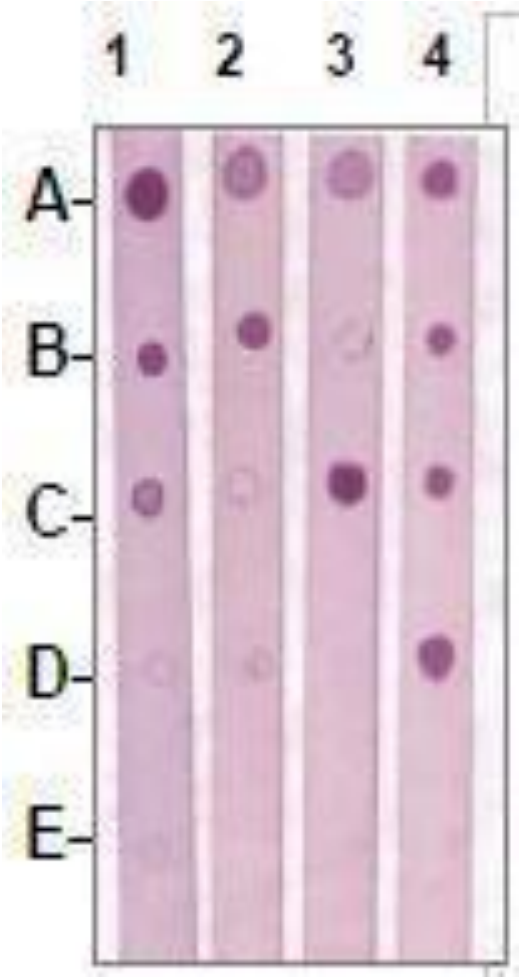
ИСПОЛЬЗОВАНИЕ А*ВИДИН-БИОТИНОВОЙ* СИСТЕМЫ В ИФА




ELISA Reagents
for laboratory use
Store at 4°C
Do not freeze
BIOREBA
BIOREBA
BIOREBA
BIOREBA

Dot-ИФА

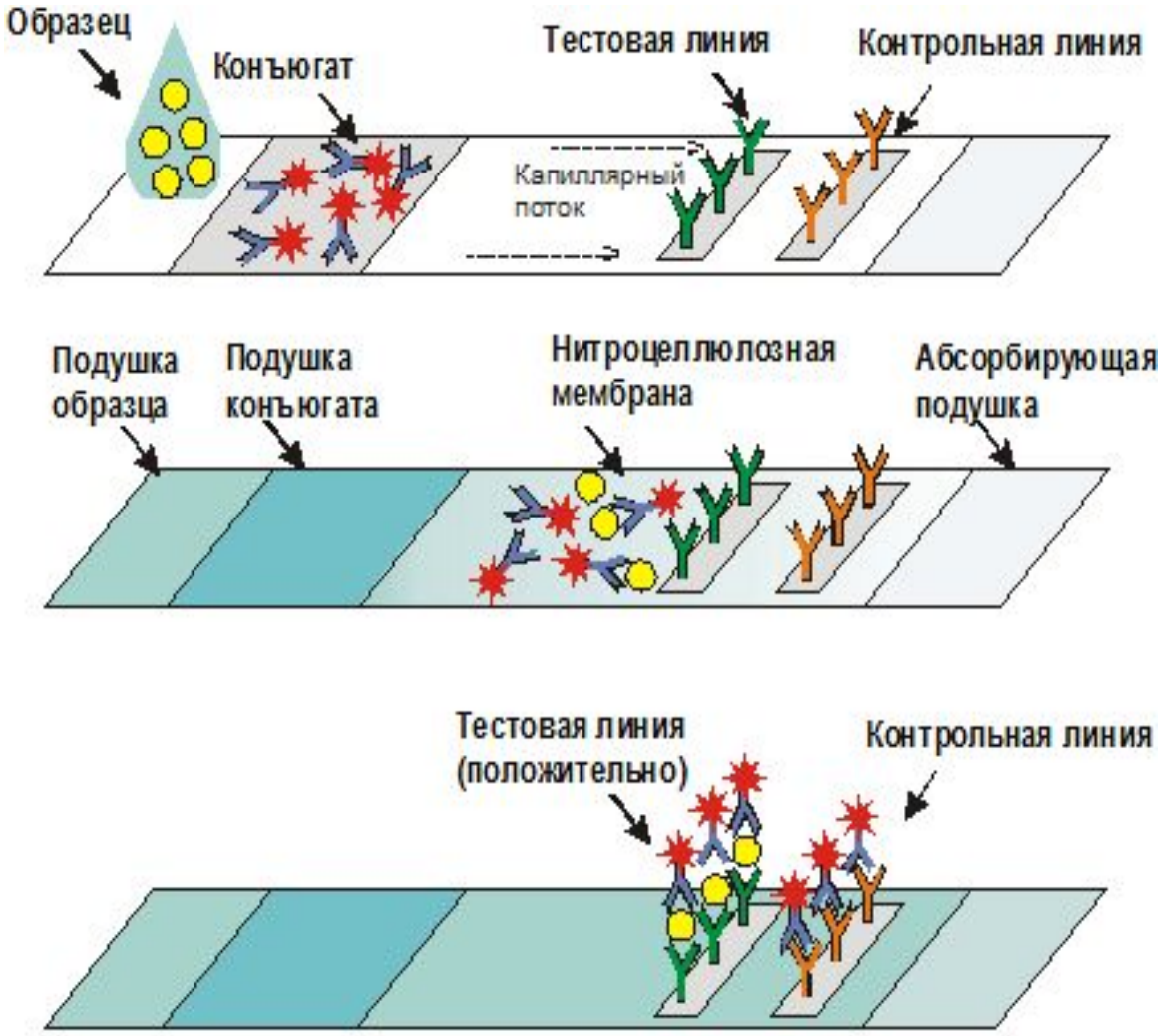




Сравнительная характеристика *dot*-ИФА и ИФА.

Показатели, характеристики	*dot*-ИФА	ИФА
Продолжительность	2 час	3-6 часов
Число этапов постановки	3 часа	4-5 часов
Число промывок	3	3
Цена анализа (в долларах США)	0,05-0,1	1-2
Спецподготовка персонала	нет	да
Применяемость в полевых условиях	да	нет
Необходимость фотометра	нет	да
Возможность непрерывной регистрации	да	неб

ПРИНЦИП ИММУНОХРОМАТОГРАФИЧЕСКОГО АНАЛИЗА



Оборудование для производства иммунохроматографической тест - системы

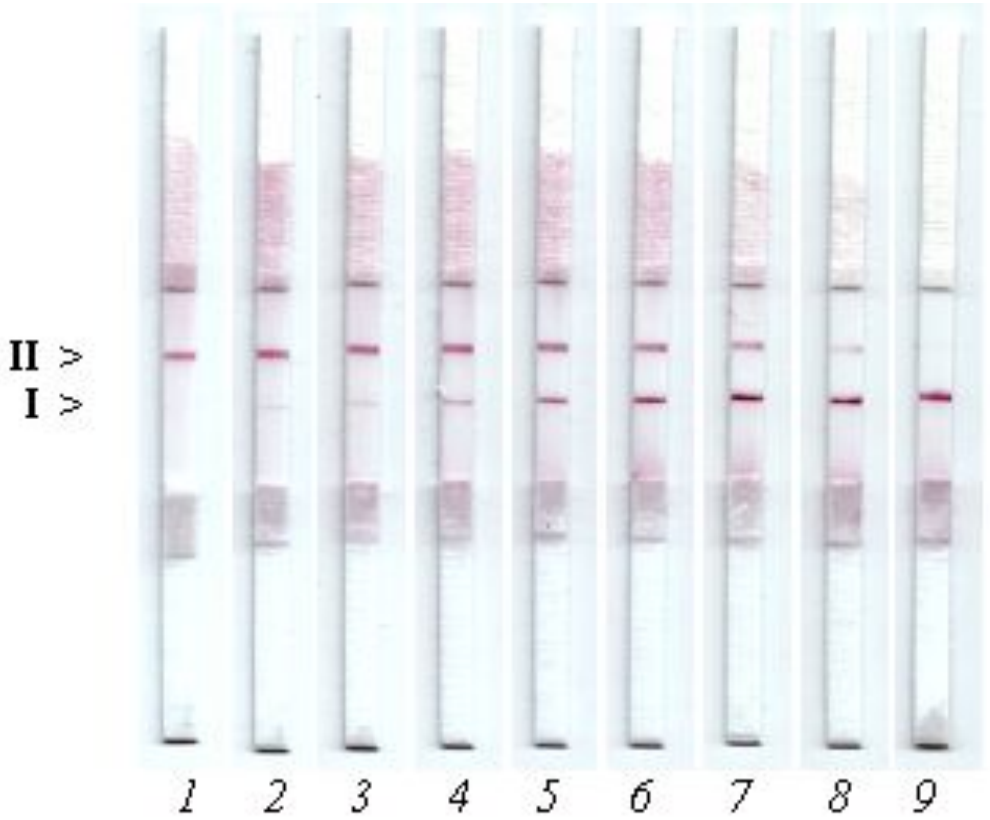
Презиционный диспенсер автоматический

Автоматический гильотинный резак

Вакуумный сушильный шкаф для упаковки тестов

Иммунохроматографическое определение антигена

(I – аналитическая зона, II – контрольная зона)



РЕЗУЛЬТАТЫ ИХА-ТЕСТА