Serological tests 2
(Antigen antibody interactions)
Lab 3

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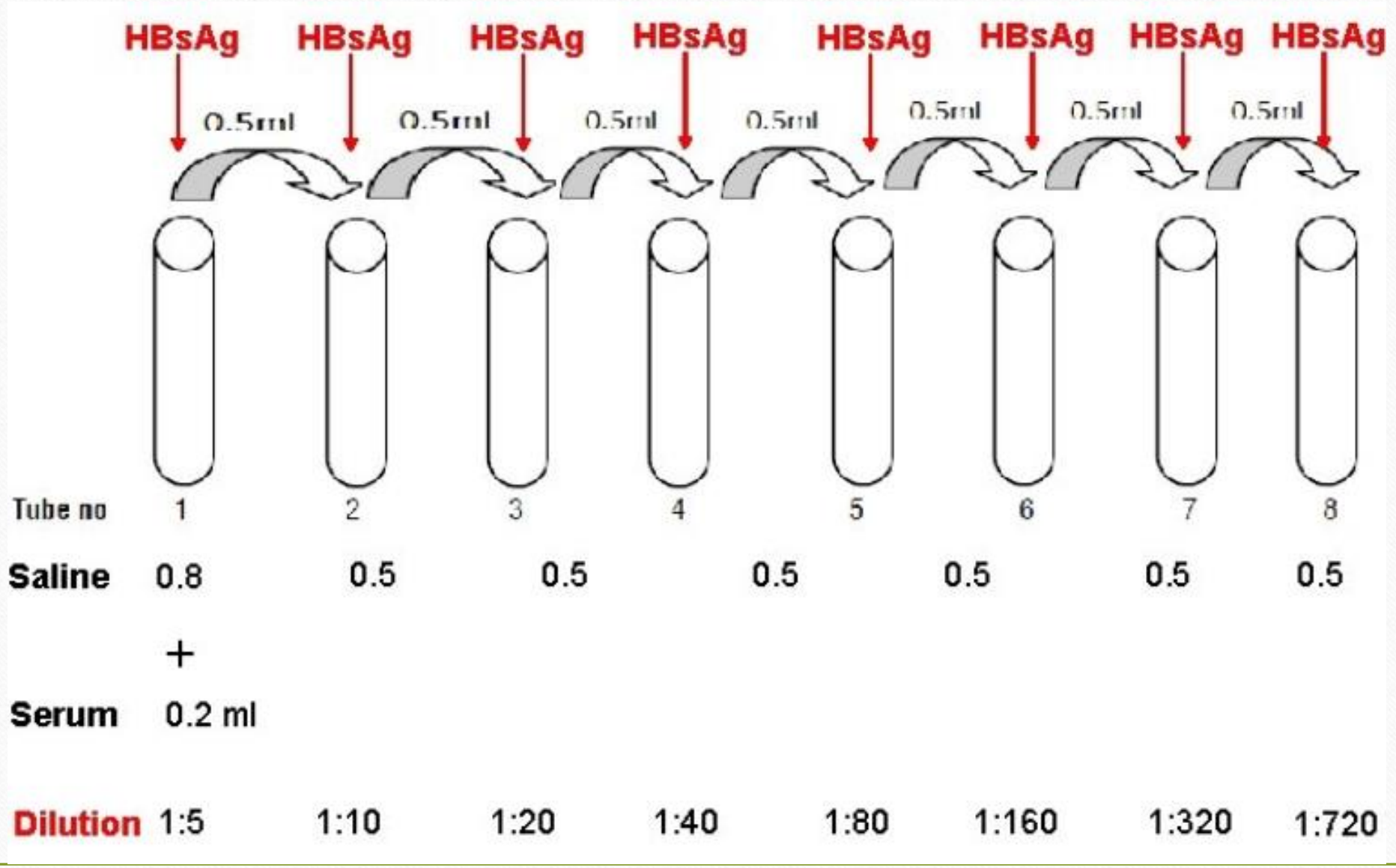
HCG Latex Agglutination Test


DIAGNOSTIC AUTOMATION, INC.
MICROWELL ELISA CE
Brucella IgG
96 Test
8C°
1501-8
BRG-196
2011-12


CODE
J79
PACK
25T
RHEUMATOID FACTOR
LATEX TEST KIT (R.A. TEST KIT)
Contents
Reagent 1 : RF Antigen (Gamma Globulin)
Reagent 2 : Positive Control
Reagent 3 : Negative Control
Accessories : Disposable Plastic Dropper, Disposable Applicator Sticks, Rubber Teat, Glass Slides
Shake well before use
CE
ISO 9001 & 13485
Certified Company
Mfg. Lic. No. G-639
BEACON
DIAGNOSTICS PVT. LTD.


ANTI – A, ANTI – B, ANTI - A
METHOD – IMMUNOAGGLUTINATION
PRODUCT CODE – AB10 | AB11
INTENDED USE: Test for qualitative detection of ABO a
INTRODUCTION
ANAMOL
BLOOD GROUPING
4 x 10 ML
Anamol Laboratories Pvt. Ltd.
ANAMOL
THE ORIGINAL MAKERS
Making Biochemical Formulations Since 1981
ANTI D
ANTI AB


HBsAg
HBsAg
HBsAg
HBsAg
HBsAg
HBsAg
HBsAg
HBsAg
0.5ml
0.5ml
0.5ml
0.5ml
0.5ml
0.5ml
0.5ml
Tube no 1 2 3 4 5 6 7 8
Saline 0.8 0.5 0.5 0.5 0.5 0.5 0.5
+
Serum 0.2 ml
Dilution 1:5 1:10 1:20 1:40 1:80 1:160 1:320 1:720

Precipitation Reaction Types

They are of mainly three types:

1. Precipitation in Solution

a) Ring Test.

b) Slide Test.

c) Tube Test

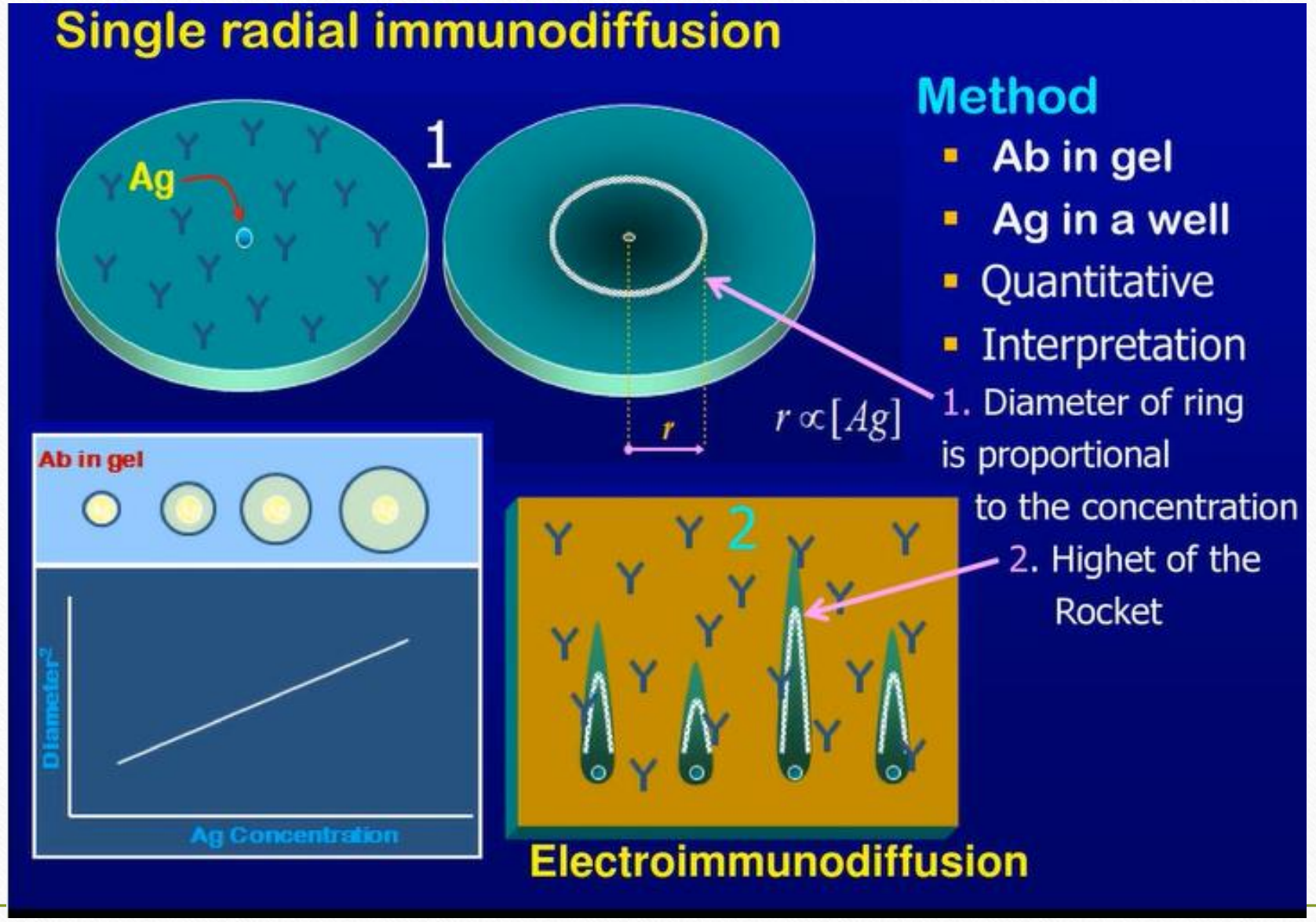
2. Precipitation in Agar.

a) Single diffusion immunodiffusion test (Mancini test)

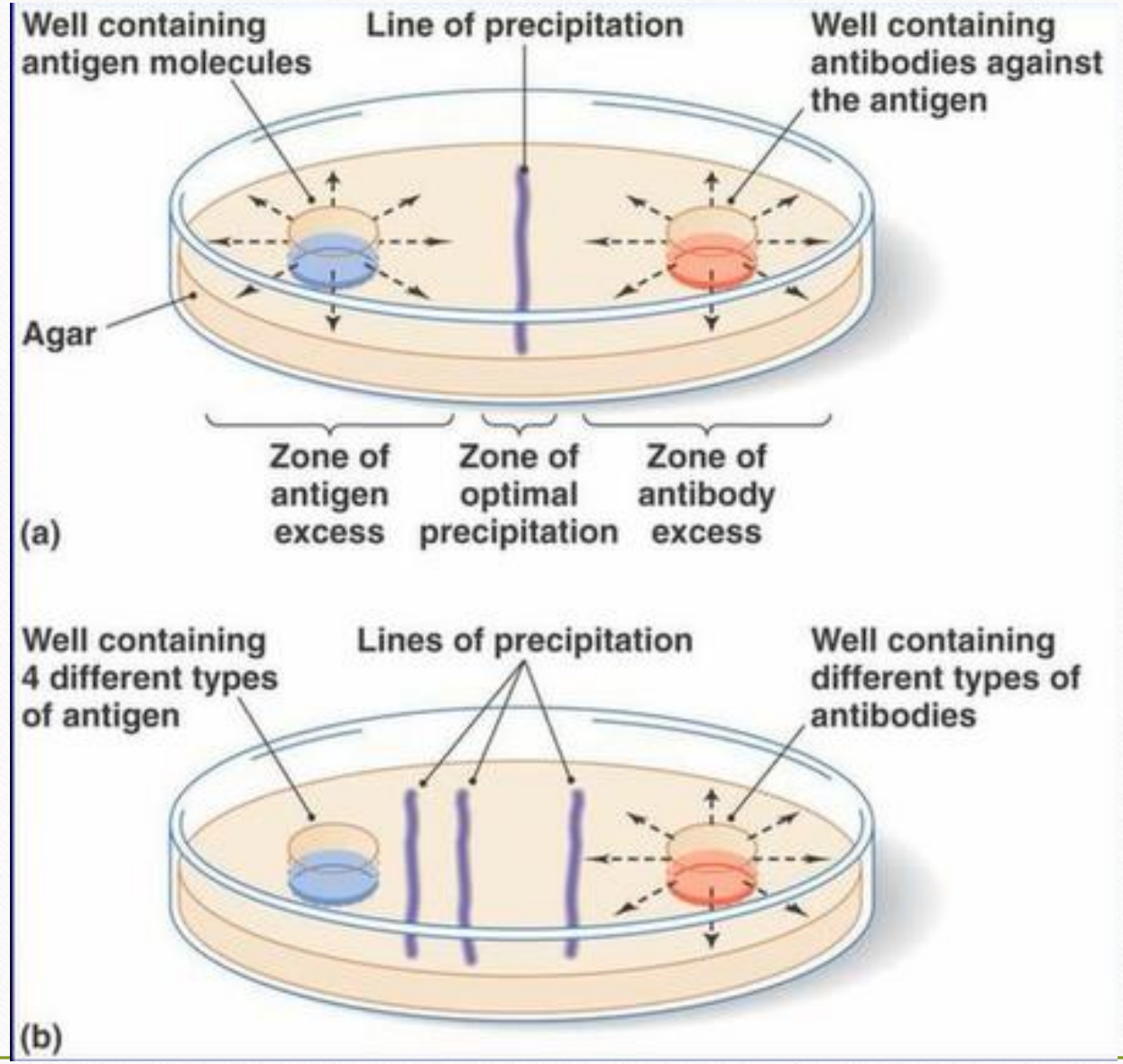
b) Double diffusion immunodiffusion test (Ouchterlony test)

3. Precipitation in Agar in an electric field (mmunoelectrophoresis).

Single

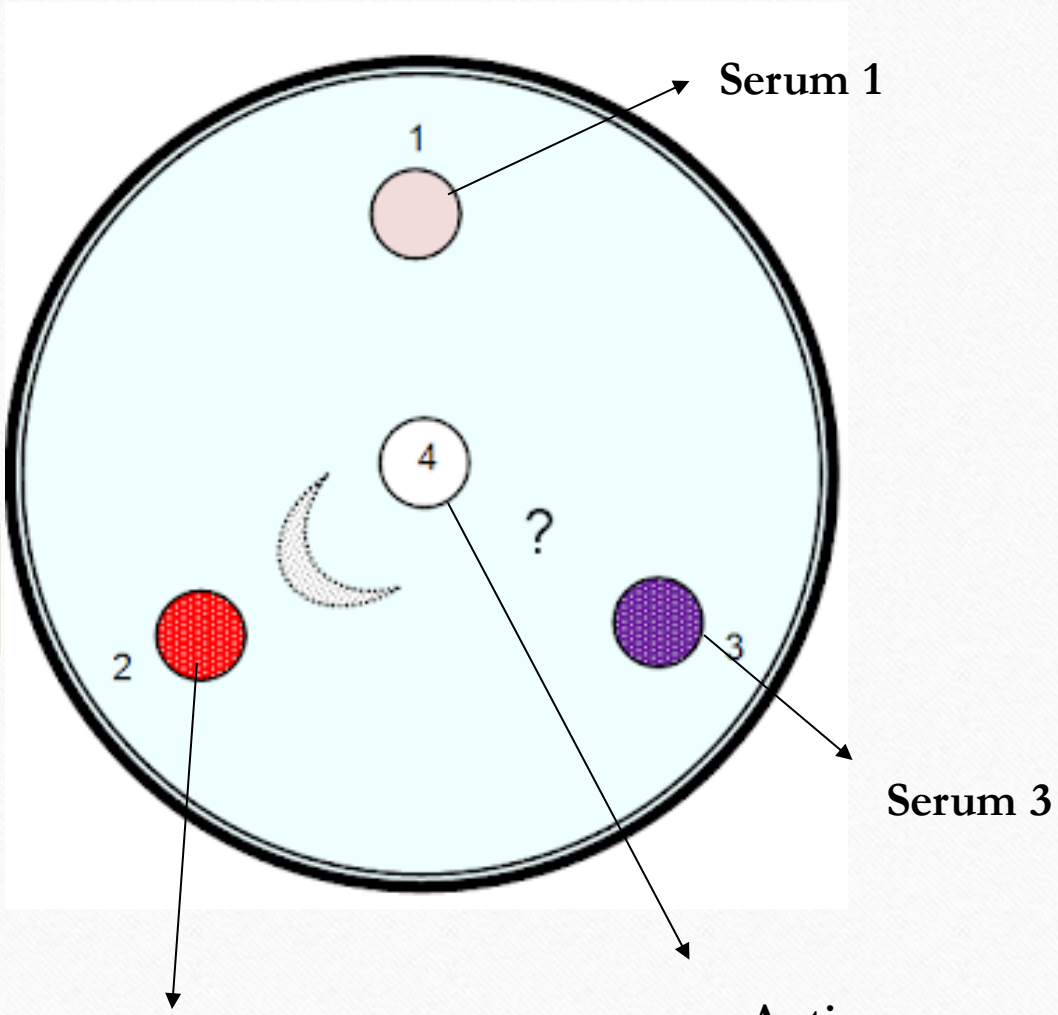

Single radial immunodiffusion
Ag
1
r
$r \propto [Ag]$
Method
Ab in gel
Ag in a well
Quantitative
Interpretation
1. Diameter of ring is proportional to the concentration
2. Highet of the Rocket
Ab in gel
Diameter²
Ag Concentration
2
Electroimmunodiffusion

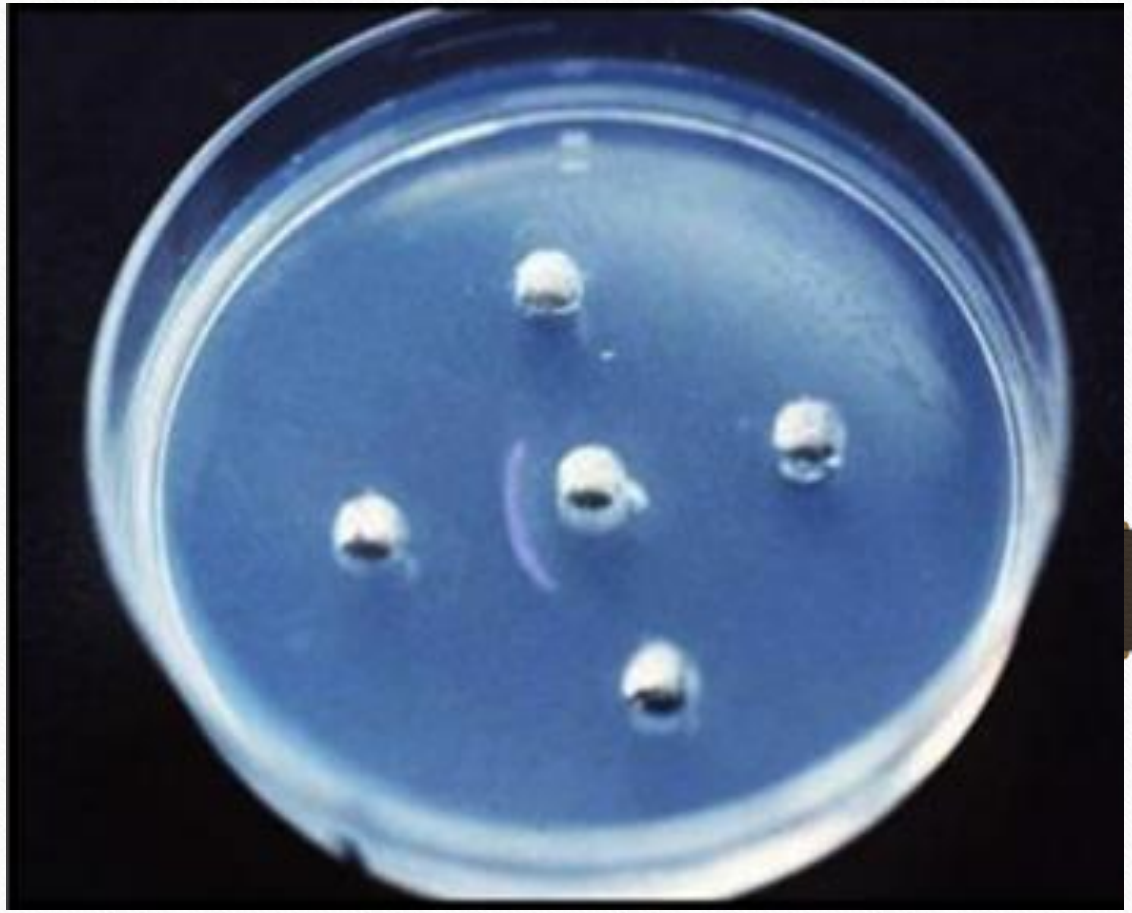
Double


Well containing antigen molecules
Line of precipitation
Well containing antibodies against the antigen
Agar
Zone of antigen excess
Zone of optimal precipitation
Zone of antibody excess
(a)
Well containing 4 different types of antigen
Lines of precipitation
Well containing different types of antibodies
(b)

Ouchterlony Gel Diffusion

- Both antigen and antibody can diffuse independently.
- Holes punched in agar.
- Known antibody or antigen added to center well.
- Known sample added to outer well.
- Unknown sample added to outer well next to unknown sample.
- Wait for bands to form.

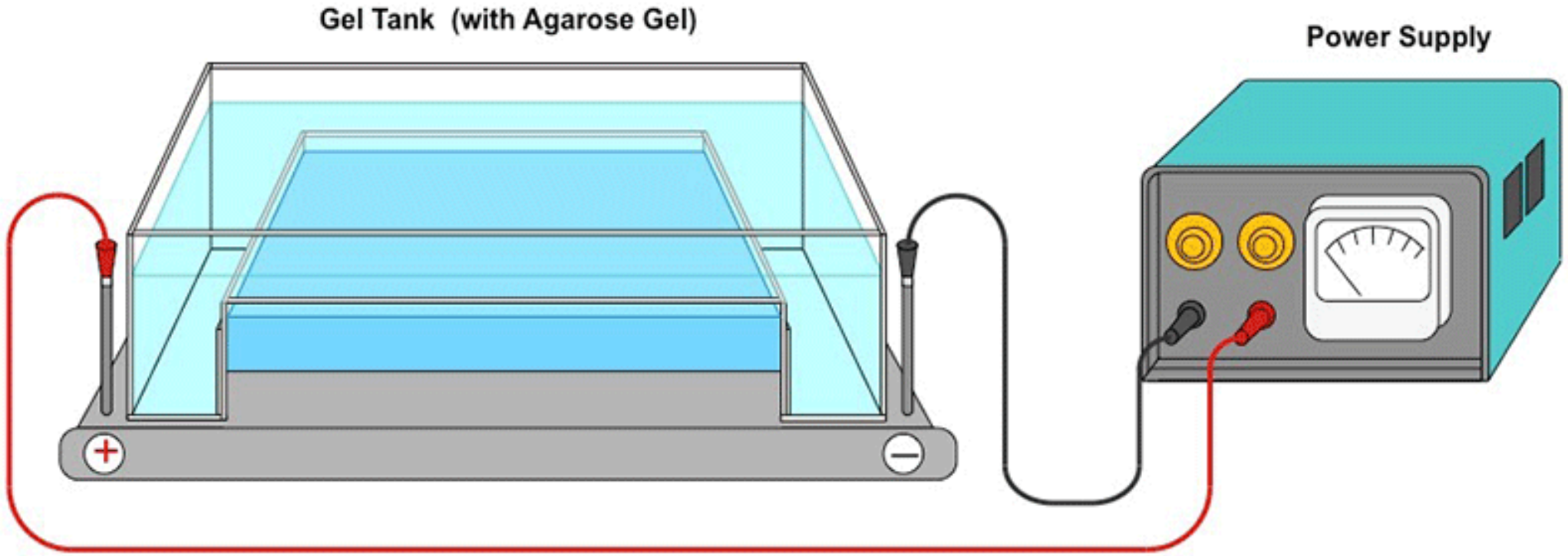

Serum 1
1
4
?
2
3
Serum 3
Antigen
Serum 2



Principle of Immunoelectrophoresis

- When an electric current is applied to a slide layered with gel, the antigen mixture placed in wells is separated into individual antigen components according to their charge and size.
- Following electrophoresis, the separated antigens are reacted with specific antisera placed in troughs parallel to the electrophoretic migration, and diffusion is allowed to occur. Antiserum present in the trough moves toward the antigen components resulting in the formation of separate precipitin lines in 18-24 hrs, each indicating reaction between individual proteins with their antibody.

Immunoelectrophoresis



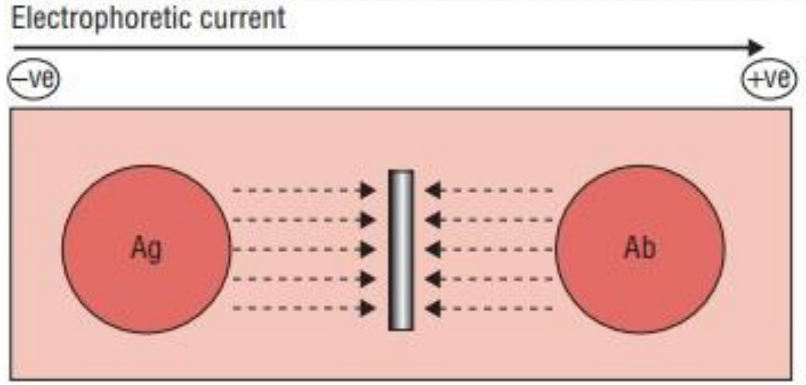


FIG. 14-6. Counter-current immunoelectrophoresis.

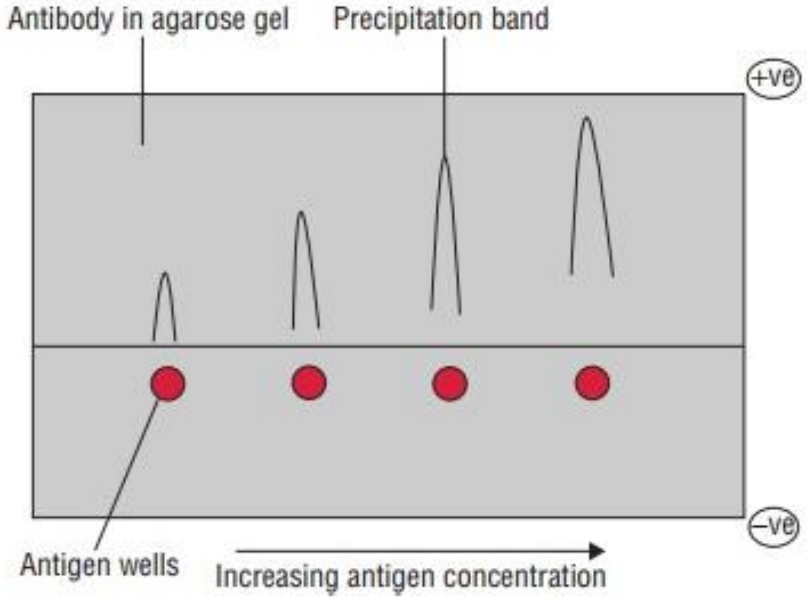


FIG. 14-7. Rocket electrophoresis.

Neutralization Tests

Neutralization is an antigen–antibody reaction in which the biological effects of viruses and toxins are neutralized by homologous antibodies known as neutralizing antibodies. These tests are broadly of two types:

(a) virus neutralization tests

(b) toxin neutralization tests.

Virus neutralization tests

- Neutralization of viruses by their specific antibodies are called virus neutralization tests. Inoculation of viruses in cell cultures, eggs, and animals results in the replication and growth of viruses. When virus-specific neutralizing antibodies are injected into these systems, replication and growth of viruses is inhibited.
- **Viral hemagglutination inhibition** test is an example of virus neutralization test frequently used in the diagnosis of viral infections, such as influenza, mumps, and measles. If patient's serum contains antibodies against certain viruses that have the property of agglutinating the red blood cells, these antibodies react with the viruses and inhibit the agglutination of the red blood cells.

Toxin neutralization tests

- Toxin neutralization tests are based on the principle that biological action of toxin is neutralized on reacting with specific neutralizing antibodies called antitoxins. Examples of neutralization tests include:
- ***In vivo***— Schick test to demonstrate immunity against diphtheria and *Clostridium welchii* toxin neutralization test in guinea pig or mice.
- ***In vitro***— (a) anti-streptolysin O test and (b) Nagler reaction used for rapid detection of *C. welchii*.

Immunofluorescence

The property of certain dyes absorbing light rays at one particular wavelength (ultraviolet light) and emitting them at a different wavelength (visible light) is known as fluorescence. Fluorescent dyes, such as fluorescein isothiocyanate and lissamine rhodamine, can be tagged with antibody molecules. They emit blue-green and orange-red fluorescence, respectively under ultraviolet (UV) rays in the fluorescence microscope. This forms the basis of the immunological test. Immunofluorescence tests have wide applications in research and diagnostics. These tests are broadly of two types:

1. Direct immunofluorescence test

2. Indirect immunofluorescence test

Direct immunofluorescence test is widely used for detection of bacteria, parasites, viruses, fungi, or other antigens in CSF, blood, stool, urine, tissues, and other specimens.

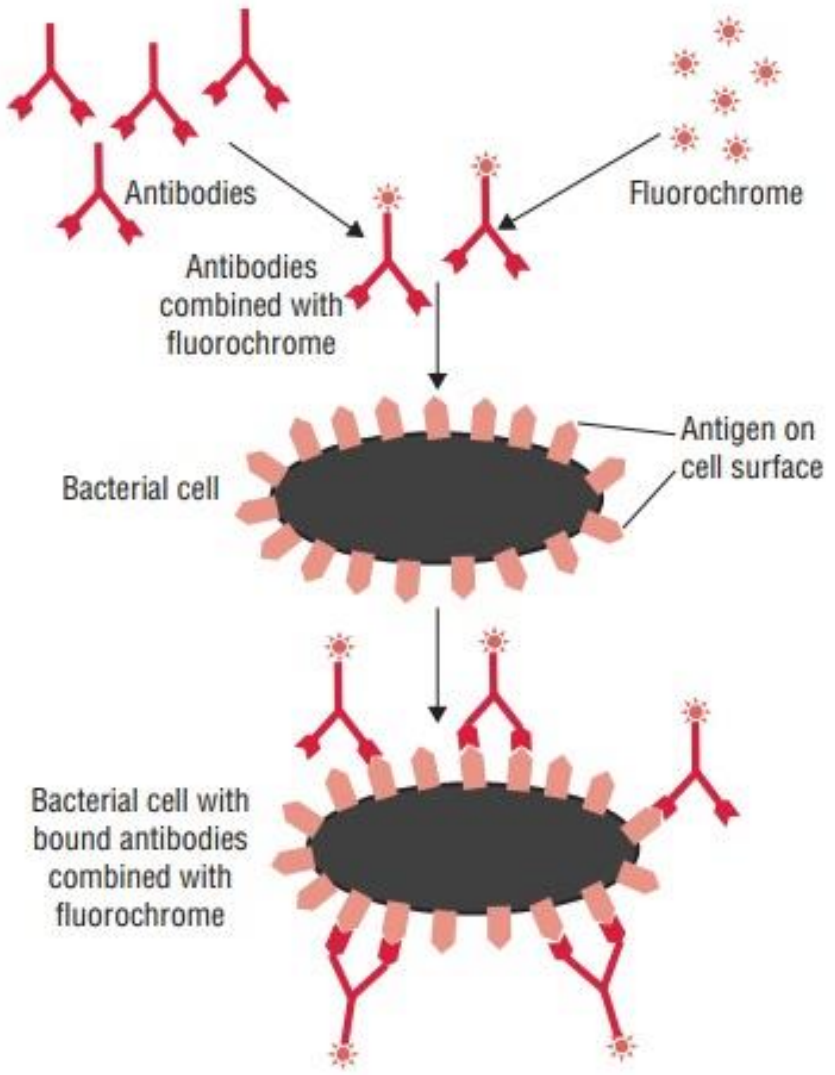


FIG. 14-13. Direct fluorescent antibody test.

Indirect immunofluorescence test

Indirect immunofluorescence is a two-stage process.

- **First stage**, a known antigen is fixed on a slide. Then the patient's serum to be tested is applied to the slide, followed by careful washing. If the patient's serum contains antibody against the antigen, it will combine with antigen on the slide.
- **Second stage**, the combination of antibody with antigen can be detected by addition of a fluorescent dye-labeled antibody to human IgG, which is examined by a fluorescence microscope.

Lab 4

Enzyme Immunoassays

- Enzyme immunoassays (EIAs) can be used for detection of either antigens or antibodies in serum and other body fluids of the patient.
- In EIA techniques, antigen or antibody labeled with enzymes are used. Alkaline phosphatase, horseradish peroxidase, and galactosidase are the enzymes used in the EIA tests.
- The commonly used EIAs are enzyme-linked immunosorbent assays (ELISAs).
- These assays involve the use of an immunosorbent specific to either the antigen or antibody. Following the antigen–antibody reaction, chromogenic substrate specific to the enzyme peroxidase, alkaline phosphatase, etc.) is added.
- The reaction is detected by reading the optical density.
- Usually, a standard curve based on known concentrations of antigen or antibody is prepared from which the unknown quantities are calculated.

There are different types of ELISA

(a) indirect ELISA,

(b) sandwich ELISA,

(c) competitive ELISA

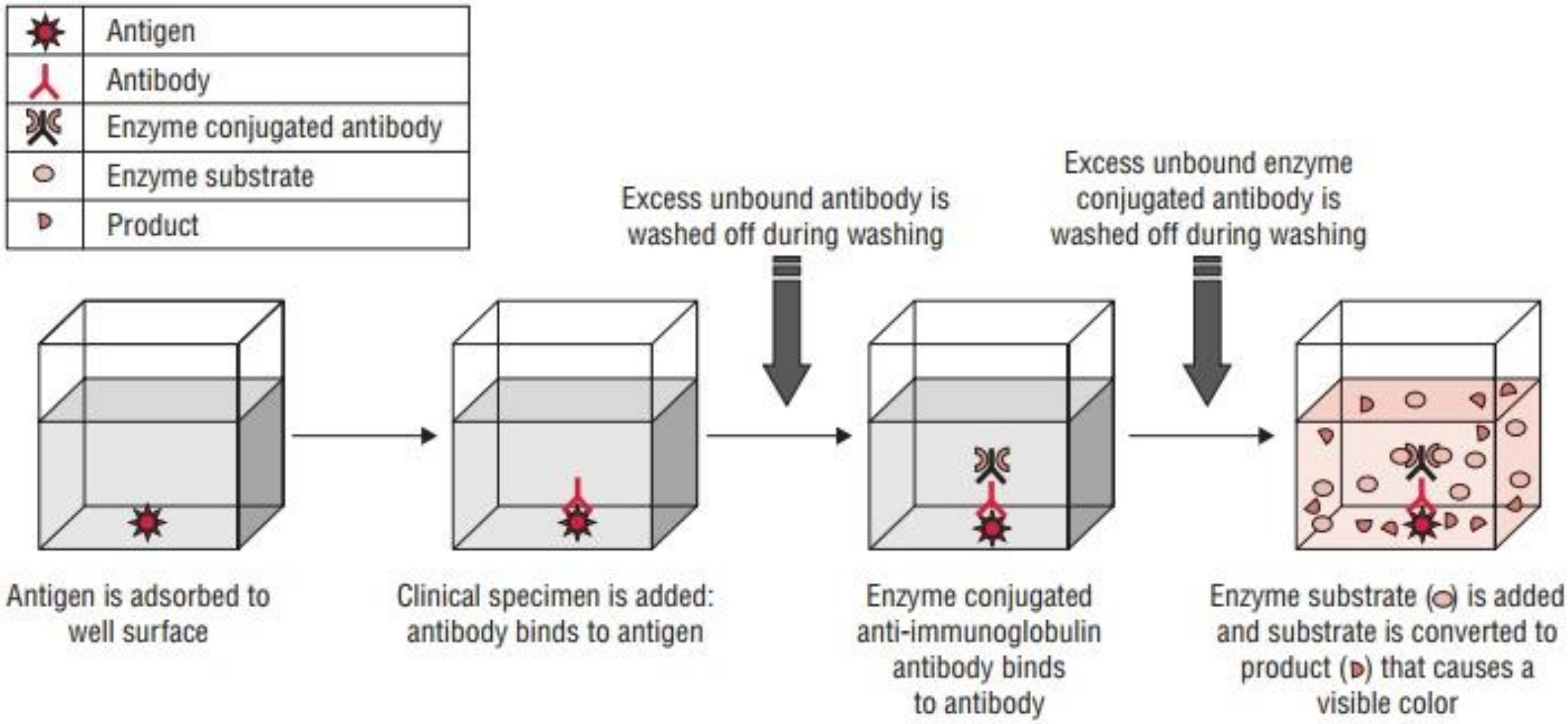


FIG. 14-14. Indirect ELISA test.

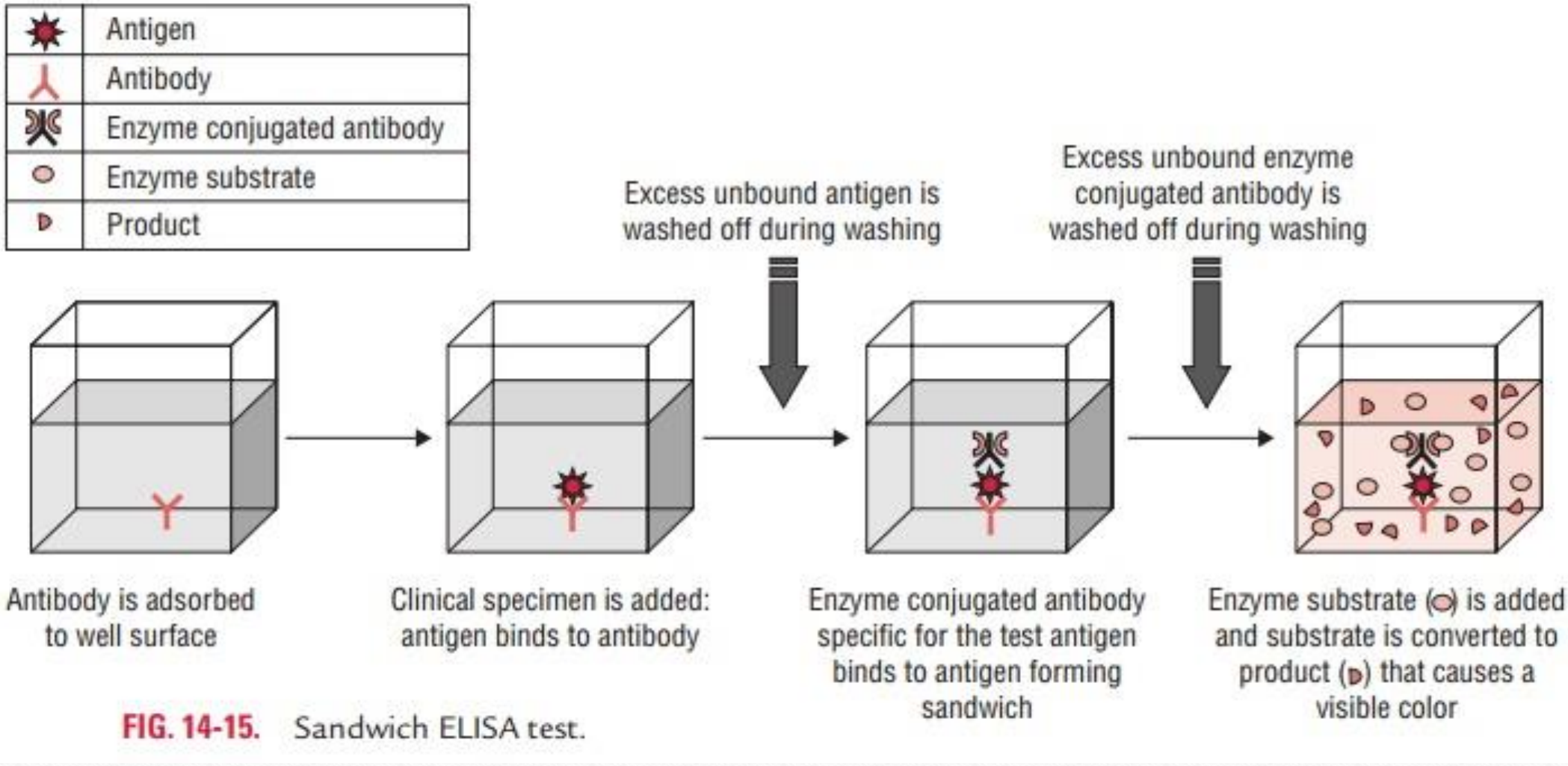


FIG. 14-15. Sandwich ELISA test.

Competitive ELISA

- Used for the estimation of antibodies present in a specimen, such as serum.
- Principle of the test is that two specific antibodies, one conjugated with enzyme and the other present in test serum (if serum is positive for antibodies), are used.
- Competition occurs between the two antibodies for the same antigen.
- Appearance of color indicates a negative test (absence of antibodies), while the absence of color indicates a positive test (presence of antibodies).

In this test, the microtiter wells are coated with HIV antigen.

- The sera to be tested are added to these wells and incubated at 37°C and then washed.
- If antibodies are present in the test serum, antigen–antibody reaction occurs.
- The antigen– antibody reaction is detected by adding enzyme-labeled-specific HIV antibodies.
- In a positive test, no antigen is left for these antibodies to act.
- Hence, the antibodies remain free and are washed away during the process of washing.
- When substrate is added, no enzyme is available to act on it.
- Therefore, positive result indicates no color reaction. In a negative test, in which no antibodies are present in the serum, antigen in the coated wells is available to combine with enzyme-conjugated antibodies and the enzyme acts on the substrate to produce color.