SEROLOGICAL DIAGNOSIS OF VIRAL INFECTIONS:

POSSIBILITIES OF SEROLOGICAL DIAGNOSIS

TYPES OF SEROLOGICAL REACTIONS
Ag-Ab reactions used for the detection of unknown Ag or Ab, in vitro

In virology:

**Viral (direct) diagnosis:**
- detection of viral antigens

**Serological (indirect) diagnosis:**
- detection of specific antiviral antibodies
POSSIBILITIES OF SEROLOGICAL DIAGNOSIS

Ø Qualitative detection of total specific antiviral antibodies

Ø Quantification of total specific antiviral antibodies (titer of Abs)
  1/2  1/4  1/8  1/16  1/32  1/64
POSSIBILITIES OF SEROLOGICAL DIAGNOSIS

Detection of Ab classes (qualitative and quantitative)
POSSIBILITIES OF SEROLOGICAL DIAGNOSIS

Ø Detection of Ab synthetized to each viral protein (Ag) of specific molecular weight - serological profile
POSSIBILITIES OF SEROLOGICAL DIAGNOSIS

Ø Determination of specific antibody avidity
Types of serological reactions

I – detection of Ab that block biological functions of the virus
   Neutralization test
   Haemagglutination Inhibition Test

II – based on biological activity of Abs in complex with Ags
   Immunoagglutination
   Complement Fixation Test (CFT)

III – based on application of labeled antibodies
   EIA (ELISA)
   Immunofluorescence
   RIA

IV – determination of serological profile
   Western blot
I – Ab that block biological functions of the virus

Neutralization Test  →  neutralizing Ab
(Abs that block viral infectivity)

neutralizing Abs bind to viral anti-receptors

viral suspension  +  patient’s serum  →  inoculation in the cell culture

No CPE!!
Titer of Ab → reciprocal value of the highest dilution of patient’s serum that gives positive reaction

Multiple (twofold, fourfold, tenfold...) serial dilutions of patient’s serum:

Titer of neutralizing Ab is ?
INHIBITION OF HAEMAGGLUTINATION

I – Ab that block biological functions of the virus

Haemagglutination Inhibition Test → neutralizing Ab
(Abs that block viral haemagglutination)

Virus + Patient’s serum + RBC → INHIBITION OF
HAEMAGGLUTINATION
Haemagglutination Inhibition Test

Titer of Ab → reciprocal value of the highest dilution of patient’s serum that gives positive reaction

Multiple (twofold, fourfold, tenfold...) serial dilutions of patient’s serum:

Titer of haemagglutinating Ab is?
**Haemagglutination Inhibition Test**

**Procedure:**

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<tr>
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<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
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**Incubation at 37°C**

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<th><strong>A1</strong></th>
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<th><strong>A3</strong></th>
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<tr>
<td>Negative Control</td>
<td>Positive Control</td>
<td>50 µl saline + 50 µl serum</td>
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</tbody>
</table>
II – based on biological activity of Abs in complex with Ags

Immunoagglutination
- Reaction between corpuscular Ag and specific Ab, manifested as forming of agglutinates

\[
\text{Latex particle coated with viral Ag} + \text{Patient's serum} = \text{Indirect immunoagglutination - qualitative}
\]
Immunoagglutination

Indirect immunoagglutination - qualitative
II – based on biological activity of Abs in complex with Ags

Complement Fixation Test (CFT)

First step

Second step

Result

= antigen

Y = antibody

= complement

No haemolysis

Haemolysis

Sensitized RBC
Complement Fixation Test (CFT)

Titer of Ab → reciprocal value of the highest dilution of patient’s serum that gives positive reaction

Multiple (twofold, fourfold, tenfold...) serial dilutions of patient’s serum:

Titer of Ab is?
III – based on application of labeled antibodies

ELISA – Enzyme Linked ImmunoSorbent Assay
ELISA

Diagram:

- Viral antigens
- IgM
- Enzyme conjugated anti-IgM Ab
IV - determination of serological profile

Western blot as serological reaction: confirmation test (HIV)

Serological profile - presence of Ab specific for different viral Ag
Western Blot for HIV

3 genes:
- GAG
- POL
- ENV

Positive

At least 1 Ab for products of all 3 major genes (3)
(CDC) Two Ab for any of the 3 major Ag: p24, gp41, gp120/160 (2)
AVIDITY OF ANTIBODIES

**Avidity** - strength of bond between Ab and Ag

* low-avidity antibodies (primary immune response)

* high-avidity antibodies (memory immune response)