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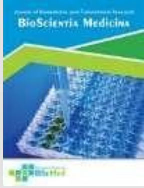

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Enzyme Linked Immunosorbent Assay (ELISA) Technique Guideline

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ABSTRACT

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ELISA is generally carried out on a *plate* containing 96 wells, where the antibody binding process with protein will occur. This very simple process makes ELISA easy to do. Ease of ELISA for washing, making it easier to clean the following materials when tested. This will make testing with ELISA specific.

The ELISA examination procedure begins with the process of attaching *(coating)* the antigen and / or antibody to the surface of the well on the *plate.* Furthermore, the blocking step is carried out (blocking) antigen and antibody bonds at the *unspecific-site* with a *blocking agent.* After incubation and washing, the plates were incubated with enzyme-bound antibodies. Next, washing the plates was carried out and continued with the addition of the substrate so that the color change would be produced and the OD *(optical density)* value was read with an ELISA reader.

The washing stage is an important one to remove antibodies that are not bound to antigens. In addition, make sure that no washing liquid is left on the *plate,*

because it is feared that it will affect the next stage of the inspection.

Terminology

- ELISA *plate*: a container for antigen-antibody collection, which generally contains 96 test wells.
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- Antibody: A protein that binds to an antigen. If we are going to assess IL-12 levels using ELISA from a serum sample, then an antibody will be added, then the antibody is a protein that will bind to IL-12, or known as anti-IL-12.
- Standard: A protein that will be graded, which has known levels. If we are going to assess IL-12 levels using ELISA from serum samples, the standard is the IL-12 protein that has known levels, for example the standard IL-12 level is 100 pg / mL. Generally, this standard will be made into several concentrations, so that you will get a graph depicting the standard VS the OD value of the standard. The graph will be used to calculate the levels in the sample.
- OD *(Optical Density)* value: a value that describes the intensity of the color change on ELISA
- ELISA reader: Tool used to get the OD value from an ELISA examination.

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There are several types of ELISA, namely: *direct, indirect, sandwich or competitive.* In the immobilization stage the antigen can be attached directly to the p*late* or indirectly to the *plate,* by binding with antibodies that have been attached to the bottom of the *plate.* Furthermore, the antigen is detected directly with the primary antibody bound to the enzyme or the antigen is detected indirectly with the secondary antibody that is bound to the primary antibody and the enzyme. Enzymes that are generally bound to antibodies include: alkaline phosphatase (AP) or horseradish peroxidase (HRP).

1. Direct ELISA

In this type, the antigen is attached to the bottom of the *plate*, then the antigen will be detected through the antibody bound to the enzyme.

Advantages:

- Examination with this method is faster
- Cross-reaction with secondary antibodies can be eliminated

Disadvantages:

- The resulting signal amplification is weak
- Lack of flexibility in selecting enzyme labeled primary antibodies
- There may be reactions between primary antibodies and enzymes bound to these primary antibodies.

2. Indirect ELISA

In this method, the antigen is attached to the base of the *plate*, then, the primary antibody which is not labeled with the enzyme is inserted. Next, put back the enzyme labeled secondary antibody, which will bind to the primary antibody.

Advantages:

- The sensitivity of the test is increased with the use of primary and secondary antibodies.

Disadvantages:

- Cross-reactions can occur with secondary antibodies which will result in a non-specific signal.
- Longer incubation time is required
- The cost required is greater than the direct method

3. ELISA sandwich

In this method, the antibody is first attached to the base of the *plate.* Next, the test sample (antigen) is inserted into the well on the *plate,* then a secondary antibody bound to the enzyme is inserted into the well on the *plate.*

Advantages:

- Has high specificity
- Suitable for use with less pure samples

Disadvantages:

- The cost is quite large because it uses two antibodies

4. Competitive ELISA

Competitive ELISA is an ELISA examination method where there is a competitive reaction between the sample antigen and antigen bond that is attached to the bottom of the *plate* well with the primary antibody. In this method, the non-sample antigen is attached to the bottom of the *plate*. Next, the sample antigen and primary antibody are inserted into the well. Then put secondary antibodies that are bound to the enzyme in the wells on the *plate*.

ELISA data interpretation

The final result of the ELISA examination will be obtained the OD value *(optical density)*, however the OD value is not the level of the ELISA examination, it is still necessary to process the OD value data. The first thing to do is to make a standard OD value curve VS standard content, so that the line equation will be obtained from the curve. The equation obtained will be used to convert the OD value of the sample into a grade value.

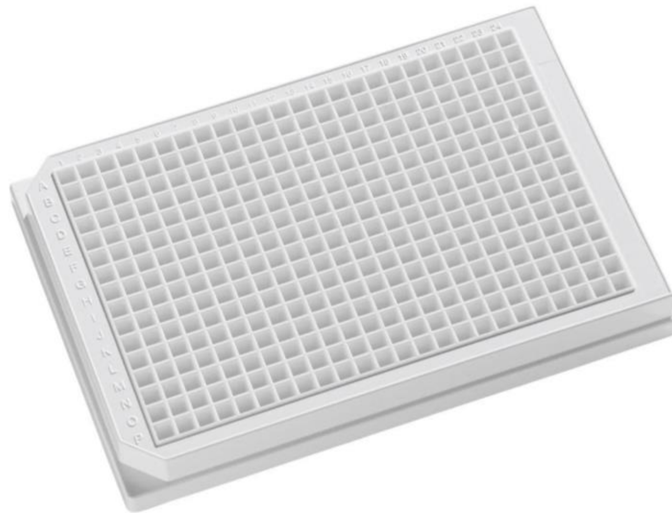
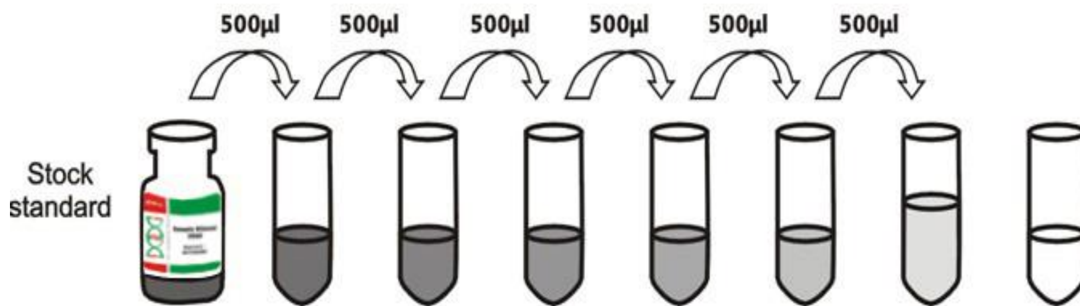


Figure 1. ELISA plate

Figure 2. ELISA antibody

Figure 3. Standard ELISA



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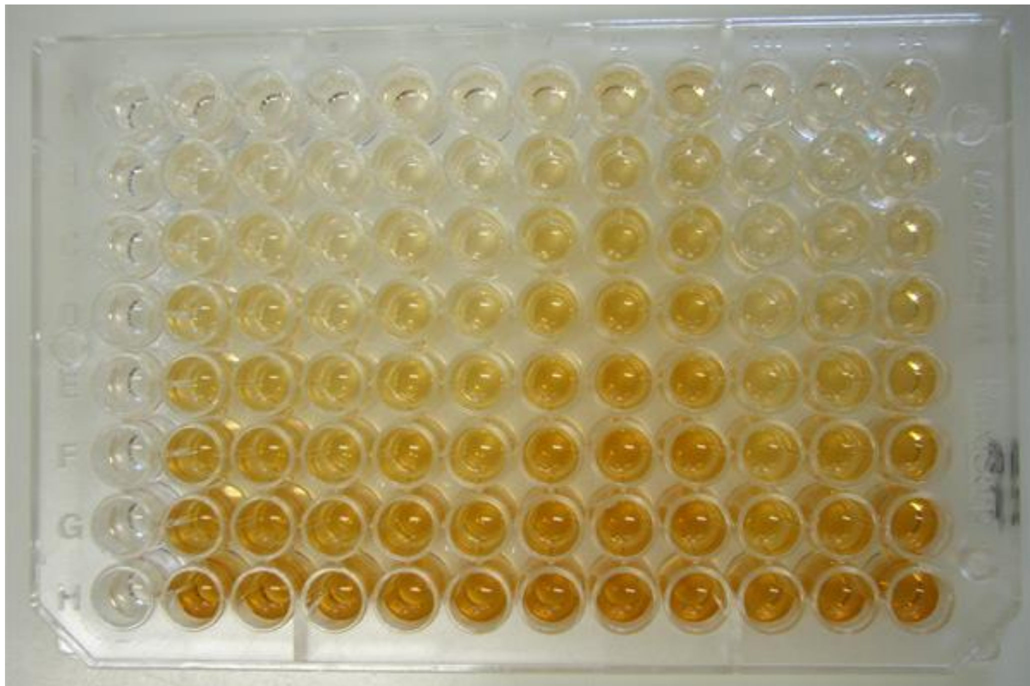

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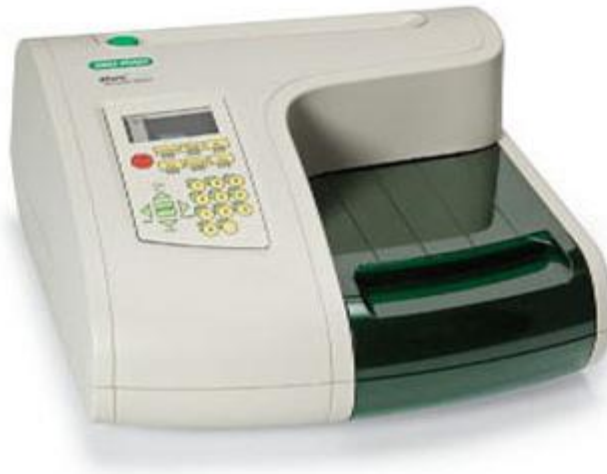
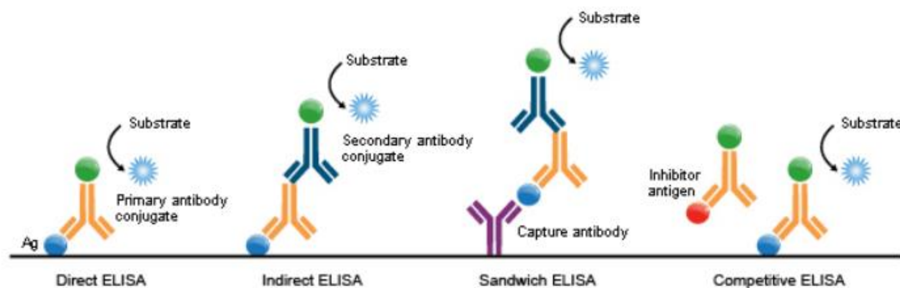


Figure 6. ELISA reader



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Submission acknowledgement

Dear author(s),

Rachmat Hidayat*, Patricia Wulandari has submitted the manuscript "Enzyme Linked Immunosorbent Assay (ELISA) Technique Guideline" to Bioscientia Medicina: Journal of Biomedicine and Translational Research. The paper will be screened by editor and reviewed by peer review.

Cordially,

Prof. Paula Magnano, PhD

Editor

(*) Corresponding author

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Peer Review Results

Dear author(s),

Rachmat Hidayat*, Patricia Wulandari has submitted the manuscript “Enzyme Linked Immunosorbent Assay (ELISA) Technique Guideline“ to Bioscientia Medicina: Journal of Biomedicine & Translational Research. The decision : Revision Required.

Cordially,

Prof. Paula Magnano, PhD

Editor

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Reviewer 1: Revision required

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- Has high specificity
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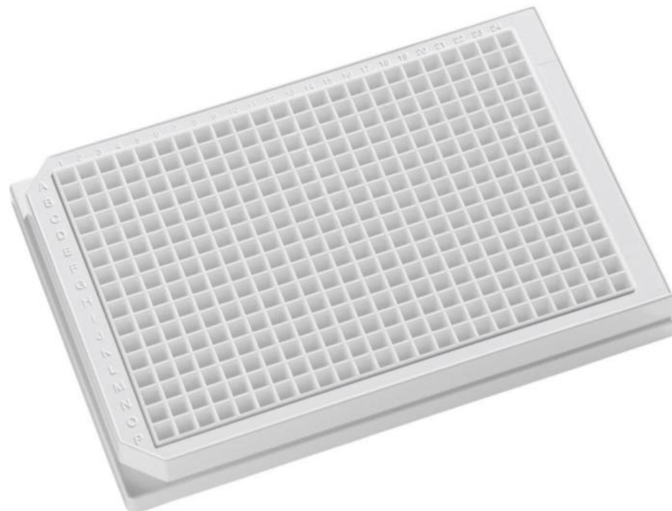

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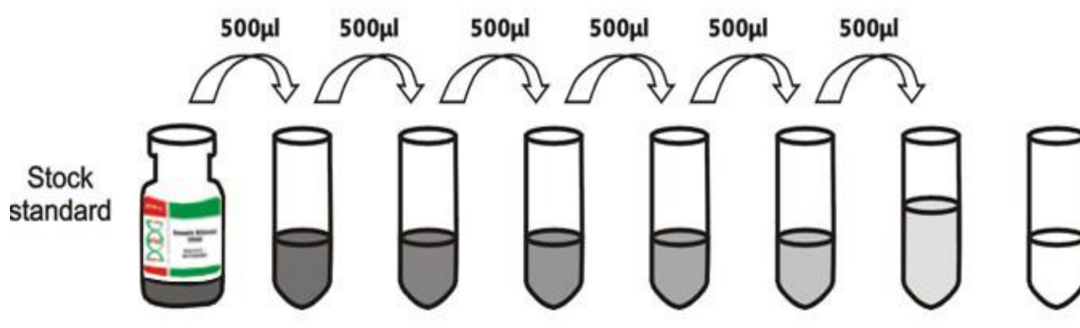
Figure 2. ELISA antibody

Figure 3. Standard ELISA



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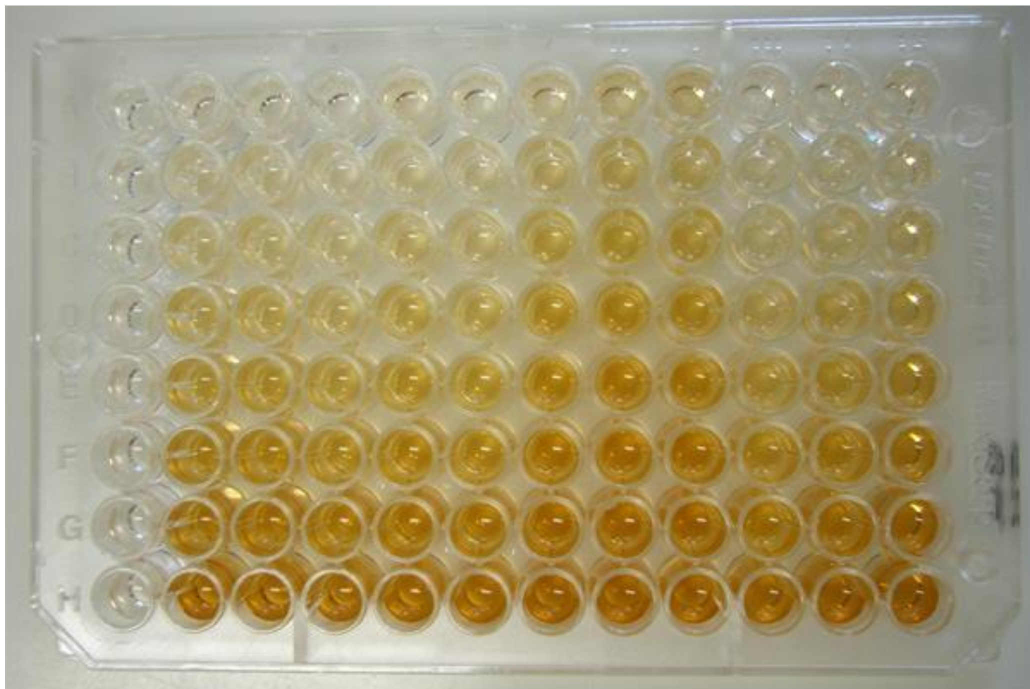

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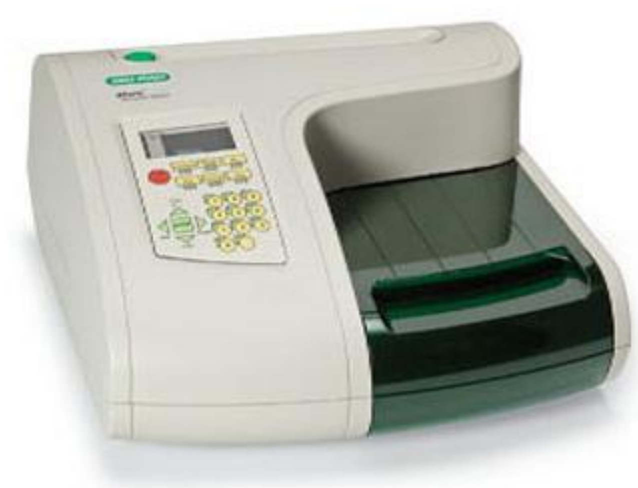
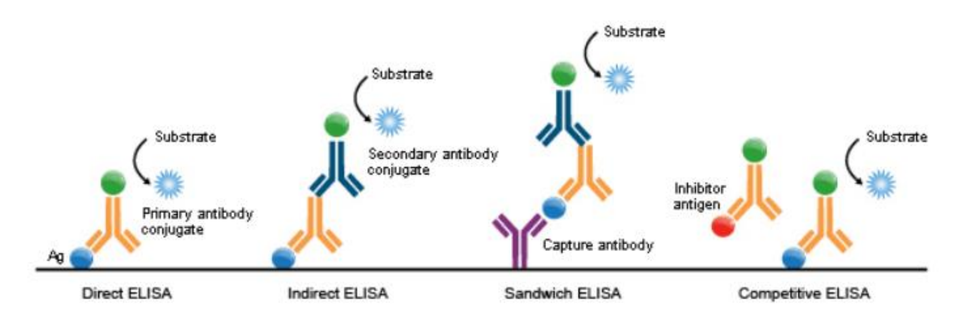


Figure 6. ELISA reader



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Reviewer Comment:

1→ Title of Manuscripts should be explained main review and declared type of literature review: narrative or systematic review.
2→ Keywords should be showed the main words of the study, the authors can use MeSH to develop keywords.
3→ Abstract should be showed the main of background, main of review and conclusion of study.
4→Introduction should be showed the urgency of study (epidemiology data), biological plausibility concept, and lack of knowledge in the study.
5→ Conclusion should more specific and not more showed more review.
6→ Authors must check the references for make update references. References should no more than 10 years.

Reviewer 2: Revision required

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Enzyme Linked Immunosorbent Assay (ELISA) Technique Guideline

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A R T I C L E I N F O

Keywords:

ELISA
Antigen
Antibody
Guideline

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A B S T R A C T

ELISA (Enzyme-linked immunosorbent assay) is a technique used to assess the quantification of peptide, protein, antibody and hormone levels, based on the principle of antigen-antibody binding. In the ELISA technique, antigen immobilization will be carried out on a solid surface, then bound with antibodies to form an antigen-antibody bond complex, where the antigen-antibody complex is bound to the enzyme. The detection signal in the form of a color change will be formed due to the reaction between the enzyme and the substrate.

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ELISA is generally carried out on a *plate* containing 96 wells, where the antibody binding process with protein will occur. This very simple process makes ELISA easy to do. Ease of ELISA for washing, making it easier to clean the following materials when tested. This will make testing with ELISA specific.

The ELISA examination procedure begins with the process of attaching *(coating)* the antigen and / or antibody to the surface of the well on the *plate.* Furthermore, the blocking step is carried out (blocking) antigen and antibody bonds at the *unspecific-site* with a *blocking agent.* After incubation and washing, the plates were incubated with enzyme-bound antibodies. Next, washing the plates was carried out and continued with the addition of the substrate so that the color change would be produced and the OD *(optical density)* value was read with an ELISA reader.

The washing stage is an important one to remove antibodies that are not bound to antigens. In addition, make sure that no washing liquid is left on the *plate,* because it is feared that it will affect the next stage of the inspection.

Terminology

- ELISA *plate*: a container for antigen-antibody collection, which generally contains 96 test wells.
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- Antibody: A protein that binds to an antigen. If we are going to assess IL-12 levels using ELISA from a serum sample, then an antibody will be added, then the antibody is a protein that will bind to IL-12, or known as anti-IL-12.
- Standard: A protein that will be graded, which has known levels. If we are going to assess IL-12 levels using ELISA from serum samples, the standard is the IL-12 protein that has known levels, for example the standard IL-12 level is 100 pg / mL. Generally, this standard will be made into several concentrations, so that you will get a graph depicting the standard VS the OD value of the standard. The graph will be used to calculate the levels in the sample.
- OD *(Optical Density)* value: a value that describes the intensity of the color change on ELISA
- ELISA reader: Tool used to get the OD value from an ELISA examination.

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There are several types of ELISA, namely: *direct, indirect, sandwich or competitive.* In the immobilization stage the antigen can be attached directly to the p*late* or indirectly to the *plate,* by binding with antibodies that have been attached to the bottom of the *plate.* Furthermore, the antigen is detected directly with the primary antibody bound to the enzyme or the antigen is detected indirectly with the secondary antibody that is bound to the primary antibody and the enzyme. Enzymes that are generally bound to antibodies include: alkaline phosphatase (AP) or horseradish peroxidase (HRP).

1. **Direct ELISA**

In this type, the antigen is attached to the bottom of the *plate*, then the antigen will be detected through the antibody bound to the enzyme.

Advantages:

- Examination with this method is faster
- Cross-reaction with secondary antibodies can be eliminated

Disadvantages:

- The resulting signal amplification is weak
- Lack of flexibility in selecting enzyme labeled primary antibodies
- There may be reactions between primary antibodies and enzymes bound to these primary antibodies.

2. **Indirect ELISA**

In this method, the antigen is attached to the base of the *plate*, then, the primary antibody which is not labeled with the enzyme is inserted. Next, put back the enzyme labeled secondary antibody, which will bind to the primary antibody.

Advantages:

- The sensitivity of the test is increased with the use of primary and secondary antibodies.

Disadvantages:

- Cross-reactions can occur with secondary antibodies which will result in a non-specific signal.
- Longer incubation time is required
- The cost required is greater than the direct method

3. **ELISA sandwich**

In this method, the antibody is first attached to the base of the *plate.* Next, the test sample (antigen) is inserted into the well on the *plate*, then a secondary antibody bound to the enzyme is inserted into the well on the *plate.*

Advantages:

- Has high specificity
- Suitable for use with less pure samples

Disadvantages:

- The cost is quite large because it uses two antibodies

4. Competitive ELISA

Competitive ELISA is an ELISA examination method where there is a competitive reaction between the sample antigen and antigen bond that is attached to the bottom of the *plate* well with the primary antibody. In this method, the non-sample antigen is attached to the bottom of the *plate.* Next, the sample antigen and primary antibody are inserted into the well. Then put secondary antibodies that are bound to the enzyme in the wells on the *plate.*

ELISA data interpretation

The final result of the ELISA examination will be obtained the OD value *(optical density),* however the OD value is not the level of the ELISA examination, it is still necessary to process the OD value data. The first thing to do is to make a standard OD value curve VS standard content, so that the line equation will be obtained from the curve. The equation obtained will be used to convert the OD value of the sample into a grade value.

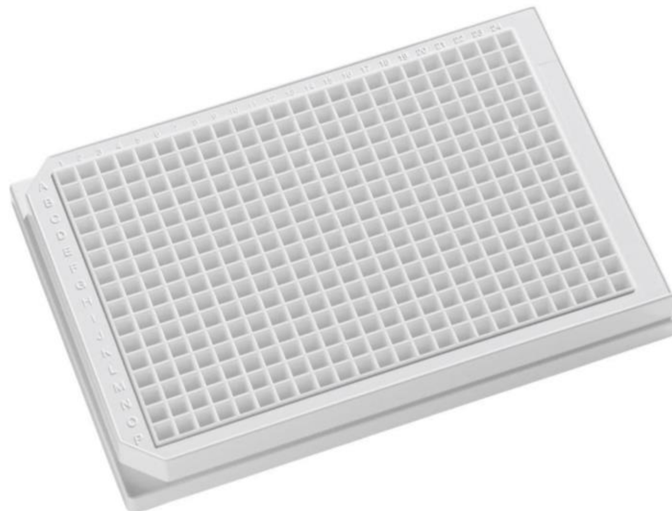

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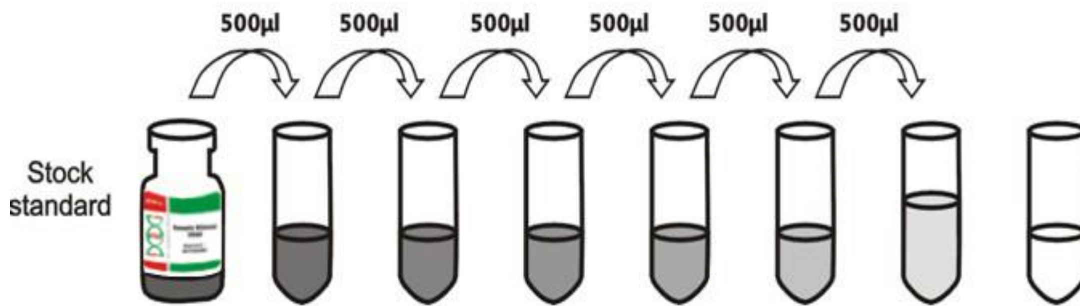
Figure 2. ELISA antibody

Figure 3. Standard ELISA



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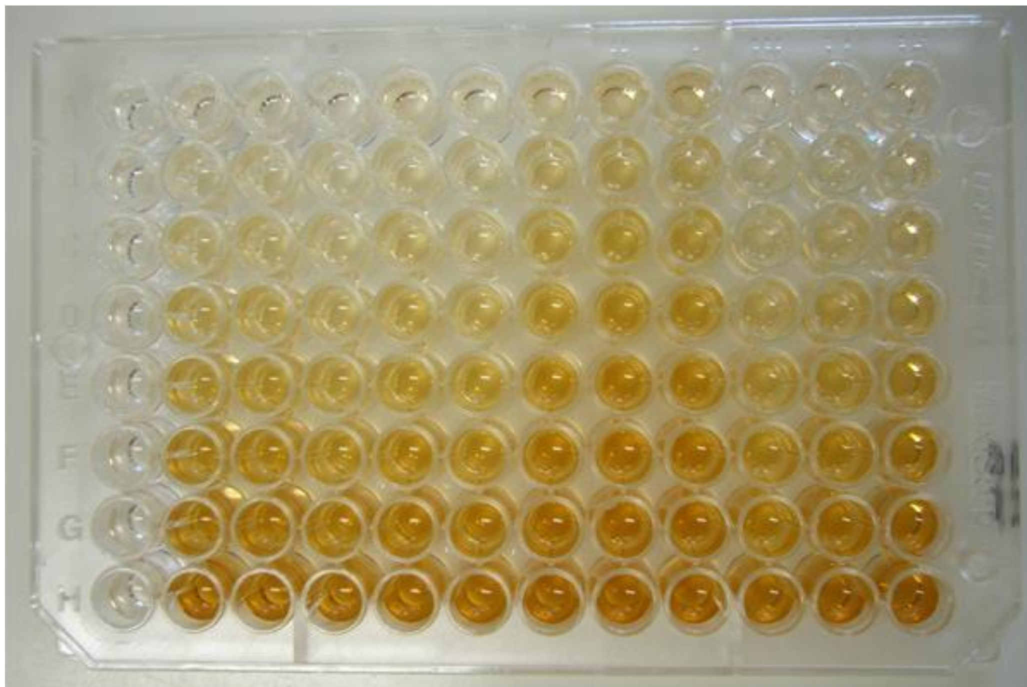


Figure 5. Changes in Color Intensity on ELISA Plate

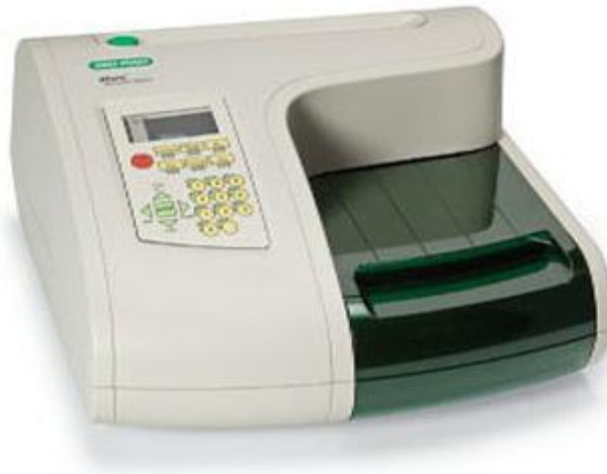
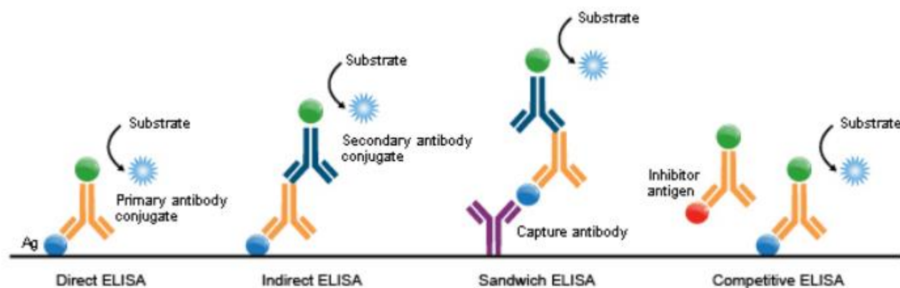


Figure 6. ELISA reader



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Reviewer Comment:

a. Authors should make more specific and attractive. Authors also should be declared the type of study clearly.
b. Authors should make abstract more specific and try to show the main concept of the review.
c. Authors should develop introduction more attractive. Authors should be tried to show the urgency by epidemiology data. Authors also should be developed main sub tittle of review in systematic, constructive and specific. Authors not only showed the standar review and no focus, try to focused in your review.
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ARTICLE INFO

Keywords:
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All authors have reviewed and approved the final version of the manuscript.

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ABSTRACT

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In this type, the antigen is attached to the bottom of the *plate*, then the antigen will be detected through the antibody bound to the enzyme.

Advantages:

- Examination with this method is faster
- Cross-reaction with secondary antibodies can be eliminated

Disadvantages:

- The resulting signal amplification is weak
- Lack of flexibility in selecting enzyme labeled primary antibodies
- There may be reactions between primary antibodies and enzymes bound to these primary antibodies.

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In this method, the antigen is attached to the base of the *plate*, then, the primary antibody which is not labeled with the enzyme is inserted. Next, put back the enzyme labeled secondary antibody, which will bind to the primary antibody.

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- Longer incubation time is required
- The cost required is greater than the direct method

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In this method, the antibody is first attached to the base of the *plate*. Next, the test sample (antigen) is inserted into the well on the *plate*, then a secondary antibody bound to the enzyme is inserted into the well on the *plate.*

Advantages:

- Has high specificity
- Suitable for use with less pure samples

Disadvantages:

- The cost is quite large because it uses two antibodies

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Competitive ELISA is an ELISA examination method where there is a competitive reaction between the sample antigen and antigen bond that is attached to the bottom of the *plate* well with the primary antibody. In this method, the non-sample antigen is attached to the bottom of the *plate.* Next, the sample antigen and primary antibody are inserted into the well. Then put secondary antibodies that are bound to the enzyme in the wells on the *plate.*

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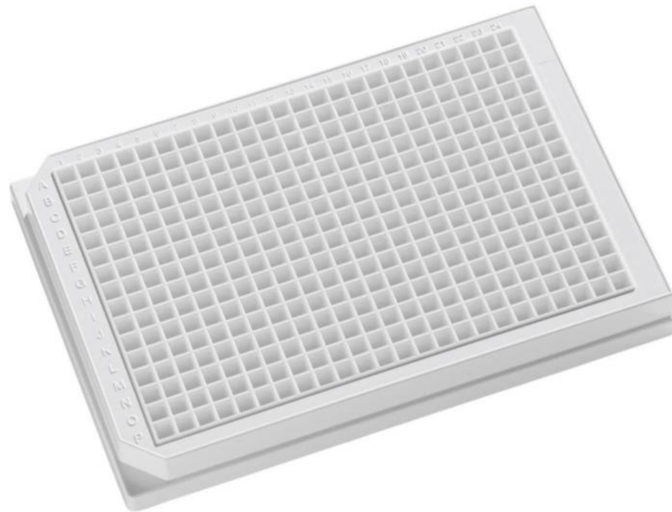


Figure 1. ELISA plate

Figure 2. ELISA antibody

Figure 3. Standard ELISA

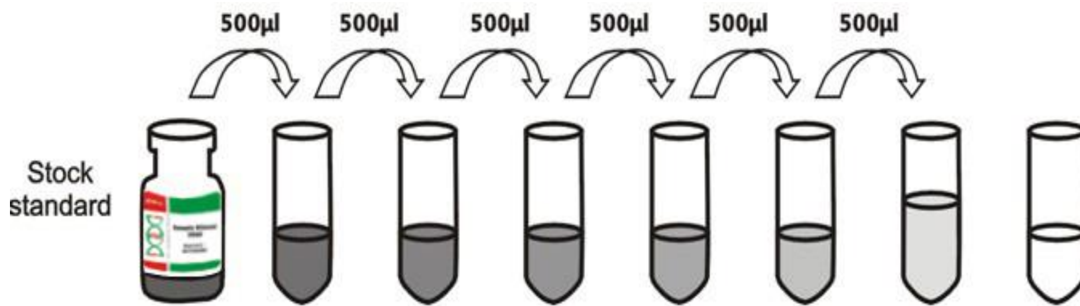


Figure 4. Making Serial Concentration Standard

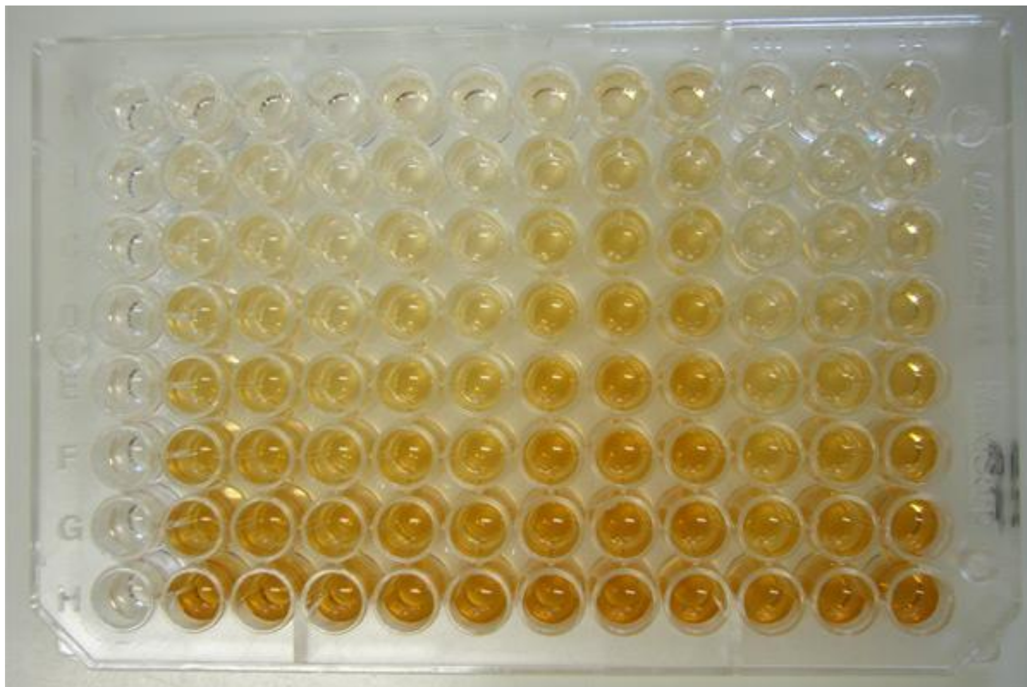


Figure 5. Changes in Color Intensity on ELISA Plate

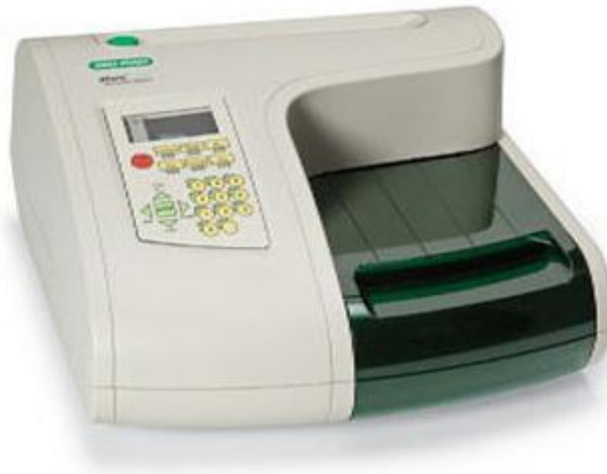
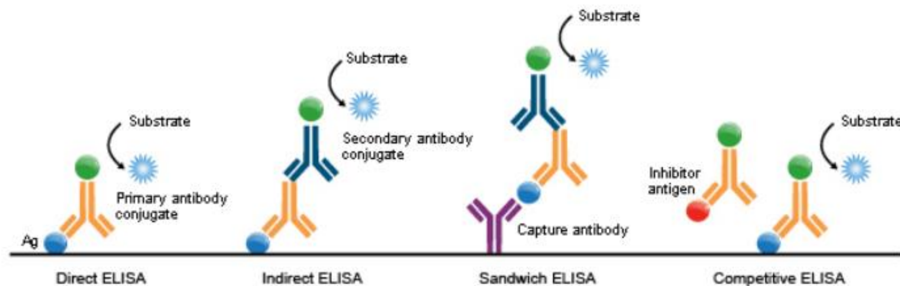


Figure 6. ELISA reader



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Enzyme Linked Immunosorbent Assay (ELISA) Technique Guideline

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A B S T R A C T

ELISA (Enzyme-linked immunosorbent assay) is a technique used to assess the quantification of peptide, protein, antibody and hormone levels, based on the principle of antigen-antibody binding. In the ELISA technique, antigen immobilization will be carried out on a solid surface, then bound with antibodies to form an antigen-antibody bond complex, where the antigen-antibody complex is bound to the enzyme. The detection signal in the form of a color change will be formed due to the reaction between the enzyme and the substrate.

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1. Introduction

ELISA is generally carried out on a *plate* containing 96 wells, where the antibody binding process with protein will occur. This very simple process makes ELISA easy to do. Ease of ELISA for washing, making it easier to clean the following materials when tested. This will make testing with ELISA specific.

The ELISA examination procedure begins with the process of attaching *(coating)* the antigen and / or antibody to the surface of the well on the *plate*. Furthermore, the blocking step is carried out (blocking) antigen and antibody bonds at the *unspecific-site* with a *blocking agent*. After incubation and washing, the plates were incubated with enzyme-bound antibodies. Next, washing the plates was carried out and continued with the addition of the substrate so that the color change would be produced and the OD *(optical density)* value was read with an ELISA reader.

The washing stage is an important one to remove antibodies that are not bound to antigens. In addition, make sure that no washing liquid is left on the *plate*, because it is feared that it will affect the next stage of the inspection.

Terminology

- ELISA *plate*: a container for antigen-antibody collection, which generally contains 96 test wells.

- Antigen: A protein to be assessed for levels, derived from the sample to be tested. If we are going to assess IL-12 levels by using ELISA from serum samples, then the IL-12 contained in our serum is termed an antigen.
- Antibody: A protein that binds to an antigen. If we are going to assess IL-12 levels using ELISA from a serum sample, then an antibody will be added, then the antibody is a protein that will bind to IL-12, or known as anti-IL-12.
- Standard: A protein that will be graded, which has known levels. If we are going to assess IL-12 levels using ELISA from serum samples, the standard is the IL-12 protein that has known levels, for example the standard IL-12 level is 100 pg / mL. Generally, this standard will be made into several concentrations, so that you will get a graph depicting the standard VS the OD value of the standard. The graph will be used to calculate the levels in the sample.
- OD *(Optical Density)* value: a value that describes the intensity of the color change on ELISA
- ELISA reader: Tool used to get the OD value from an ELISA examination.

ELISA type

There are several types of ELISA, namely: *direct, indirect, sandwich or competitive.* In the immobilization stage the antigen can be attached directly to the p*late* or indirectly to the *plate,* by binding with antibodies that have been attached to the bottom of the *plate.* Furthermore, the antigen is detected directly with the primary antibody bound to the enzyme or the antigen is detected indirectly with the secondary antibody that is bound to the primary antibody and the enzyme. Enzymes that are generally bound to antibodies include: alkaline phosphatase (AP) or horseradish peroxidase (HRP).

1. Direct ELISA

In this type, the antigen is attached to the bottom of the *plate*, then the antigen will be detected through the antibody bound to the enzyme.

Advantages:

- Examination with this method is faster
- Cross-reaction with secondary antibodies can be eliminated

Disadvantages:

- The resulting signal amplification is weak
- Lack of flexibility in selecting enzyme labeled primary antibodies
- There may be reactions between primary antibodies and enzymes bound to these primary antibodies.

2. Indirect ELISA

In this method, the antigen is attached to the base of the *plate*, then, the primary antibody which is not labeled with the enzyme is inserted. Next, put back the enzyme labeled secondary antibody, which will bind to the primary antibody.

Advantages:

- The sensitivity of the test is increased with the use of primary and secondary antibodies.

Disadvantages:

- Cross-reactions can occur with secondary antibodies which will result in a non-specific signal.
- Longer incubation time is required
- The cost required is greater than the direct method

3. ELISA sandwich

In this method, the antibody is first attached to the base of the *plate*. Next, the test sample (antigen) is inserted into the well on the *plate*, then a secondary antibody bound to the enzyme is inserted into the well on the *plate.*

Advantages:

- Has high specificity
- Suitable for use with less pure samples

Disadvantages:

- The cost is quite large because it uses two antibodies

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4. Competitive ELISA

Competitive ELISA is an ELISA examination method where there is a competitive reaction between the sample antigen and antigen bond that is attached to the bottom of the *plate* well with the primary antibody. In this method, the non-sample antigen is attached to the bottom of the *plate.* Next, the sample antigen and primary antibody are inserted into the well. Then put secondary antibodies that are bound to the enzyme in the wells on the *plate.*

ELISA data interpretation

The final result of the ELISA examination will be obtained the OD value *(optical density),* however the OD value is not the level of the ELISA examination, it is still necessary to process the OD value data. The first thing to do is to make a standard OD value curve VS standard content, so that the line equation will be obtained from the curve. The equation obtained will be used to convert the OD value of the sample into a grade value.

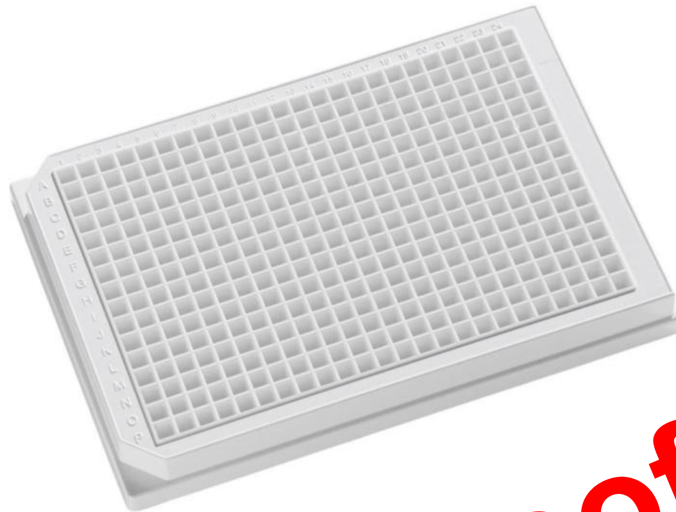

Figure 1. ELISA plate

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Figure 2. ELISA antibody

Figure 3. Standard ELISA

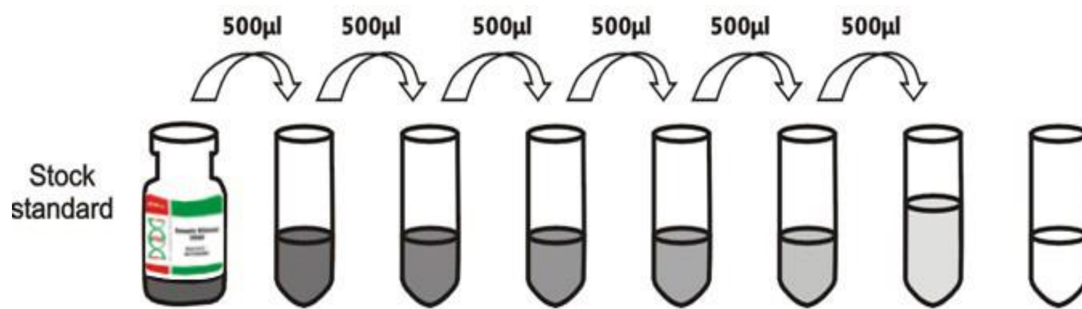

Figure 4. Making Serial Concentration Standard

Figure 5. Changes in Color Intensity on ELISA Plate

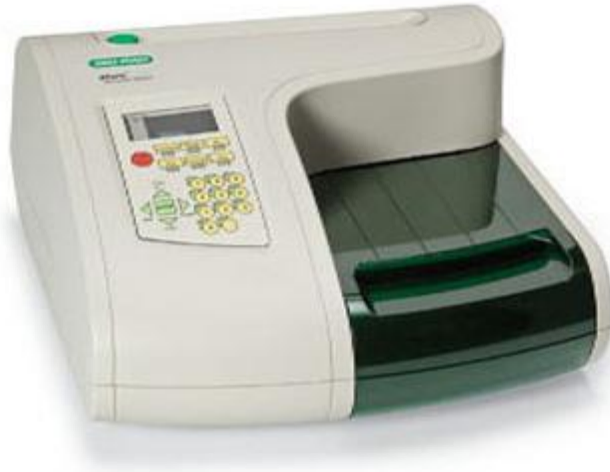

Figure 6. ELISA reader

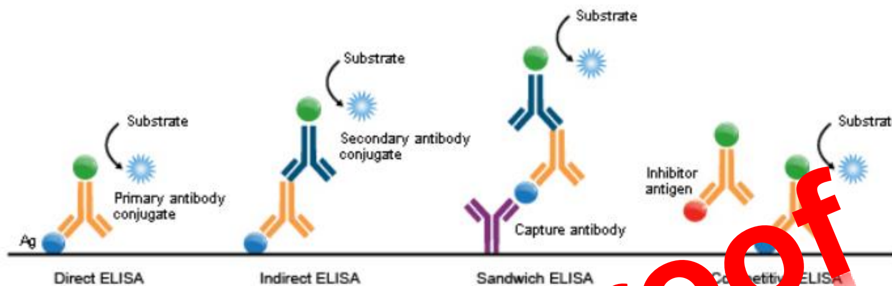

Figure 7. ELISA types

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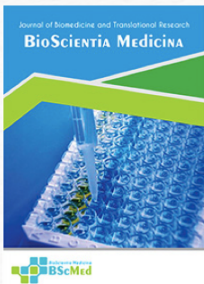

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