The People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

Mila University Center.

Faculty of Science and Technology Department

of Natural and Life Sciences

TD 06 Immunology correction

Exercise 1

To determine ABO blood grouping, two tests can be used: **the globular test** (Beth-Vincent) and **the plasma test** (Simonin-Michon).

➢ **The globular test** (Beth-Vincent) makes it possible to determine the antigenic phenotype of an individual, that is to say the antigens carried by his red blood cells.

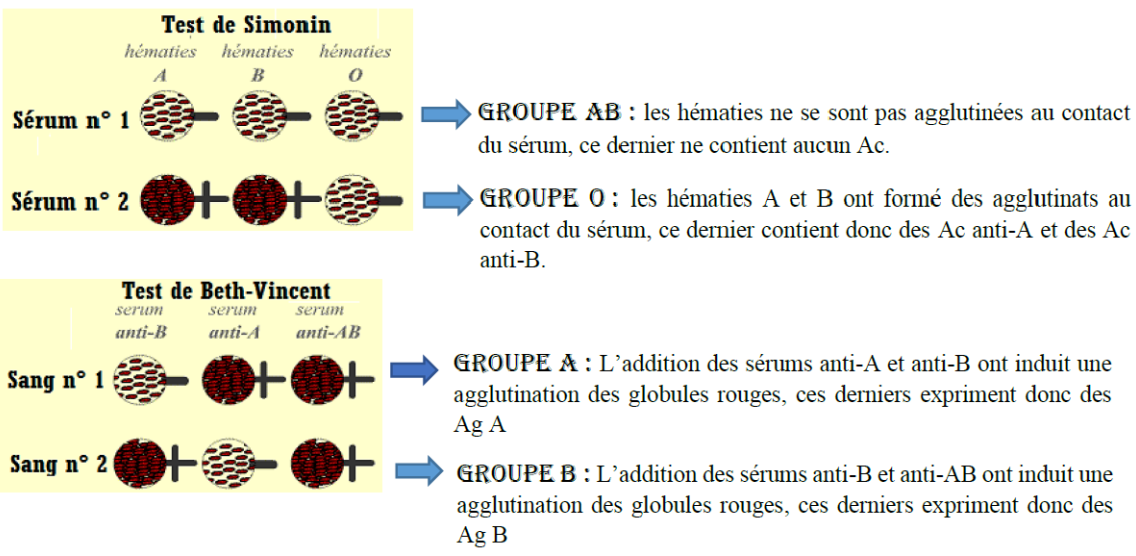
➢ **The plasma test** (Simonin-Michon) makes it possible to carry out the complementary study, that is to say the determination of the circulating natural antibodies present in the serum of an individual.

Performance of the tests

❖ **Beth-Vincent test**. The blood of the individual, containing his red blood cells, is placed in the presence of test sera, each possessing a specific type of antibody, directed against an antigen of the **ABO system.** One
is therefore a red blood cell agglutination test with test sera.

❖ **Simonin-Michon test.** The individual's serum, containing his circulating antibodies, is placed in the presence of test red blood cells, each belonging to a specific antigenic group of the ABO system. It is therefore a serum agglutination test with red blood cells tests.

Blood group deduction from processed samples:



Exo 2

A- The Enzyme-Linked Immuno Assay (ELISA) technique is mainly used in immunology to detect and/or assay the presence of
proteins, antibodies or antigens, in a sample. In particular, it is used for HIV screening, and makes it possible to determine the serum concentration of antibodies directed against the virus. The test used in this case is called: indirect ELISA.

Indirect ELISA:

The principle of indirect ELISA is to detect the presence of a specific antibody in a sample. To do that we need:

- A known antigen specific to the antibody sought (gp41 or p24 proteins for HIV-1 and gp36 for HIV-2)
- A sample to be analyzed (Serum likely to contain anti-HIV Acs)
- An anti IgG secondary antibody coupled to a peroxidase (Horseradish peroxidase). This antibody will specifically recognize IgG antibodies)

B- Carrying out the test: It consists of four main steps:

• • **Antigen binding:** The known antigen, specific to the antibody sought, is incubated on a microtiter plate. The antigen will electrostatically attach to the bottom of the wells. They are then washed to remove unattached antigens.

• • **Fixation of the antibody to be assayed:** We incubate our sample to be assayed (serum containing the antibody), as well as our standards (solution containing known concentrations of antibodies). The specific antibodies will bind to the antigens. Well washing is required to remove unbound antibodies.

• **Fixation of the detection antibody:** A secondary antibody coupled to a
peroxidase It is an anti IgG that will therefore recognize the primary antibody.
|||UNTRANSLATED_CONTENT_START|||Un lavage des puits est nécessaire pour enlever les anticorps secondaires non fixés.|||UNTRANSLATED_CONTENT_END|||

• • **Revelation:** An enzyme-specific substrate is incubated which, if the reaction is positive (presence of the desired antibody), will be transformed and induce a blue coloration. The intensity of the coloration is proportional to the amount of enzyme present and therefore to the concentration of antibodies sought.

• The main steps in performing the test are:

• **Step One:**

• Separation of HIV proteins by SDS-PAGE electrophoresis. Separation of proteins according to molecular weight

• **Step Two:**

Transfer of proteins from the gel to a nitrocellulose or PolyVinyliDene Fluoride (PVDF) membrane brought into contact with the gel according to their same arrangement on the gel (application of an electric current).
This transfer makes the proteins accessible for detection by antibodies.

• These membranes are then cut into strips for use.

• **Step Three:**

• Application of the sera to be tested to the protein bands. If it contains antibodies specific to HIV proteins, they bind to these proteins.

• The immune complexes formed are revealed by an enzyme-labeled anti-globulin after addition of its substrate.

• A test is considered positive if it reveals the presence in the serum of:

• Either 3 antibodies: 2 directed against the envelope proteins gp41, gp120 or gp160 and 1 against a core protein (p55, p40, p24 or p17) or an enzyme protein (p66, p51 or p31).

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• Either 2 antibodies: one directed against the membrane protein gp 160 and the other against the core protein p24

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