- **Lymphocytes isolation:**
  - **Total lymphocyte separation (B+T lymphocytes).**
 - **B or T lymphocytes separation (alone).**
- **Comparison between serum and plasma:**
  - **Serum**: a blood sample is taken → no anticoagulant is added → centrifuge → blood will clot and precipitate in the bottom while serum is left behind.
  - **Plasma**: a blood sample is taken → an anticoagulant is added → centrifuge → RBCs will precipitate at the bottom while plasma is at top and between them is the Buffy coat (composed of WBCs and platelets).
- **Separation of total lymphocytes:**
  - Lymphocytes (B and T) are present in the blood sample which will be taken from the patient.
  - This blood sample will be added to a media called ficoll.
  - After centrifugation → a buffy coat of lymphocytes will appear in the middle between RBCs which will precipitate in ficoll and plasma at the top.
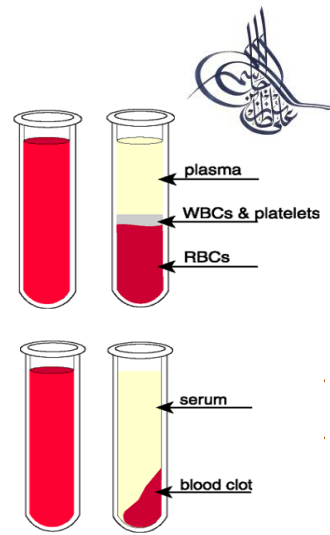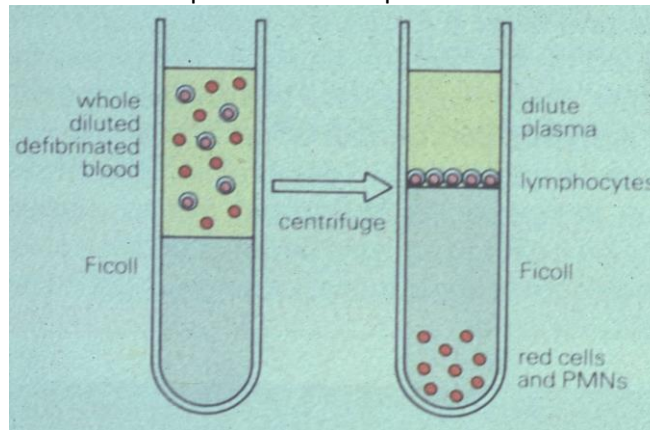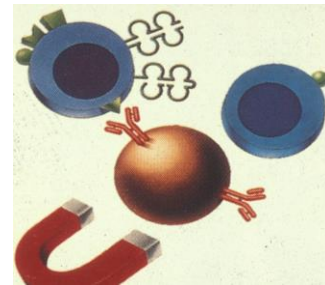

- **Isolation of B-lymphocytes:**
  - This is done by using immunomagnetic beads which have monoclonal antibodies against CD19 or CD20 which are normally present on the surface of B-lymphocytes.
- **Isolation of T-cells:**
  - This is done by using immunomagnetic beads which have monoclonal antibodies against CD3 or CD2 which are normally present on the surface of T-cells.
- **HLA-typing (tissue-typing):**
  - **Done by**: serological method (microcytotoxicity).
  - **Sample**: blood → for class-I (total or T lymphocytes) are used while for class-II (B-lymphocytes are used).
  - **Procedure**: The sample will be added in a plate which has pre-dropped anti-HLA is its wells (1µl blood/well) → complement will mediate cell lysis when it is added to the lymphocyte which is reacting with the anti-HLA found in the well.
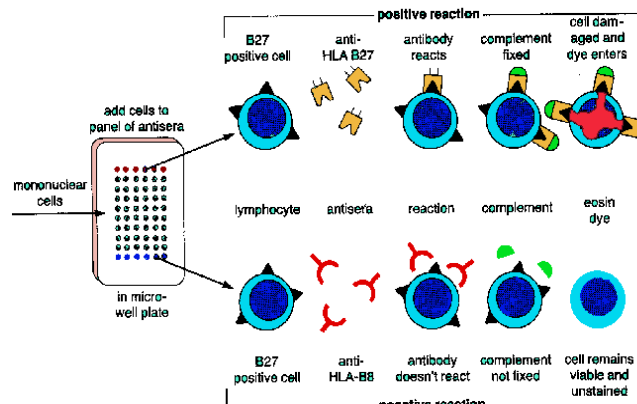
- **Inheritance of HLA types:**
  - A child will inherit 50% from his mother and 50% from his father. So the parent are considered haploidentical to their child.
  - **Between siblings:**
    - ✓ 25% chance of being totally identical.
    - ✓ 50% chance of being haploidentical.
    - ✓ 25% chance of being totally different.
- **HLA-report interpretation:**

	PATIENT	DONOR 1	DONOR 2	DONOR 3
NAME **Age** **Sex** **Relationship** **Blood Group**	-------- M B+ve	-------- F SISTER B+ve	---------- M FATHER O+ve	---------- F MOTHER B+ve
HLA-CLASS I **HLA-A** **HLA-B** **HLA-BW(4/6)** **HLA-C**	A30 – A32 B7 – B49 BW4 – BW6 CW6 – CW7	A30-A32 B7- B49 BW4 – BW6 CW6 – CW7	A30 -A24 B49 – B5 BW4 CW6	A2 – A32 B7 – B41 BW4 – BW6 CW2 – CW7
HLA-CLASS II **HLA-DR** **HLA-DRW** **HLA-DQ**	DR8 – DR13 DR52 DQ6 – DQ3	DR8 – DR13 DR52 DQ6– DQ3	DR8 – DR12 DR52 DQ3	DR7 – DR13 DR52 – DR53 DQ6 – DQ2

- **How to interpret?**

  For example, look to HLA-A (in HLA class-I) → the patient is (A30-A32) → his sister is identical to him (she also has A30-A32) → while his father is haploidentical to him (the patient inherited A30 from his father → the mother is also haploidentical to him (the patient inherited A32 from his mother.

  (COMPARE OTHER HLA TYPES OF THE PATIENT WITH THE 3 DONORS).

- **Final comments:**
  - ✓ The patient is HLA identical to the sister.
  - ✓ The mother and father are haploidentical to the patient.
  - ✓ For bone marrow transplantation, identical HLA donor is required.