Flow Cytometry

1. To know the basic principles behind flow cytometry
   - Flow cytometry is a technological process that allows for individual measurements of cell fluorescence & light scattering
   - The process is performed at rates of thousands of cells per second
   - This information can be used to individually sort or separate subpopulations of cells
   - Pre-requisites for flow cytometry: cells in single cell suspension, fluorescent probes & cytometer.
   - Principles of flow cytometry:
     - Light scattered by laser or arc lamp
     - Scatter can be Forward (FSC) – parallel or perpendicular or Side (SSC)
     - FSC- some similarities to size; SSC- some similarities to granularity & complexity
     - Specific florescence detection
     - Florescent & SSC detectors; FSC detector
     - Hydrodynamically focused stream of particles
     - Electrostatic particle separation for sorting
     - Multivariate data analysis capability

2. To understand the purpose of using fluorescent probes in flow cytometry
   - Florescence may be used in the detection of:
     - protein, RNA & DNA
     - DNA synthesis
     - Dye efflux
     - Organelle activity
     - Change in pH
     - Protein interactions
     - Cell movement & diversion
   - Mechanism of fluorescence:
     - the florescent molecule is excited by the excitation of laser.
     - this imparts E to electrons in the molecule which then released as the molecule relaxes.
     - the E is released as light.

3. To know the basic application of flow cytometry in clinical immunology/haematology

<table>
<thead>
<tr>
<th>Differential leukocyte counting</th>
<th>To measure population of specific WBC types. Example: T cell numbers in AIDS analysis or to determine a particular immune disorder</th>
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<tbody>
<tr>
<td>Immunophenotyping</td>
<td>Identifies &amp; quantifies populations of cells in heterogenous sample-blood, bone marrow or lymph. Useful in diagnosing hematological malignancies such as lymphomas &amp; leukaemia</td>
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<tr>
<td>Cell-cycle analysis</td>
<td>Analyse replication states of the 4 distinct phases. Useful to look for cell aneuploidy associated with chromosomal abnormalities</td>
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