12.6: Flow Cytometry, Cytogenetics and Molecular Genetics

Classification of Leukemia

The classification of Acute Leukemia relies on the use of a variety of laboratory results, including morphology, immunophenotyping, genetic features, and clinical features. Classification allows for appropriate disease management, treatment, prognosis, and monitoring to occur. The laboratory is crucial in this aspect. The following is a brief summary of the type of laboratory testing involved in the classification of Acute Leukemia in addition to what has already been discussed.

Flow Cytometry

Flow Cytometry, also known as immunophenotyping, is a technique that can be used to help determine a cell’s lineage based on cell markers (e.g Cluster of Differentiation/CD Markers) present and the stage of maturation of a cell.¹

Principle:

Monoclonal antibodies with fluorescent labels that are specific for the surface antigen of interest are incubated with the sample. Samples are taken up by the flow cytometer and injected into a stream of sheath fluid to allow cells to be positioned centrally, this process is called hydrodynamic focusing. A laser is directed at the cells and the bound antibodies fluoresce. Fluorescence detectors are used to detect the fluorescence and a scatter graph is produced based on the antibodies bound.² Other properties such as light scatter (in the forward and side direction) are combined with fluorescence intensity measurements to distinguish cell populations.
Flow cytometry can be used to help determine what cells are present to help diagnose acute leukemias and other hematological disorders.

Table 1. Common Surface Markers for Blood Cells.\(^2\)

<table>
<thead>
<tr>
<th>Cell Lineage</th>
<th>Surface Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature Cells</td>
<td>CD34, CD117</td>
</tr>
<tr>
<td>Granulocytes, Monocytes</td>
<td>CD13, CD14, CD15, CD33</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>CD71, Glycophorin A</td>
</tr>
<tr>
<td>Megakaryocytes</td>
<td>CD41, CD42, CD61</td>
</tr>
<tr>
<td>T Lymphocytes</td>
<td>CD2, CD3, CD4, CD5, CD7, CD8</td>
</tr>
<tr>
<td>B Lymphocytes</td>
<td>CD19, CD20, CD22</td>
</tr>
</tbody>
</table>

Cytogenetics

Cytogenetics involve the identification of abnormal karyotypes which may be characteristic to a related disorder.\(^1\)

Fluorescence In Situ Hybridization (FISH)

FISH is a molecular method that is a cytogenetic tool that is used to detect chromosomal abnormalities such as translocations, deletions, inversion, and duplications.\(^3\)

The method involves using a fluorescently labelled DNA or RNA probe that is complementary to a specific target sequence. After denaturing double stranded DNA to single stranded DNA, the labelled probe is allowed to incubate and hybridize with the DNA. After incubation, the sample is washed to remove any unbound probes and then a counterstain is added to assist examination. Samples are examined with a fluorescent microscope to look for any chromosomal abnormalities in the cells.\(^3\)

Molecular Genetics

Molecular genetics involve the use of molecular techniques to identify specific genetic sequences and mutations that
can be characteristic for a diagnosis.¹

**Polymerase Chain Reaction (PCR)**

PCR is commonly used to amplify a specific target sequence such as a mutation.⁴

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**References:**

