Hematological analyzers:

1. ***Measurements available***-The various broad category of measurements provided by the analyzers are shown in the table

|  |  |  |  |
| --- | --- | --- | --- |
| Name | **Advia** | **Sysmex** | **Abbott** |
| CBC | Yes | Yes | Yes |
| WBC (diff) | Yes | Yes | Yes |
| RET analysis | Yes | Yes | Yes |
| HGB | Yes | Yes | Yes |

* 1. Measured parameters: Parameters obtained from the different analyzers

**RBC characterization**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | **Advia** | **Sysmex** | **Abbott** |
| RBC | Y | Y | Y |
| HGB | Y | Y | Y |
| HCT | Y | Y | Y |
| MCV | Y | Y | Y |
| MCH | Y | Y | Y |
| MCHC | Y | Y | Y |
| RDW | Y | Y (SD +CV) | Y |
| HDW | Y | - | - |
| NRBC | - | Y | Y |
| NR/W | - | Y | Y |
| RETC | - | Y | Y |
| %RET | - | Y | Y |
| RET-He | - | Y | - |
| IRF | Y | Y | Y |
| HFR | Y | Y | - |
| MFR | - | Y | - |
| LFR | - | Y | - |
| MCVr | Y | - | - |
| CHr | Y | - | - |
| CHCMr | Y | - | - |

**WBC characterization**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | **Advia** | **Sysmex** | **Abbott** |
| WBC | Y | Y | Y |
| NEU | Y | Y | Y |
| %NEU | Y | Y | Y |
| LYM | Y | Y | Y |
| %LYM | Y | Y | Y |
| MONO | Y | Y | Y |
| %MONO | Y | Y | Y |
| EOS | Y | Y | Y |
| %EOS | Y | Y | Y |
| BASO | Y | Y | Y |
| %BASO | Y | Y | Y |
| CD3T | - | - | Y |
| %CD3 | - | - | Y |
| CD4T | - | - | Y |
| %CD4 | - | - | Y |
| CD8T | - | - | Y |
| %CD8 | - | - | Y |
| LUC | Y | - | - |
| %LUC | Y | - | - |
| % Blast | Y | - | - |
| MN | - | Y | - |
| % MN (mononuclear cells) | Y | - | - |
| PMN | - | Y | - |
| %PMN (Poly MN cells) | Y | - | - |
| % BASO suspect | Y | - | - |
| Ratio of T-helper /inducer cells to T-suppressor/ cytotoxic cells | - | - | Y |

**Platelet characterization**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | **Advia** | **Sysmex** | **Abbott** |
| PLT | Y | Y (multiple measurements) | Y |
| MPV | Y | Y | Y |
| CD61 | - | - | Y |
| Large LPLT | Y | Y (ratio) | - |
| PCT | - | Y | - |
| PDW | - | Y | - |

1. ***Techniques for measurement:***

|  |  |  |  |
| --- | --- | --- | --- |
| Name | **Advia** | **Sysmex** | **Abbott** |
| CBC | Laser flow cytometry | Electrical impedance | Electrical impedance (cross checked light scat) |
| WBC (diff) | Laser flow cytometry | Laser flow cytometry | Laser flow cytometry |
| RET analysis | Laser flow cytometry | Laser flow cytometry | Laser flow cytometry |
| HGB | Absorption spectrophotometry | Absorption spectrophotometry | Absorption spectrophotometry |
| Platelet analysis | Laser flow cytometry | Electrical impedance | Light scattering (crosschecked Elec. Imp) |

1. ***Principles of operation: Discussion of the various techniques***

**Laser flow cytometry-** It is carried out by passing a liquid along the walls of the channel/tube through which the sample is passed while the sample is sent through the inner core of the tube (and is surrounded by the sheath fluid). The sheath fluid and sample greatly vary in density and flow velocity. This fluid ensures hydrodynamic focusing and also enables the flow of single cells through the tube (by narrowing the diameter of the core of the tube). Single cells are necessary for accurate counting and characterization of each cell within the sample mixture (blood). A laser source is placed near the tube and filters are placed at 1800 and 900 of the light source. The screen placed at 180 deg from the light source measures the forward scattering while the screen placed perpendicular to the light source measures the side scatter. There is a coincidence correction applied for the overlap of multiple cells through the tube at the same time (both light scattering and electrical impedance).

Abbott: Multiple filters are placed perpendicular to the light source in order to obtain the various differential metrics. Various observations utilized for the characterization of the sample are:

* 00 light loss-measure of cell size
* 70 light scatter- measure cellular complexity
* 900 depolarized scatter- differentiates neutrophils from eosinophils (eosinophils tend to depolarize light- measures granularity)
* 900 scatter – lobularity of the cells
* FL1-RETCs (contains more RNA due to granular endoplastic reticulum-RNA is stained).
* FL3- NRBCs, non-viable WBCs and fragile WBCs (staining the DNA)

Advia: Various reagents are mixed with the sample in order to achieve the specific characterization goals

*Reagents:*

RBC-Sodium dodecyl sulfate, glutaraldehyde (also used for platelets)

RET- zwitterionic detergent and cationic dye (Oxazine 750)

Perox 1- Sodium dodecyl sulfate, Sodium chloride, Sorbitol, formaldehyde

Perox 2- 4-Chloro-1- naphthol, Diethylene glycol

Perox 3- Stabilizer, hydrogen peroxide

Baso- Hydrochloric acid, Pthalic acid, preservative, surfactant

*Measurement:*

Sysmex: Methods for measuring various quantities are discussed below.

Basophils- Lysercell is added which lyses the cell membrane of erythrocytes and leukocytes except basophil. From forward scattering vs fluorescent intensity for each cell, leukocyte count, differential counts of basophils and nucleated erythrocytes measured.

Leukocyte differentiation-Addition of surfactants damages the cell membrane of leukocytes. Morphology of the cells change based on the type of leukocyte. Fluorescent dye stains the cell nuclei and other cell organelles. WDF scattergram utilized to detect the type of leukocyte cell. Staining intensity reveals: monocytes > lymphocytes > neutrophils > eosinophils. Based on the strength of the light scattered: eosinophils > neutrophils > monocytes > lymphocytes

Blast and abnormal cells from lymphocyte series- Detected using forward scattering and fluorescence (abnormal lymphocytes are in the area of higher fluorescent intensity). Abnormal cells differ from normal cells in their cell membrane characteristics, nucleic acid content.

RET detection- Nucleic acids in immature erythrocytes are stained by Fluorocell (detected by flow cytometry). High and medium fluorescence ratio depicts the immature RET fractions.

Platelet differentiation- Fluorescent dye stains platelets (not erythrocytes) and based on intensity of the fluorescence, the mature and immature platelets can be differentiated. Immature platelets have strong forward scattering.

**Electrical impedance-** It is used to count the number of cells in the sample by passing a single cell through a channel and applying a voltage across the domain. As a cell passes through the voltage source, there is an associated change in the resistance, which brings about a change in the current. This is converted into signals, which are used for characterizing the number and volume of the cells. Bigger cells apply greater resistance, thereby enabling the calculation of volume from this technique. A coincidence correction approach is incorporated within the measurement technique in order to reduce the errors owing to overlap of multiple cells while applying the electrical voltage. It utilizes a DC current.

**Absorption spectrophotometry-** Based on the concentration of hemoglobin within the blood sample, the absorbance spectrum of the sample varies (Beer’s law). In the Advia (cyanisation) and Sysmex instrument, hemoglobin characterization is brought about by the formation of a complex with an external reagent. The advantage of forming a complex is attributed to the stable peak obtained from it. The wavelengths at which the absorbance spectrum are measured for the various instruments are: Advia-546 nm, Abbott- 544nm, Sysmex- peak is 535 nm and shoulder at 560 nm.