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| CD45 – Gating Cytometric Analysis | | | | |
| **Purpose** | Hematopoietic cell populations can be gated based on their CD45 staining and side scatter properties, allowing for further phenotypic analysis. This is useful for the identification and characterization of malignant and normal blood cells and their precursors. | | | |
| **Policy Statements** | This procedure applies to all laboratory technologists performing Flow Cytometry testing, the section  supervisor, and section pathologist. | | | |
| **Principle and Clinical Significance** | Blood, bone marrow, body fluids and tissues contain a very complex mixture of cell populations. Among the different lineages represented there are significant variations of morphologic parameters such as cell size, nuclear size and shape, and cytoplasmic granularity. Even within the same lineage there may be profound differences, particularly in the myeloid line. Cell size and granularity are important factors which may be analyzed by flow cytometry through intrinsic light scatter analysis. Forward light angle scatter (FSC) correlates with cell size and right angle (or side) scatter (SSC) correlates with cell granularity. Immunoflourescence assays may be performed based on the fluorescence intensity of cells stained with fluorochrome tagged monoclonal antibodies directed toward a particular cell surface antigen. Cell surface markers which demonstrate variable expression dependent upon cell lineage and/or maturation stage are a valuable tool for cytometric analysis of a complex mixture of heterogeneous cells. CD45, the common leukocyte antigen, is expressed by all cells of hematopoietic origin. By pairing CD45 fluorescence measurement with the intrinsic light scatter parameters for granularity, SSC, significant discrimination of the complex populations of cells within the specimen can be resolved. | | | |
|  | Using the CD45 vs SSC display for gating the flow cytometric data, the positions are highly reproducible and easily recognizable. These patterns permit the identification of normal cells allowing attention to be drawn to abnormal populations when present, and therefore the characterization of abnormal cells in hematopoietic malignancies. CD45 is added to each tube of the monoclonal antibody panel to allow for CD45 gating and multi–color analysis of the bone marrow cells.  [Attachment I - CD45 - Gating Cytometric Analysis](http://khan.childrensmn.org/Manuals/Lab/SOP/Flow/Res/208506.pdf) | | | |
| **Materials** | See specific procedures for materials needed. | | | |
| **Sample Analysis and**  **Result Reporting** | The goal of flow cytometric analysis is to enumerate and characterize the subsets of leukocytes. The flow cytometric data are analyzed using FACSDiva software. Each leukocyte subset has unique clustering characteristics as described below. The cell population(s) of interest is identified on the basis of its right angle light scatter and CD45 fluorescence. The single or multi-color fluorescence parameters are then analyzed for each tube to determine the phenotype of the population of interest. Normal bone marrow populations are as follows. | | | |
|  | * **Lymphocytes**: Mature lymphocytes have the highest CD45 flourescence intensity and lowest SSC. This gate contains mature B, T, and NK cells. * **Hematogones**: Normal immature B cells. Hematogones have slightly dimmer expression of CD45 compared to mature lymphocytes, but can be distinguished from leukemic blasts by their expression of other markers (CD34, CD10, CD38, TdT, CD9) in a normal maturation continuum. As they mature, SSC becomes lower and CD45 expression becomes brighter. Hematogones do not express aberrant markers from other cell lineages as may be seen in leukemic blasts. * **Monocytes**: Mature monocytes in bone marrow express almost the same amount of CD45 as lymphocytes, but are distinguished by their higher SSC. * **Segmented Neutrophils and Bands**: The neutrophil lineage can be subdivided into four compartments based on CD45 expression and SSC. The most mature cells among the neutrophil lineage have higher CD45 expression than do other neutrophilic cells. * **Myelocytes and** **Metamyelocytes**: The intermediate stages of neutrophil maturation are in close proximity to the mature neutrophils but have a lower intensity of CD45 expression. * **Promyelocytes**: Promyelocytes display the same level of CD45 flourescence as the myeloblasts, myelocytes and metamyelocytes, but have a higher SSC because of their increased granularity. * **Myeloblasts**: These have the lowest CD45 expression of any of the cells in the myeloid series. They also have the lowest SSC, which is approximately equivalent to monocytes but greater than lymphoblasts or hematogones. * **Nucleated Erythroid Cells**: The cells in the erythroid lineage have decreased amounts of CD45 as compared to the other lineages in the bone marrow. Some types of ALL have lymphoblasts which lack or very weakly express CD45 in which case the population may fall in the area of normal erythroid cells. | | | |
| **Procedure Notes** | Logical relationships between subsets should exist when analyzing specimens. For instance, it would be expected that B-cell markers would have similar levels of expression or the T-cell subsets would have a logical relationship (CD4 + CD8, etc.)Normal antigens may be overexpressed, underexpressed, or absent in abnormal cell populations. Malignant cells may also aberrantly express antigens associated with other cell lineages. Care must be taken to evaluate results in view of the morphologic and clinical picture. When both normal and abnormal populations appear in the specimen, the normal populations may serve as a built in quality control measure to assure that the antibodies and the test system are working properly.An appropriate isotypic control should be run to measure auto-fluorescence or non-specific binding and for guidance in distinguishing fluorescence negative and fluorescence positive cell populations. Isotypic controls may not be needed when the unlabeled cell populations are clearly separated from the labeled cells. Markers should be set between in the area of clear separation. Isotypic controls may be necessary for monoclonal antibodies that do not have a distinct negative population. In such cases the cursors should be set on the isotypic control so that <2% of the cells are positive and should not be moved for tubes where fluorescence distributions are continuous with no clear demarcation between positively and negatively labeled cells.List mode data of the flow cytometry on each patient is exported to the G:Flow Lab network drive and stored according to state and accreditation agency regulations. | | | |
| **References** | 1. Clinical Applications of Flow Cytometry: Immunophenotyping of Leukemic Cells: Proposed Guideline. NCCLS Document H43-P. December 1993. 2. Flow Cytometry Principles for Clinical Laboratory Practice. Owens MA, Loken MR. 1995. 3. CD45 Gating for routine Cytometric Analysis of Human Bone Marrow Specimens. Steltzer GT, Shults KE, Loken MR. Clinical Flow Cytometry, Vol 667. 1993. 4. FACSComp Software users guide: Overview BDIS. January 1996. | | | |
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Colleen Berglund | 08/11/1997 | Initial Version |
| 2 | Colleen Berglund | 01/13/2000 | Updated Initial version |
| 3 | Jim Berger | 10/29/2009 |  |
|  | 4 | Jim Berger | 03/30/2011 | Updated for new location of Flow Cytometry Lab |  |  |
|  | 5 | Al Quigley | 03/31/2013 | Updated for CMS Web |  |  |
|  | 6 | Amanda McCaustland | 08/28/20 | Added hematogones, minor updates and reformatting |  |  |