How does flow cytometry help diagnose LGLL?
Flow cytometry from sample to results

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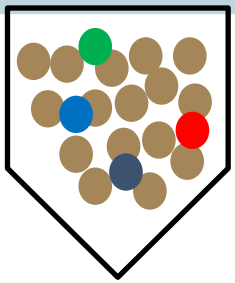
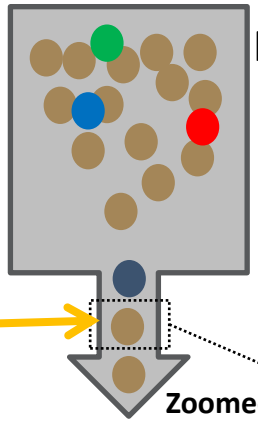
1



Tube with blood sample

Note: see next slide for detailed description of this diagram. A subsequent flow cytometry tutorial explains this with an analogy.

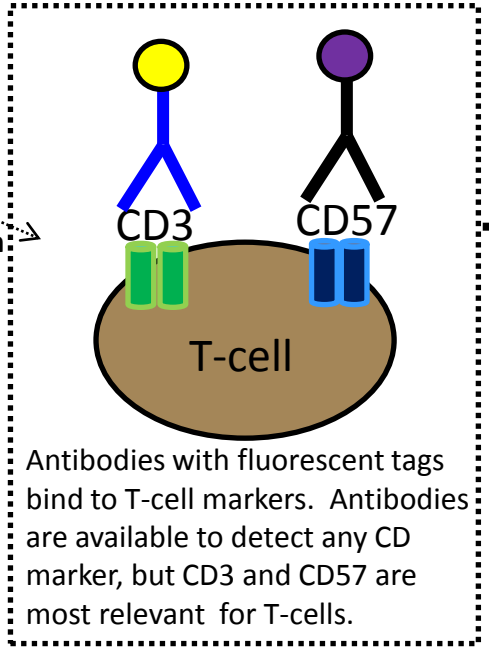
2



Flow cytometer

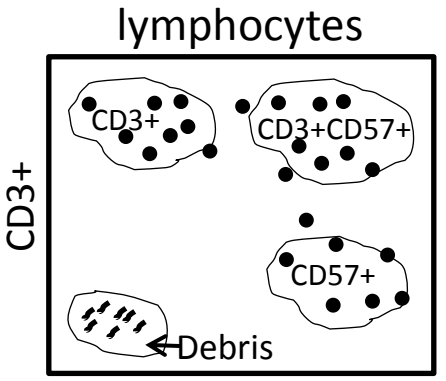
Zoomed-in view

3

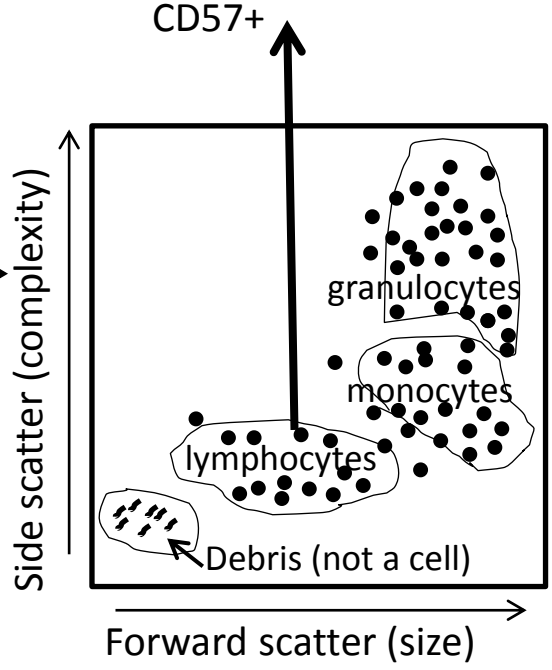


Antibodies with fluorescent tags bind to T-cell markers. Antibodies are available to detect any CD marker, but CD3 and CD57 are most relevant for T-cells.

4



In this graph, a population of CD3+CD57+ (T) cells are circled. Whatever total number this is compared to all the cells present gives a % value.



This gives us an idea of the size and shape of the cells being monitored.

Description:

1. The patient's sample, containing all blood cells is the starting point. The blood is mixed with antibodies for an amount of time and then excess antibodies are washed away.
2. The flow cytometer draws up a sample of blood cells. In this example, we are looking at a T-cell (brown colored cells). The cells flow single file past a laser; antibodies will have bound to cells depending on which markers are present, and give a fluorescent signal.
3. The upside-down Y signifies an antibody and the circle attached to it is the fluorescent probe. The CD3-specific antibody will bind to CD3 on the outside of the T-cell. The laser will shine onto the sample causing detection of the CD3 marker. A different antibody will bind CD57 and have its own fluorescent probe that can emit a signal. The cell in this example has both CD3 and CD57 markers present and would be therefore identified as a T-cell. Sometimes T-cells have other markers, but these are two widely-used markers to identify a T-LGL cell.
4. A general/example readout is shown. Scatter refers to how the cells scatter the light from the laser. Forward scatter is based on cell size and side scatter is based on granularity of the cells. Cells can have a varying amount of granularity (granule compartments that contain toxic compounds to kill other cells). These parameters allow the cells to be divided into different types of populations. This graph has each type of population circled. From that, another graph can be made which shows the lymphocyte population. In this case, the CD3 and CD57 markers are on each axis of the graph. The cell populations are circled, with the CD3+CD57+ cells (T cells having both markers on their surface) in the upper right quadrant.

Overall Summary:

- In basic terms, flow cytometry simply means that you are flowing a sample of your blood cells through a chamber to find out which cells are present. Antibodies that specifically identify certain CD markers will bind, and their fluorescent probe will emit a signal. This allows flow cytometry to report which markers are present on the cell and then overall how many cells of each type are present.
- Typically, CD3, CD8, and CD57 can be used to identify a T-cell. In comparison, CD16, CD56, and CD94 are usually used to identify an NK-cell. In some cases, a T-cell may have the CD56 marker. This is unusual and the reason for it is unknown. The cells depicted in this diagram do not show all the markers typically present on the cell surface. Flow cytometry will gather data on other surface markers but the most important ones in diagnosing T-LGLL are depicted here.
- In this example, the brown cells (all T-cells) are abundant in the sample (16 of the 20 cells) and all express CD3 and CD57. This would suggest a T-LGL leukemia diagnosis. This test will tell us the percentage of T-cells (in this case) present, relative to the other blood cells.