

Assessing Bacterial Invasion

Summary of: Edwards, RA and Maloy, SR (2001) Inside or Outside: Detecting the Cellular Location of Bacterial Pathogens. *BioTechniques* 30(2):304-311.

A novel method is described using two fluorescent proteins to determine location (intracellular or extracellular) of bacteria in macrophage infection assays. This is a real-time quantitative assay that is not toxic to either the bacteria or the host cell. Previously, all the methods available were finite end-point assays that resulted in the destruction of the sample and could interfere with the process of infection.

Edwards and Maloy introduce an assay that inserts two different fluorescent gene reporter markers into the bacterial cell: red fluorescence to detect extracellular bacteria and green fluorescence to detect intracellular bacteria. The cells are incubated with macrophages and then bacterial cell location is detected based on fluorescence. Evaluation by flow cytometry determines the number of macrophages and scores the number of fluorescent bacteria present.

The advantages of this new assay are: 1) it can be performed in real time with no fixing or staining, 2) it does not alter macrophage or bacterial cell surfaces, and 3) it can be applied to other systems in which intracellular gene expression has been characterized (e.g. Salmonella, Legionella). Another clear advantage of this new assay is sensitivity: FACS analysis allows rapid sampling of large volumes and dilute solutions that eliminate the need for a high level of infection.



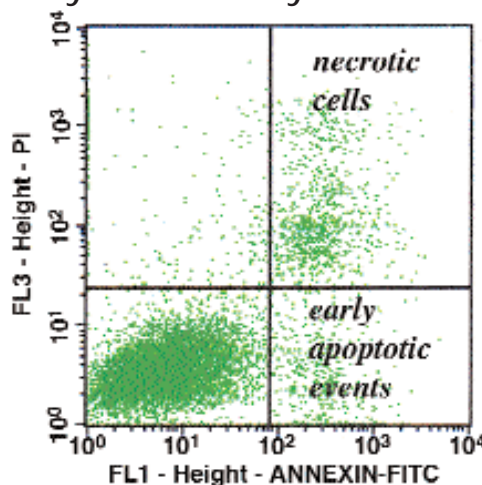
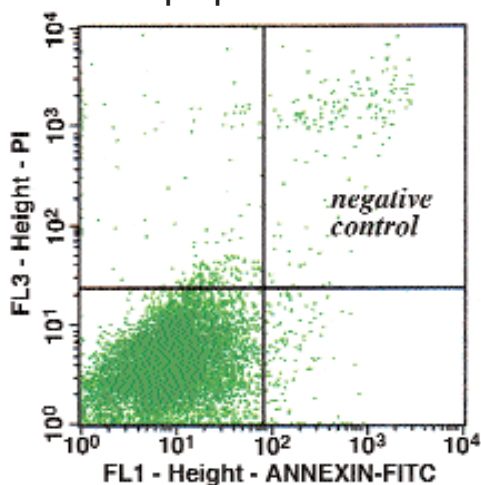
Kristina Majer coordinates labeling and other tissue culture services for Cytometry Research customers.

Measuring Cell Death

Summary of: Merchant, SH, Gonchoroff, NJ and Hutchison, RE (2001) Apoptotic Index by Annexin V Flow Cytometry: Adjunct to Morphologic and Cytogenetic Diagnosis of Myelodysplastic Syndrome. *Cytometry* 46:28-32.

The implications of apoptosis or "programmed cell death" on physiological and pathological development of adult organisms has greatly expanded the need to better understand this phenomenon. Flow cytometry has proven to be an invaluable tool in the study of apoptosis. The most common method for distinguishing necrotic cells from apoptotic cells involves a two-color experiment utilizing FITC-conjugated Annexin V with the DNA binding protein compound, propidium iodide (PI). The calcium-dependent phospholipid-binding protein Annexin V has a high affinity for phosphatidylserine, a phospholipid that translocates from the inner surface to the outer leaflet of the plasma membrane upon cell death. This can be readily measured by the FITC fluorescent label. To distinguish apoptotic cells from necrotic cells the red fluorescent nucleic acid dye PI is used. PI can only be introduced into the DNA of necrotic cells that have lost their plasma membrane integrity.

Apoptosis Detection by FACS Analysis



Legend.

Quadrants:

Lower left = viable cells that exclude PI and are negative for Annexin V-FITC (AF) binding.
Upper right = nonviable necrotic cells AF+ and PI+.
Lower right = apoptotic cells AF+, PI negative, with cytoplasmic membrane integrity.

Left panel: control sample not induced for apoptosis with <1% Annexin V-FITC binding.

Right panel: induced for apoptosis with ~5% expression.

Annexin V-FITC Binding Expression