PROPIDIUM IODIDE SOLUTION
REF.: CYT-PIR-25

INTRODUCTION

Flow cytometric studies of cell DNA content have been increasingly applied in the clinical settings to the study of solid tumors in addition to lymphoreticular and hematopoietic neoplasms where this has been shown to be of value in predicting clinical behavior. These kind of measurements provide rapid and objective information on the biology of neoplastic cells: it allows the analysis of either the existence or the absence of clonal abnormalities in the overall DNA content of tumour cells (DNA aneuploidy), and on the distribution of a specific neoplastic cell population throughout the different cell cycle phases.

CLINICAL UTILITY

The detection of **DNA aneuploidies** by flow cytometry offers relevant clinical information for prognostic evaluation and follow-up of patients with solid tumours and/or hematological malignancies. It has been demostrated that, except for thyroid benign diseases or essential monoclonal gammopathies, the existence of DNA aneuploidy is characteristic of malignant neoplasms and in general (except for acute lymphoblastic leukemia, multiple myeloma and neuroblastoma) is associated with a worse prognosis.

The studies on the distribution of tumor cells throughout the different **cell cycle** phases have been shown to be especially relevant regarding its clinical and prognostic implications. In this sense, a high proportion of S-phase cells in solid tumors and hematological neoplasms is assotiated to a histopathologic diagnosis of malignance with clinical, biological and histological characteristics of poor prognosis and with a worse disease outcome and a shorter survival.

REAGENTS

- DNA labelling buffer: 25 ml vial containing detergent, propidium iodide and RNAse for DNA staining. 25 Test (1 ml/Test).

PROTOCOL

1.- In case of using bone marrow samples, pass them 3 or 4 times through a syringe in order to disaggregate the sample. Perform a white blood cell count of the sample and take 10^6 cells in a volume of 100-150 µl.
In case of solid tumor samples, disaggregate the piece and wash it in citrate buffer for DNA*. Centrifuge at 540 g for 15 minutes, resuspend the cell pellet with 1-2 ml of citrate buffer for DNA and pipette from the cell suspension a volume of 30-40µl.

2.- Add 2ml of erythrocyte lysing solution to lyse mature red cells present in the sample. Mix gently and incubate in an horizontal position during 10' at room temperature in the dark.

3.-Wash out the lysing solution: Centrifuge for 5' at 540g. Discard the supernatant and placed each tube inverted in a vertical position over a filter paper in order to eliminate the possible remaining lysing buffer from the tube. Resuspend the cell pellet.

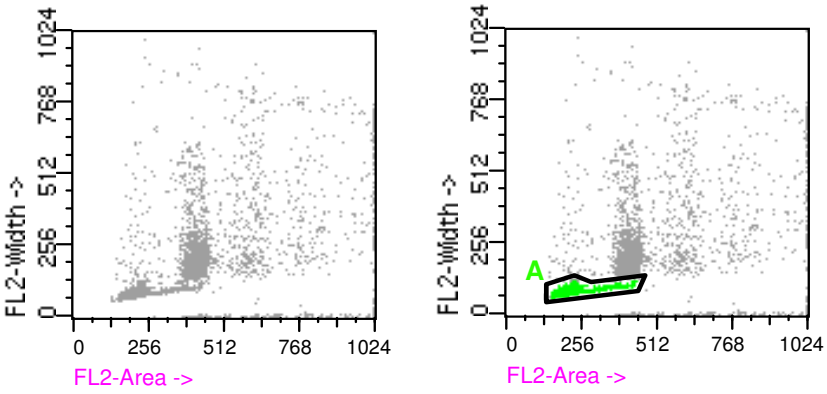
4.- Add 1 ml of DNA labelling solution. Incubate in the dark for 10 minutes at room temperature in horizontal position (maximum 3 hours).

5.- Acquire data in a flow cytometer (low speed position).

**Composition of the citrate buffer for DNA: Sucrose 85.5 g (250mM) and trisodium citrate $2H_2O$ 11.76 g (40mM) were dissolved in approximately 800 ml distilled water. Dimeththylsulfoxide 50 ml was added. Distilled water was added to a total volume of 1000 ml and the pH was adjusted to 7.6.*

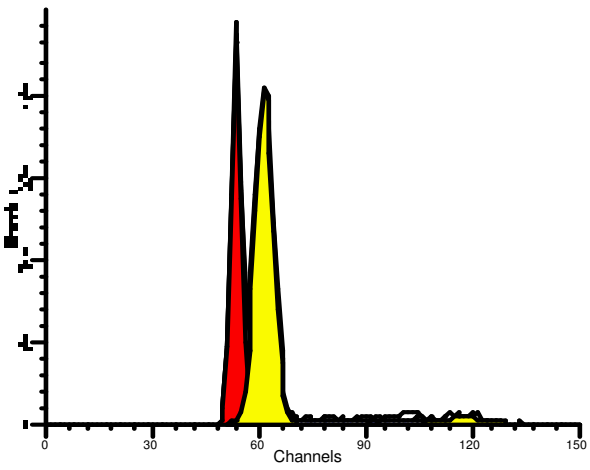
DATA ANALYSIS

1.- Gate singlets in either an FL2-Area/FL2-Width dot plot (in a Becton/Dickinson flow cytometer), or an FL2-Area/FL2-Peak dot plot (instruments from Coulter Corporation and Ortho Diagnostic Systems) following the instructions shown below (dot plots):



2.- Cell cycle estimation. This kind of study is based on the identification of two groups of cells, the major corresponds to G_0/G_1 DNA peak representing cells in the resting (G_0) or the cycling presynthetic (G_1) phases of the cell cycle, and the second DNA peak (G_2/M) represents the fraction of cells that have twice as much DNA as the events in G_0/G_1. Events in the fluorescence channels of the histogram between G_0/G_1 and G_2/M represent cells containing intermediate DNA levels, and in principle correspond to cells at the DNA synthesis phase of the cell cycle. Calculate the percentage of cells in each cell cycle phase using the mathematical models included in the specific software programs available in the laboratory.

3.- Explore the possible existence of DNA aneuploid after comparing the relative distribution of the G_0/G_1 DNA peak. In the case there would be a DNA aneuploidy, calculate the DNA index by dividing the mode of the DNA fluorescence intensity of the G_0/G_1 problem cells by that of the G_0/G_1 normal cells.



Analysis type: Manual analysis
Prep: Fresh/Frozen

DIPLOID: 43.44 %
Dip G0-G1: 84.04 % at 53.51
Dip G2-M: 0.90 % at 107.01
Dip S: 15.07 % G2/G1: 2.00
Dip %CV: 2.53
ANEUPLOID 1: 56.56 %
An1 G0-G1: 96.06 % at 61.36
An1 G2-M: 3.60 % at 118.30
An1 S: 0.33 % G2/G1: 1.93
An1 %CV: 4.04
An1 DI: 1.15

Total S-Phase: 6.73 %

Extra Pop: %
Debris: 0.04 %
Aggregates: 0.00 %
Modeled Events: 4208

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