CYCLOSCOPE SCREENING BLADDER CANCER

Ref: CYT-CS-SBC 20 TEST

CYCLOSCOPE-SBC is a kit used for the analysis of DNA in epithelial cells for the detection of bladder carcinoma in voided urine.

INTRODUCTION

Bladder cancer is the seventh most common malignancy worldwide, accounting for 3.2% of all cancers [(1)].

The analysis of cell DNA contents in urine samples by flow cytometry together with conventional histopathologic and cytologic studies has been shown to be of great diagnostic and prognostic value in bladder carcinoma. The presence of DNA aneuploidy and/or a high proliferative rate of tumor cells are associated with malignant histology and a poor clinical outcome [(2-4)].

PROCEDURE

This kit has been optimized in order to identify epithelial cells in patients suspected of having bladder cancer with the purpose of studying the existence of DNA aneuploidy and the proliferative rate (proportion of cells in G_0/G_1, S and G_2/M phases of cell cycle) of this cell population in urine samples.

The proposed method is based on the measurements of cell DNA contents on epithelial cells specifically identified by the expression of cytokeratins. Cytokeratins are intermediate filaments present in epithelial cells including those from the urothelium [(5)]. These proteins show differential expression patterns between normal and neoplastic urothelial cells [(6)]. Anti-cytokeratin antibodies have been already used to immunogate urothelial transitional cells for further analysis of their cell DNA contents enabling the specific recognition of bladder cancer cells in tumour samples [(2)].

This double staining method (DNA/cytokeratin) allows the identification of neoplastic cells present in the sample in order to perform a specific analysis of their DNA contents separately from that of normal cells present in the same sample.

GOALS

1.-To study the cell cycle distribution (proportion of cells in G_0/G_1, S and G_2/M phases of cell cycle) of epithelial cells in urine samples.

2.- Detection of DNA aneuploidy as an specific parameter for the presence of a bladder tumour.

Both parameters can be of clinical utility for the diagnosis and the detection of tumour recurrence in patients with bladder carcinoma.

REAGENTS

- Mixture of Primary Antibodies: Vial including a mixture of purified murine monoclonal antibodies specific for the detection of citokeratins present in the cytoplasm of epithelia cells. Antibodies are diluted in PBS with 0,1% NaN_3 as preservative. Ready to use. Add 20 µl / test. Presentation: 400 µl / vial.

- Secondary Antibody: FITC labelled IgG goat anti mouse IgG F(ab)'2. Antibodies are diluted in PBS with 0,1% NaN_3 as preservative. Ready to use. Add 10 µl / test. Presentation: 200 µl / vial.

- DNA Labelling Buffer: 20 ml vial containing detergent, propidium iodide and RNase for DNA staining. Add 1ml / test.

PROTOCOL

1.- Concentrate the cells and wash out the fixing solution

- Centrifuge the fixed urine samples at 540g during 5 minutes in order to concentrate the cells.

- Discard the supernatant and pass it to 5ml polystyrene tubes. Add to the residual volume 3ml of sodium citrate buffer. Centrifuge for 5' at 540g.
- Discard the supernatant obtaining a residual volume of 300µl.

2.- Labelling of epithelial cells characteristic antigens:
- Add 20 µl of the primary antibody mixture to each tube and 1ml of DNA labelling solution. Mix gently.
- Incubate 15' at room temperature in the dark.
- In order to wash out the excess of primary antibodies:
  - Centrifuge for 5' at 540g.
  - Discard the supernatant.
  - Resuspend the cell pellet.
- Add 10 µl of the secondary antibody reagent to each tube. Mix gently.
- Incubate 15' at room temperature in the dark.

3.-Acquire data in a flow cytometer (low speed position). Data acquisition must be performed within the first three hours after sample preparation is finished. Keep tubes at 4 ^{o}C until data acquisition is performed.

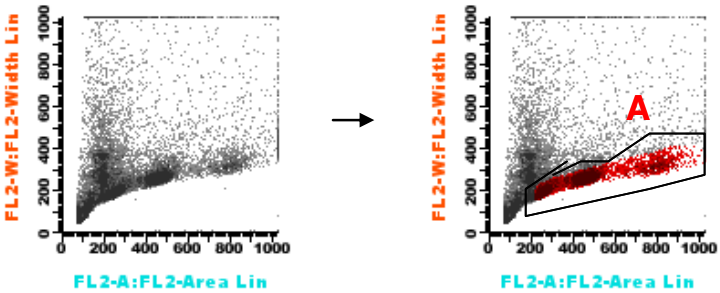
DATA ACQUISITION

Acquire a minimum of 50000 total events. Some samples will not have enough cells, in these cases acquire until the volume of the sample is finished.

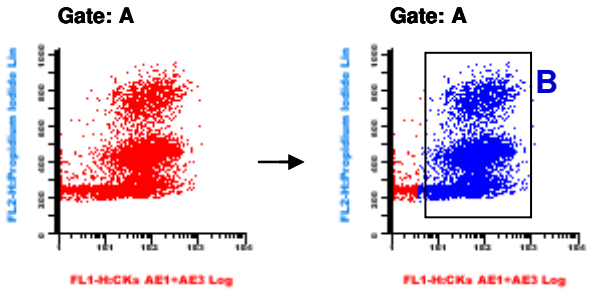
Check that the FL2-Area peak is around 200values in the FL2-Area histogram.

DATA ANALYSIS

1.- Gate cells, excluding the aggregates cellular, in a FL2-Area/FL2-Width dot plot or in a FL2-Area/FL2-Peak dot plot.



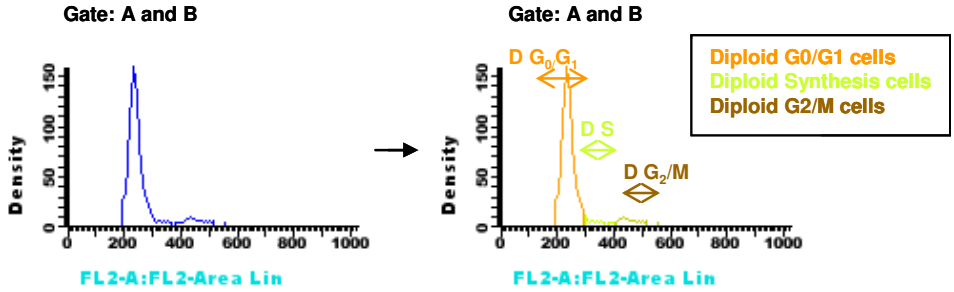
2.- After selecting the cells (Gate A), create a region to select the epithelia cells according to a high FL1-FITC fluorescence intensity.



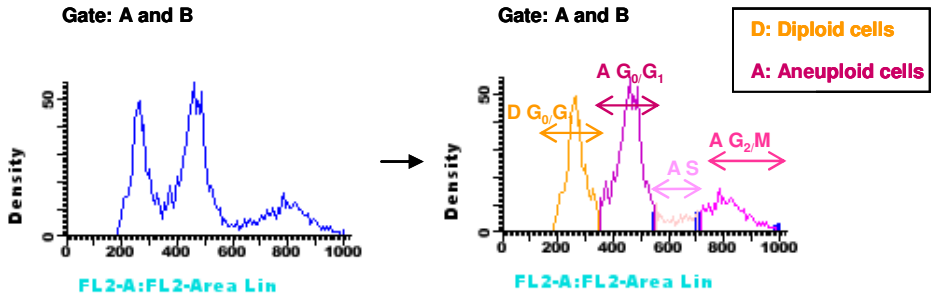
3a.- Cell cycle estimation. Calculate the percentage of epithelial cells in each cell cycle phase using mathematical models included in the specific software programs available in the laboratory.

3b.- Explore the possible existence of DNA aneuploidy after comparing the relative distribution of the G_0/G_1 DNA peak. An aneuploid sample shows at least two different peaks of G_0/G_1 with different cell DNA content corresponding to a heterogeneous sample with normal and aneuploid cells.

*Note. For a correct calculation of the cell cycle distribution of a cell population, the coefficient of variation in G_0/G_1 phase of total cells, must be lower than 15%.



Diploid normal sample



Pathological sample with one aneuploidy

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