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CYCLOSCOPE-MM is a kit used for the analysis of DNA in plasma cells. It focuses on DNA studies of myeloma plasma cells in bone marrow samples from patients with multiple myeloma (MM) and monoclonal gammapathies of undetermined significance (MGUS).

INTRODUCTION

MM is a lymphoproliferative disorder characterized by a clonal expansion of B cells in the last step of differentiation (plasma cells). An accumulation of myeloma plasma cells coming from one or more clones is observed in the bone marrow of patients with MM.

Cell cycle studies in MM have demonstrated to have an important prognostic impact; also the analysis of DNA aneuploidy has been considered to be a powerful tool for the detection of residual disease in these patients and to contribute to the differential diagnosis between MM and MGUS (1, 2).

PROCEDURE

This kit has been optimized in order to identify plasma cells in patients with MM and MGUS with the purpose of studying the existence of DNA aneuploidy and the proliferative rate (proportion of cells in G0/G1, S and G2/M phases of cell cycle) of this cell population.

The proposed method is sensitive and specific, allowing the identification of more than 95% of myelomatosus plasma cells (3). The possible contamination with residual plasma cells (non myelomatosus plasma cells) is irrelevant since the percentage of cells expressing CD38 with high intensity (CD38+++ is usually less than 1% in normal bone marrow (in general around 0,25%) (4).

GOALS

1.-To study the cell cycle distribution in myelomatosus plasma cells.

2.-To detect minimal residual disease in patients with MGUS and MM in complete remission. Aneuploid criteria is defined by the presence of two or more different peaks of G0/G1 cells (5).

CLINICAL UTILITY

1.- Cell cycle

1a.- Prognostic evaluation of MM patients. Recently, it has been described that the

number of bone marrow plasma cells in S-phase (S) in patients with MM is an independent prognostic factor (6).

1b.- To monitorize disease progression.

2.- Minimal residual disease

2a.- Enumeration of clonal plasma cells in bone marrow samples from patients in complete remission, including samples which will be used for autologous transplantation.

2b.- Detection of aneuploid plasma cells in peripheral blood and/or apheresis samples which will be used for autologous transplantation.

SENSITIVITY

The lowest level for detection of aneuploid plasma cells in order to detect minimal residual disease is 10-4 (one cell among 10.000 normal cells) in bone marrow samples.

REAGENTS

- Mixture of Primary Antibodies: Vial including a mixture of purified murine monoclonal antibodies specific for the detection of antigens present in human plasma cells. Antibodies are diluted in PBS with 0,1% NaN3 as preservative. Ready to use. Add 20 µl/test. Presentation: 0,5 ml/vial.

- Secondary Antibody: FITC labelled IgG goat anti mouse IgG F(ab)'2. Antibodies are diluted in PBS with 0,1% NaN3 as preservative. Ready to use. Add 20 µl/test. Presentation: 0,5 ml/vial.

- Lysing Solution: 50 ml vial containing an erythrocyte lysing solution. Add 2 ml/test.

- DNA Labelling Buffer: 30 ml vial containing detergent, propidium iodide and RNase for DNA staining. Add 1ml/test.

PROTOCOL

1.-Perform a white blood cell count of the sample. In case of using bone marrow samples, prior to cell enumeration pass them 3 or 4 times through a syringe in order to disggregate the sample. Take 10^6 cells from the sample, in a volume of 100-150 µl.

2.-Labelling of surface antigens characteristic of human plasma cells:

- Add 20 µl of the primary antibody mixture to each tube. Mix gently.
- Incubate 15' at room temperature (darkness is not necessary).
- In order to wash out the excess of primary antibodies:
  Fill each tube with PBS (2ml/tube).
  Centrifuge for 5' at 540g.
  Discard the supernatant.
  Resuspend the cell pellet.
- Add 20 µl of the secondary antibody reagent to each tube. Mix gently.
- Incubate 15' at room temperature in the dark.
- In order to wash out the excess of secondary antibody:
  Fill each tube with PBS.
  Centrifuge for 5' at 540g.

Discard the supernatant.
Resuspend the cell pellet.

3.-Add 2 ml 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.

4.-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.

5.-Add 1ml of DNA labelling solution. Incubate in the dark for 10' at room temperature (horizontal position).

6.-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 ºC until data acquisition in performed.

DATA ACQUISITION

Data acquisition can be developed using two different procedures as follows:

Procedure 1 (two steps acquisition)

-Acquisition of a minimum of 5000 events (all cells).

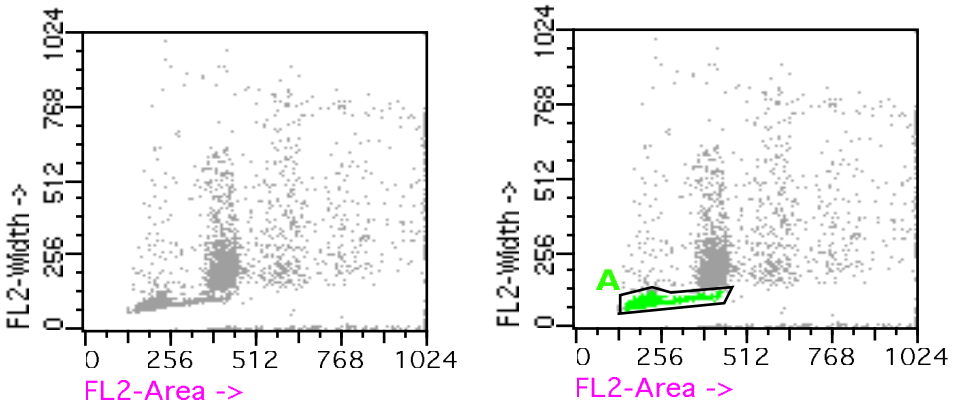
-Acquisition with an activated live gate including those cells displaying a high FL1-FITC fluorescence intensity plasma cells). Acquire a minimum of 5000 plasma cells.

Procedure 2. Acquisition in a single step of a number of events (all cells) including a minimum of 5000 plasma cells.

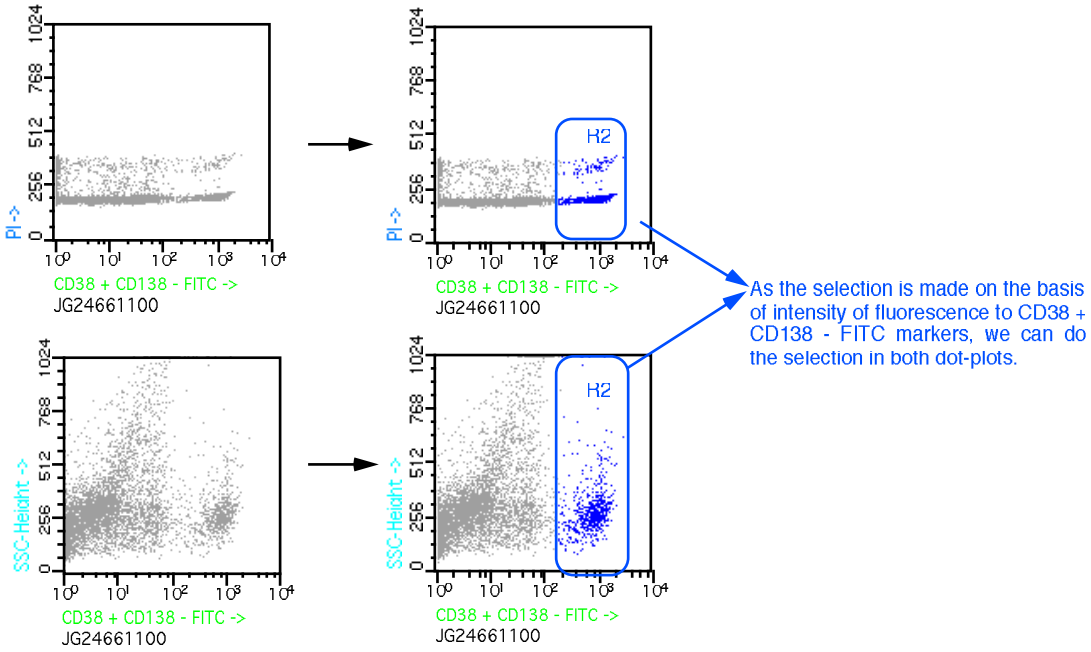
DATA ANALYSIS

General Procedure:

1.- Gate singlets in a FL2-Area/FL2-Width dot plot (in a Becton/Dickinson flow cytometer), in a FL2-Area/FL2-Peak dot plot (instruments from Coulter Corporation) in a FL2-Area/FL2-Peak dot plot (in a Ortho Diagnostic Systems flow cytometer.)



2.- Afler selecting of singlets, paint a gate on plasma cells according to a high FL1-FITC fluorescence intensity. Using two different colours discriminate between those cells displaying high intensity for FLl-FITC (plasma cells) and the normal bone marrow hematopoietic cells (FL1-FITC$^{-/+}$-$^{/+}$).



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

3b.- Calculate the percentage of aneuploid plasma cells in those cases in which detection of minimal residual diseases is requested.

*Note. For a correct calculation of the cell cycle distribution of a cell population, the coefficient of variation in G0/G1, phase of total cells, must be lower than 5%.

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