



Clonalpath™

INSTRUCTION MANUAL

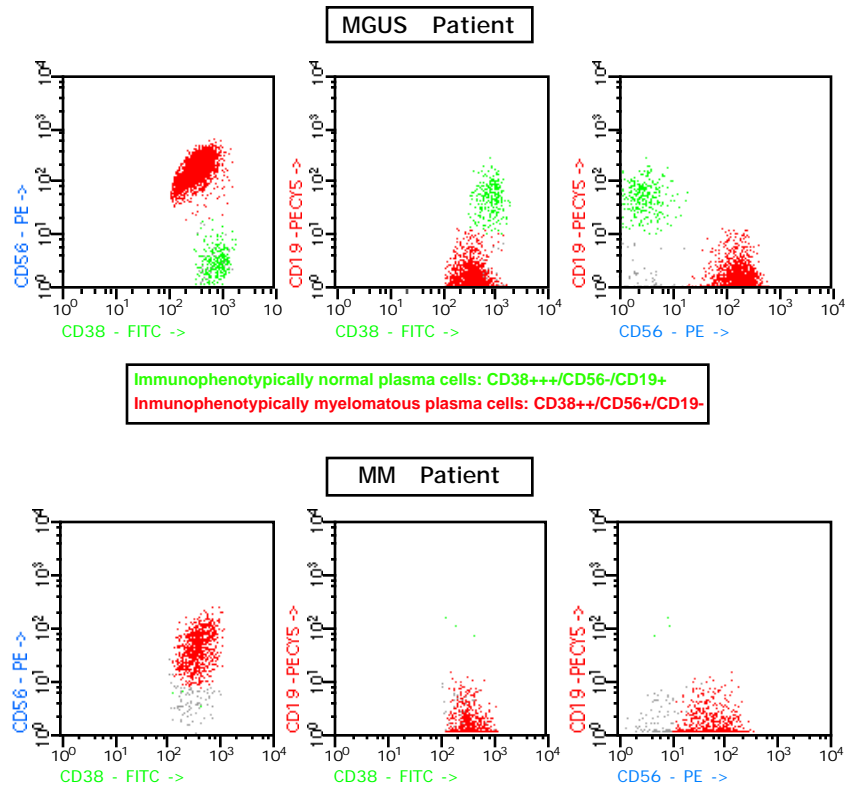
CATALOG NUMBER X1053 (20 TESTS/KIT)

INTRODUCTION

Monoclonal gammopathies are a heterogeneous group of diseases characterized by the expansion of monoclonal plasma cells that produce a monoclonal immunoglobulin (M-component), which is detectable in serum and/or urine. Although multiple myeloma (MM) represents the prototype of monoclonal gammopathy, the most common plasma cell disorder is the monoclonal gammopathy of undetermined significance (MGUS) (1). The importance of accurate differential diagnosis between MM and MGUS is clear. Inappropriate delay of treatment for multiple myeloma permits the development of clinical symptoms and complications, including bone fracture and renal failure. On the other hand, chemotherapy for MGUS exposes patients to an unnecessary treatment. Clonalpath is a reagent which is focused on contributing to establish the differential diagnosis between MGUS and MM based on the determination of the number of residual normal bone marrow plasma cells (BMPC), identified by immunophenotype. Recently, it has been demonstrated that this parameter is the most powerful single criterium for the differential diagnosis between MGUS and MM (2).

PROCEDURE

There are two different PC subpopulations in the bone marrow from MGUS patients (2, 3). One of these PC subpopulations shows phenotypic characteristics identical to those of normal PC and they correspond to residual immunophenotypically normal PC (INPC). This PC subpopulation displays very strong reactivity for the CD38 antigen, low FSC/SSC and positivity for CD19, while it is generally negative for the CD56 antigen. In contrast, the second -and predominant- PC subpopulation shows the opposite pattern, which is typical of myelomatous PC (4, 5): the antigen CD56 is strongly positive, CD19 is constantly negative and shows a lower CD38 expression and higher FSC/SSC values. The presence of immunophenotypically normal PC, which is a constant finding in MGUS patients, is a rare event in MM and when it is present, its frequency is significantly lower than that observed in MGUS (2).



For research use only. Not for use in human diagnostics or therapeutics.

GOALS

This kit has been optimized in order to perform the differential diagnosis between MGUS and MM on the basis of the number of immunophenotypically normal PC from total BMPC. The cut-off point has been suggested to be useful at a value of 3%: MGUS cases display a percentage of immunophenotypically normal plasma cells (CD38⁺⁺⁺CD56⁻CD19⁺) from total BMPC higher than 3% while MM patients present a number of immunophenotypically normal plasma cells lower or equal than 3%.

REAGENT

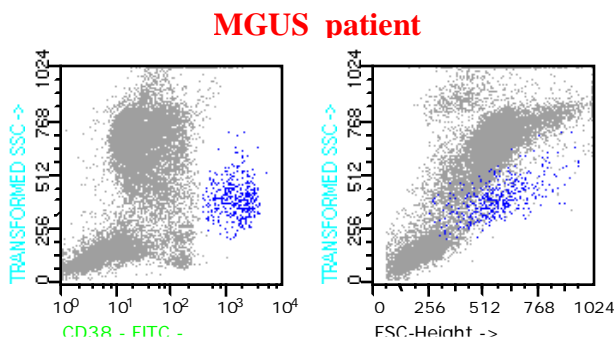
One vial containing a mixture of murine monoclonal antibodies: CD38 conjugated with fluorescein-isothiocyanate, CD 56 conjugated with phycoerythrin and CD19 conjugated with phycoerythrin-cyanine 5. Presentation of the vial 25 test.

PROTOCOL

1. Pass the bone marrow sample 3 or 4 times through a syringe in order to disaggregate cell clumps. Perform a white blood cell count of the sample and take $1-1.5 \cdot 10^6$ cells in a volume of 100-150 μ l.
2. Add 15 μ l of Clonalpath to each tube. Mix gently.
3. Incubate 10' at room temperature in the dark.
4. Add 2 ml of an erythrocyte lysing solution to each tube. Mix gently and incubate the sample according to the general protocol of the laboratory (usually 10 minutes at room temperature).
5. Wash out the lysing solution*:
 - Centrifuge for 5' at 540g.
 - Discard the supernatant and place 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.
6. Add 2 ml of PBS and wash the cells following the same steps detailed in point 5.
7. Acquire data in a flow cytometer. Data acquisition should preferentially be performed within the first three hours after sample preparation is finished. Keep tubes at 4 °C until data acquisition is performed.

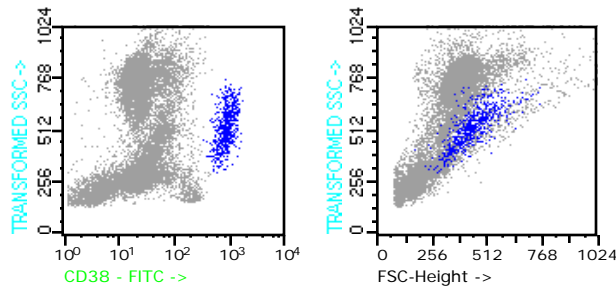
DATA ANALYSIS

1. Gate plasma cells according to their highest fluorescence intensity for CD38-FITC and their FSC/SSC distribution as it is shown in the following diagrams:

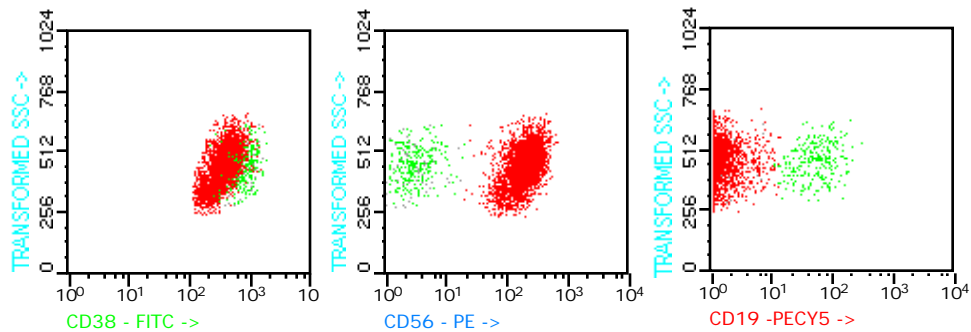


For research use only. Not for use in human diagnostics or therapeutics.

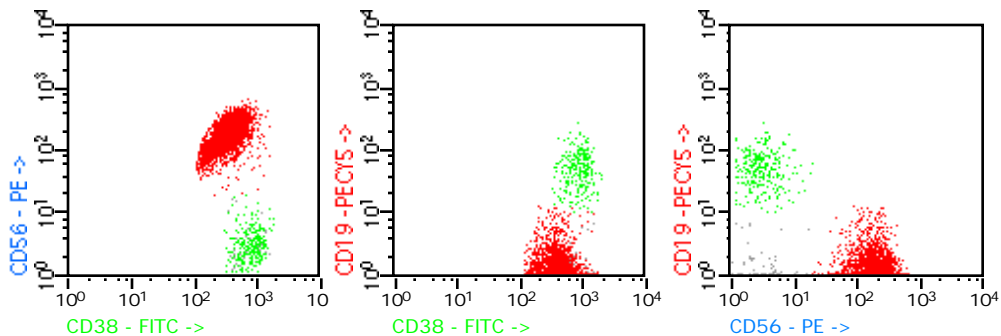
MM patient



2. Discriminate immunophenotypically normal plasma cells and myelomatous plasma cells present in the sample:
- **Immunophenotypically normal plasma cells**, painted in green in the following figures, **display positivity for CD19, they are generally negative for the CD56 antigen and they show a very strong reactivity for the CD38 antigen with low FSC/SSC.**
 - Myelomatous plasma cells, painted in red in the figures shown before, displays the opposite pattern: the antigen CD56 is strongly positive, CD19 is constantly negative and shows a lower CD38 expression and higher FSC/SSC values.



Immunophenotypically normal plasma cells: CD38+++ / CD56- / CD19+
Immunophenotypically myelomatous plasma cells: CD38++ / CD56+ / CD19-



3. When from the total BMPC the percentage of immunophenotypically normal plasma cell is higher than 3%, it is compatible with a MGUS while MM patients usually present a number of immunophenotypically normal plasma cells lower or equal than 3%. with respect to the total number of BMPC.

CLINICAL UTILITY

With the prolongation of life expectancy the probability of recognizing monoclonal components increases, leading to a significant problem for health systems in terms of both the number of patients and the attending costs. Most of these patients will have MGUS and it is well known that in at least a quarter of these patients earlier or later on MGUS will develop into a multiple myeloma, usually after a long period of stability (1, 6). Clonalpath is a reagent that allows the direct

For research use only. Not for use in human diagnostics or therapeutics.

measurement of the proportion of normal and clonal PC present in the bone marrow from these patients, which has recently been shown to be the most powerful single criterium for the differential diagnosis between MGUS and MM (2). The availability of Clonalpath for the discrimination between MGUS and MM has a great clinical value, mainly for border-line cases.

For research use only. Not for use in human diagnostics or therapeutics.

Exalpha Biologicals, Inc., 86 Rosedale Rd. Watertown, MA 02472
Tel: 800.395.1137 or 617.924.3400, Fax: 866.924.5100 or 617.924.5100, Web: www.exalpha.com

REFERENCES

1. Kyle RA. Monoclonal gammopathy of undetermined significance. *Baillier's Clinical Haematology*, 1995; 8: 761-81.
2. Ocqueteau M, Orfao A, Almeida J, Blade J, Gonzalez M, García-Sanz R, López Berges C, Moro J, Hernández J, Escribano L, Caballero MD, Rozman M, San Miguel JF. Immunophenotypic characterization of plasma cells from monoclonal gammopathy of undetermined significance (MGUS) patients. Implications for the differential diagnosis between MGUS and multiple myeloma. *The American Journal of Pathology*, 1998; (in press).
3. Harada H, Kawano MM, Huang N, Harada Y, Iwato K, Tanabe O, Tanaka H, Sakai A, Asaoku H, Kuramoto A. Phenotypic difference of normal plasma cells from mature myeloma cells. *Blood*, 1993; 81: 2658-63.
4. San Miguel JF, García Sanz R, González M, Orfao A. Immunophenotype and DNA cell content in multiple myeloma. *Baillier's Clinical Haematology*, 1995; 8: 735-59.
5. Orfao A, García-Sanz R, López-Berges MC. A new method for the analysis of plasma cell DNA content in multiple myeloma samples using a CD38/Propidium iodide double staining technique. *Cytometry*, 1994; 17: 332-9.
6. Baldini L, Guffanti A, Cesana BM, Colombi M, Chiorboli O, Damilano Y, Maiolo AT. Role of different hematopoietic variables in defining the risk of malignant transformation in monoclonal gammopathy. *Blood*, 1996; 87: 912-18.

For research use only. Not for use in human diagnostics or therapeutics.