

MM MRD

Multiple Myeloma Minimal Residual Disease

Panel



drop-in BV421	drop-in BV510	FITC	PE	PerCP- Cyanine5.5	PE- Cyanine7	APC	APC-C750™
CD138	CD27	CD38	CD56	CD45	CD19	CD117	CD81
CD138	CD27	CD38	CD56	CD45	CD19	CyIgκ	CyIgλ

CYT- MM-MRD8



For In Vitro Diagnostic Use

INTENDED PURPOSE

Multiple Myeloma Minimal Residual Disease Panel (MM-MRD) is a pre-mixed 6-color 8-antibody combination and two antibody drop-ins (anti-CD138-BV421 and anti-CD27-BV510). The panel is designed for the qualitative and semi-quantitative identification and discrimination of malignant and normal plasma cells by flow cytometry using BD FACSCanto™ II or BD FACSLyric™ Flow Cytometers. It is intended to be used with bone marrow specimens of Multiple Myeloma treated patients. This kit must be used by flow cytometry qualified personnel.

The Multiple Myeloma Minimal Residual Disease Panel is designed to recognize the following antigens: CD138, CD27, CD38, CD45, CD56, CD19, CD81, CD117, cytoplasmic IgKappa and IgLambda.

PRINCIPLES OF THE PROCEDURE

Flow cytometry is a technology that allows the simultaneous evaluation of different characteristics of a single cell. Flow cytometers use hydrodynamic focusing to force individual cells past the laser beams. As cells are intercepted by light, a set of detectors recovers two types of signals: those generated by dispersed light (FSC/SSC), which mainly reflect cell size and internal complexity, and those related to light emission when cells are labelled with fluorochromes. Recovered signals are then amplified by a series of linear and logarithmic amplifiers and converted into electrical signals processed and plotted in a graphical representation for a proper interpretation.

The fluorochrome-labeled antibodies specifically bind to the antigens they are directed against, allowing the detection by flow cytometry of the different cell phenotypes.

The erythrocyte population, which could hinder the detection of the target population, should be lysed by using a red blood cell lysing solution.

Minimal Residual Disease (MRD) is defined by the presence, at low frequencies, of residual malignant cells post therapeutic intervention.¹ In the case of multiple myeloma (MM), studies have demonstrated that the presence of 0.01% aberrant plasma cells is associated with a poor outcome.¹



The 2008 European Myeloma Network report¹ together with recent publications^{2,3} establish CD38, CD138 and CD45 analysis in combination with CD19, CD56, CD27, CD81 and CD117 as consensus on the best suited method for MRD detection in MM.^{2,3}

It was also established that cytoplasmic kappa(κ)/lambda(λ) light chain staining is helpful in determining the clonality of abnormal plasma cells.^{3,4} This consensus is translated and improved in the EuroFlow™ MM-MRD multicolor flow cytometry panel.

KIT COMPOSITION

The Multiple Myeloma Minimal Residual Disease Panel (MM-MRD) is composed of two tubes, each containing six fluorochrome-conjugated antibodies in an optimized lyophilized formulation to preserve the stability of the pre-mixed combination of antibodies. Two conjugated antibodies are included as drop-in reagents in a liquid form.

- **Lyophilized Antibody Combination Tube 1:**
 - **Four (4) lyophilized vials of 5 tests for surface staining that contain:**
 - Anti-human CD38-FITC antibody, multi-epitope.
 - Anti-human CD56-PE antibody, clone: C5.9, isotype: IgG2b.
 - Anti-human CD45-PerCP-Cyanine 5.5 antibody, clone: EO1, isotype: IgG2b.
 - Anti-human CD19-PE-Cyanine7 antibody, clone: SA287, isotype: IgG1.
 - Anti-human CD117-APC antibody, clone: 104D2, isotype: IgG1.
 - Anti-human CD81-APC-C750™ antibody, clone: M38, isotype: IgG1.
- **Lyophilized Antibody Combination Tube 2:**
 - **Four (4) lyophilized vials of 5 tests each for surface staining that contain:**
 - Anti-human CD38-FITC antibody, multi-epitope.
 - Anti-human CD56-PE antibody, clone: C5.9, isotype: IgG2b.
 - Anti-human CD45-PerCP-Cyanine 5.5 antibody, clone: EO1, isotype: IgG2b.
 - Anti-human CD19-PE-Cyanine7 antibody, clone: SA287, isotype: IgG1.
 - **Four (4) lyophilized vials of 5 tests each for cytoplasmic staining that contain:**
 - Anti-human Cylgκ-APC goat antibody, clone: polyclonal.
 - Anti-human Cylgλ-APC-C750™ goat antibody, clone: polyclonal.
- **Liquid Form Drop ins:**
 - Anti-human CD138-BV421 antibody, clone: MI15, isotype: IgG1: enough for 50 tests.
 - Anti-human CD27-BV510 antibody, clone: O323, isotype: IgG1: enough for 50 tests.

Table 1: Multiple Myeloma Minimal Residual Disease antibody panel composition

	Fluorochrome	Brilliant Violet™ 421	Brilliant Violet™ 510	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750™
TUBE 1	Antibody	CD138*	CD27*	CD38	CD56	CD45	CD19	CD117	CD81
	Clone	MI15	O323	multiepitope**	C5.9	EO1	SA287	104D2	M38
	Isotype	IgG1	IgG1	multiepitope**	IgG2b	IgG2a	IgG1	IgG1	IgG1
TUBE 2	Antibody	CD138*	CD27*	CD38	CD56	CD45	CD19	Cy-Igκ	Cy-Igλ
	Clone	MI15	O323	multiepitope**	C5.9	EO1	SA287	N/A	N/A
	Isotype	IgG1	IgG1	multiepitope**	IgG2b	IgG2a	IgG1	N/A	N/A

* For lot specific compensation of CD27 BV510 and CD138 BV421, use the liquid reagents available in the kit.

** Proprietary

- **Lyophilized compensation tubes:** Four (4) lyophilized tubes of one (1) test each to compensate:
 - CD45-PerCP-Cyanine5.5
 - CD19-PE-Cyanine7
 - CD81-APC-C750™
 - Lambda APC-C750™
- **Fixative-free ammonium chloride erythrocyte lysing solution** 10X (Bulklysis™).
- **Fixation and permeabilization solutions** (Fix&Perm®, Nordic-MUBio BV, The Netherlands).



STORAGE CONDITIONS

- The reagent is stable until the expiration date shown on the label, when stored at 2–8°C.
- The reagents should not be frozen or exposed to direct light during storage or during incubation with the specimen.
- Keep all vials in a dry place. Once opened, the vials must be stored in a vertical position to avoid any possible spillage.
- Unused reagent remaining in the vial after reconstitution is stable for four (4) weeks from the reconstitution date if stored at 2–8°C, not frozen or exposed to direct light.
- Reagents are not considered sterile.

WARNINGS AND RECOMMENDATIONS

1. For *in vitro* diagnostic use.
2. Alteration in the appearance of the reagent, such as precipitation or discoloration indicates instability or deterioration. In such cases, the reagent should not be used.
3. All components contain sodium azide (NaN₃, CAS-Nr. 26628-22-8). Although sodium azide is used as a preservative, care should be taken to avoid microbial contamination of reagents.
4. For information regarding identification and classification of hazards and precautionary statements on chemical substances of the product, please, refer to Material Safety Data Sheet (MSDS) available at www.cytognos.com. Follow local disposal regulations for your location along with recommendations in the Material Safety Data Sheet to determine the safe disposal of this product.
5. All biological specimens and materials are considered biohazards. Handle as if capable of transmitting infection^{5,6} and use proper precautions for disposal in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.
6. Use of reagent with dilutions, incubation times or temperatures different from those recommended may cause erroneous results. Any such changes must be validated by the user.
7. The reagent is stable until the expiration date shown on the label if it is properly stored. Do not use after the expiration date shown on the label.
8. Acquire samples immediately after staining. If it cannot be done, store them at 4°C for one (1) hour maximum. Do not use samples which have not been kept under these conditions.
9. The Multiple Myeloma Minimal Residual Disease Panel (MM-MRD) contains lyophilized vials. Carefully read the following instructions for reconstitution before beginning. These instructions are included in the appropriate sequence of the recommended procedure.

SPECIMEN COLLECTION AND PREPARATION

Collect 2-5 mL of bone marrow aspirates. First-pull aspirates are highly recommended.

Specimen must be collected in commercially available anticoagulant-treated tube (not included). Use of EDTA is highly recommended. Make sure the sample is well mixed with the anticoagulant.

All specimen collection and transport steps should be performed at room temperature (RT).

Specimens shall be stored at RT for a maximum of 36 hours post-collection. They shall be acquired immediately after staining or within 1 hour from the final re-suspension if kept at 2–8°C, protected from light.

PROCEDURE

The MM-MRD assay was designed in collaboration with the EuroFlow™ Consortium and the protocol was developed following EuroFlow™ standardized recommendations and SOPs.

Materials required but not included

- BD FACSCanto™ II or BD FACSLyric™ Flow Cytometers
- Infinicyt™ Software
- SPHERO™ Rainbow Calibration Particles, 8 peaks, 3.0-3.4 µm (Catalog No: SPH-RCP-30-5A)
- For BD FACSLyric™; use BD CS&T Beads (Catalogue No: 656504 or 656505)



- For BD FACSCanto™ II; use FACSDiva CS&T IVD Beads (Catalogue No: 656046 or 656047)
- Disposable 12 x 75 mm flow cytometry test tubes (5 mL tubes)
- Automatic pipette and tips
- 50 mL centrifuge tubes (e.g., Falcon™ tubes)
- Chronometer
- Pasteur pipettes or vacuum system
- Vortex mixer
- Roller mixer or sample-rocking device
- Centrifuge
- BD FACS™ Lysing solution (Catalog No: 349202)
 - o See the *BD FACS™ Lysing Solution* instructions for use (IFU) for precautions and warnings.
- Wash buffer (filtered solution of phosphate buffered saline [PBS] containing 0.09% [w/v] NaN₃, 0.2% [w/v] bovine serum albumin [BSA] and 2 mM ethylenediaminetetraacetic acid [EDTA]).
- Acquisition buffer (filtered PBS solution containing 0.2% [w/v] BSA and 2 mM EDTA [NaN₃-free])
- Distilled water
- Deionized water

Setting up the Instrument

The MM-MRD reagent was designed in collaboration with the EuroFlow™ Consortium and the protocol was developed following EuroFlow™ standardized recommendations and SOPs.

Use BD FACSCanto™ II or BD FACSLytic™ flow cytometers for sample acquisition.

Follow instructions on Cytognos' website (www.cytognos.com/products/cyt-mm-mrd8/) for instrument set-up recommendations.

Reconstituting the Lyophilized Reagents

To reconstitute the lyophilized vials:

1. Add distilled water to each lyophilized reagent vial
 - o Tube 1 - 180 µL
 - o Tube 2 (surface staining) - 120 µL
 - o Tube 2 (cytoplasmic staining) - 70 µL
2. Place each vial in a "roller" mixer and mix thoroughly for 30 minutes at RT.
3. Spin down the reconstituted vials before each use.

To reconstitute the lyophilized compensation tubes:

1. Add the corresponding volume of peripheral blood intended for compensation directly into each tube.
2. Follow the protocol indicated below (Staining steps for specific compensation controls).

Diluting the Lysing Solutions

Dilute sufficient 10X Bulklysis™ solution to lyse the specimens. Dilute the 10X Bulklysis™ solution 1:10 with RT distilled water to make a 1X solution.

Dilute the BD FACS™ Lysing Solution 10X concentrate 1:10 with RT deionized water. The prepared solution is stable for 1 month when stored in a glass or high-density polyethylene (HDPE) container at RT.

Lysing the Specimen:

1. Determine the absolute count of nucleated cells per µL of the specimen.
2. Lyse sufficient volume of sample such that after bulk lysis, 10x10⁶ nucleated cells can be stained per tube. Do not use more than 2 mL of specimen per 50 mL of 1X Bulklysis™ solution. If larger volumes of specimen are needed, use several 50 mL tubes

Note: During the lysing process, an unselective loss of cells occurs, and 10 x 10⁶ cells per each MM-MRD tube to be stained (total of 2 tubes) are needed.

Note: Stain at least 10 x 10⁶ nucleated cells for each tube and acquire at least 5 x 10⁶ cells/tube.



3. Add 1X Bulklysis™ solution to give a total of 50 mL in each tube.
4. Mix well by manually inverting the tube and incubate for 15 min on a roller or sample-rocking device.
5. Centrifuge at 800g for 10 minutes and remove supernatant using a Pasteur pipette or a vacuum system without disturbing the cell pellet (approximately 300 µL of cell suspension should remain in the tube).
6. Add 2 mL of wash buffer and resuspend the cell pellet vigorously, preferably by vortex mixing.
7. Add wash buffer to the tube containing the cell suspension up to 50 mL.
8. Mix well by manually inverting the tube.
9. Centrifuge at 800g for 5 minutes and remove supernatant using a Pasteur pipette or a vacuum system, without disturbing the cell pellet.
10. Resuspend the cell pellet in 2 mL of wash buffer. Mix well and transfer this volume to a 5 mL flow cytometry tube.
11. Add 2 mL of wash buffer to the 50 mL tube to recuperate any cells that might be left in the bottom.
12. Transfer the cells to the 5 mL flow cytometry tube containing the rest of the sample from step 10.
13. Centrifuge at 540g for 5 min and remove the supernatant by decanting or using a Pasteur pipette.
Note: If multiple 50 mL tubes were used, combine the cell suspensions from all the tubes into one tube before adjusting the cell concentration. Keep the final volume low so that, when the cell concentration needs to be adjusted as indicated in the next step, it can be done by diluting with wash buffer.
14. Resuspend the pellet with wash buffer to give a final cell concentration of 1×10^5 cells/µL.
Note: 100 µL containing 10×10^6 cells of the lysed sample (1×10^5 cells/ µL) will be needed for each tube to be stained.

Staining steps for surface membrane markers only (MM-MRD Tube 1):

1. Reconstitute the lyophilized vial following steps in “Reconstituting the Lyophilized Reagents” section.
2. Add 30 µL of reconstituted antibody mixture in a 5 mL flow cytometry tube.
3. Add 10µL of CD27 BV510 and 3µL of CD138 BV421 to the tube.
4. Add 100 µL of cell suspension containing 10×10^6 cells to the tube.
5. Mix well by vortexing.
6. Bring the final volume to 200 µL with wash buffer.
7. Incubate for 30 minutes at RT protected from light.
8. Add 2 mL of 1X BD FACS™ Lysing solution.
9. Mix well by vortexing.
10. Incubate for 10 minutes at RT protected from light.
11. Centrifuge for 5 minutes at 540g.
12. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leaving approximately 100 µL of residual volume in the tube.
13. Add 2 mL of wash buffer.
14. Mix well by vortexing.
15. Centrifuge for 5 minutes at 540g.
16. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leaving approximately 100 µL of residual volume in the 5 mL tube.
17. Resuspend the cell pellet with acquisition buffer in a total volume of 500 µL.
18. Acquire a minimum of 5×10^6 cells immediately after staining or store at 4°C (for 1 hour maximum) until acquisition.

Staining steps for combined staining of surface membrane and cytoplasmic markers (MM-MRD Tube 2):

1. Reconstitute the lyophilized vial for surface staining following steps in “Reconstituting the Lyophilized Reagents” section.
2. Add 20 µL of reconstituted surface staining antibody mixture to a 5 mL flow cytometry tube.
3. Add 10µL of CD27 BV510 and 3µL of CD138 BV421 to the tube.
4. Add 100 µL of cell suspension containing 10×10^6 cells to the tube.
5. Mix well by vortexing.
6. Bring the final volume to 200 µL with wash buffer.
7. Incubate for 30 minutes at RT protected from light.

8. Add 2 mL of wash buffer to the tube containing the cell pellet.
9. Mix well by vortexing.
10. Centrifuge for 5 minutes at 540g.
11. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leaving approximately 100 μ L of residual volume in the tube.
12. Resuspend the cell pellet by mixing gently.
13. Add 100 μ L of Fix&Perm[®] Solution A (Fix&Perm[®], Nordic-MUBio BV, The Netherlands) and mix thoroughly by vortexing for 1-2 seconds.
14. Incubate for 15 minutes at RT protected from light.
15. Add 2 mL of wash buffer to the tube containing the cell pellet.
16. Mix well by vortexing.
17. Centrifuge for 5 minutes at 540g.
18. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leaving approximately 100 μ L residual volume in the 5 mL tube.
19. Vortex vigorously to resuspend the cell pellet.
20. Add 100 μ L of Fix&Perm[®] Solution B (Fix&Perm[®], Nordic-MUBio BV, The Netherlands).
21. Mix well by vortexing.
22. Reconstitute the lyophilized vial for cytoplasmic staining following steps in "Reconstituting the Lyophilized Reagents" section.
23. Add 10 μ L of reconstituted cytoplasmic staining antibody mixture to the tube containing the 200 μ L of cell suspension in Fix&Perm[®] Solution B (Fix&Perm[®], Nordic-MUBio BV, The Netherlands) and cell suspension.
24. Mix well by vortexing.
25. Incubate for 15 minutes at RT protected from light.
26. Add 2 mL of wash buffer to the tube containing the cell pellet.
27. Mix well by vortexing.
28. Centrifuge for 5 minutes at 540g.
29. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leaving approximately 100 μ L of residual volume in the tube.
30. Resuspend the cell pellet with acquisition buffer in a total volume of 500 μ L.
31. Acquire a minimum of 5×10^6 cells immediately after staining or store at 4°C (for 1 hour maximum) until acquisition.

Staining steps for specific compensation controls:

Ensure that the lyophilized drop from the monotube is on the bottom of the tube. Spin down is recommended after adding specimens.

For CD45-PerCP-Cyanine5.5, CD19-PE-Cyanine7 and CD81-APC-C750[™] compensation control tubes, add 50 μ L of normal peripheral blood into the compensation tube. Add wash buffer to reach a final volume of 100 μ L per tube.

For Lambda-APC-C750[™] compensation with peripheral blood, 3 washing steps are required prior to staining to remove soluble light chains:

- a) Mix 100 μ L of peripheral blood with 4 mL of wash buffer in a 5mL tube.
- b) Centrifuge for 5 minutes at 540g.
- c) Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet.
- d) Add 4 mL of wash buffer to the tube.
- e) Centrifuge for 5 minutes at 540g.
- f) Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet.
- g) Add 4 mL of wash buffer to the tube.
- h) Centrifuge for 5 minutes at 540g.
- i) Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet,
- j) Add 100 μ L of wash buffer and resuspend the cell pellet.

- k) Add 50 μ L of the resuspended cell pellet obtained into the Lambda-APC-C750™ compensation control tube.
 1. Mix gently by vortexing.
 2. Incubate for 30 minutes at RT, protected from light.
 3. Add 2 mL of BD FACS™ Lysing Solution.
 4. Mix gently by vortexing.
 5. Incubate for 10 minutes at RT protected from light.
 6. Centrifuge for 5 minutes at 540g.
 7. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leaving approximately 100 μ L of residual volume in the 5 mL tube.
 8. Add 2 mL of wash buffer.
 9. Mix gently by vortexing.
 10. Centrifuge for 5 minutes at 540g.
 11. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leaving approximately 100 μ L of residual volume in the 5 mL tube.
 12. Resuspend the cell pellet with acquisition buffer in a total volume of 300 μ L.
 13. Acquire the sample immediately after staining. If the sample cannot be acquired immediately after staining it can be stored at 4°C for 1 hour maximum prior to acquisition.

Note: The compensation control acquisition should be performed according to cytometer manufacturer instructions for use.

For the compensation of drop in liquid reagents, follow laboratory routine compensation procedure according to cytometer manufacturer instructions for use.

Flow cytometry analysis

The use of Infinicyt™ software for analysis allows data integration and multidimensional analysis of flow cytometry data. This analysis software was developed as part of the EuroFlow™ project “Flow cytometry for fast and sensitive diagnosis and follow-up of hematological malignancies”. Complete information about Infinicyt™ is available on the website: www.cytognos.com/infinicyt.

EuroFlow™ has created a MM MRD database using normal specimens that allows for the automated identification of populations using this panel.⁸ MM MRD FCS files can be analyzed using the database or manually using a pre-established gating strategy. A full description of the steps required for both analyses is listed below.

Files generated can be analyzed with Infinicyt™ using the EuroFlow™ database which automatically analyzes the sample and gives a standard report in different languages.

1. Go to Profile > Configure > Databases > Language and select your preferred language for MM-MRD reporting.
2. Click on Databases in the main window and select the two files to be analyzed (MM-MRD Tube 1 and Tube 2).
3. Select type of sample> Bone Marrow.
4. Select MM-MRD database.
5. Click OK.
6. Confirm all marker labels are correct by comparing them to the ones in the database.
7. After the automatic gating and identification process is finished, verify all events in the Check Populations to find the abnormal PCs.
8. Open the report.

Note: contact support@cytognos.com for any question regarding MM-MRD analysis using the EuroFlow™ databases.

Files can also be analyzed manually using the EuroFlow™ MM-MRD Profile.

1. Load the MM-MRD Profile from the Infinicyt™ profile manager.
2. Perform manual gating according to the international recommendation.³

3. Note that samples treated with CD38 monoclonal antibody therapies might show a dimmer expression of this marker.
4. You can load the normal reference image from the profile. Classify for each of the three normal plasma cells subpopulations (based on CD19 and CD56) and kappa and lambda clonality.
5. Verify that the ratio is within normal.
6. Classify the abnormal PC population according to its differential expression from normal plasma cells.

PERFORMANCE CHARACTERISTICS

Specificity

- The CD19 antigen is expressed on the cell surface of normal and neoplastic B cells, and it is not expressed by T cells, monocytes and granulocytes.
- The Anti-Kappa antibodies react with free kappa light chains, as well as intact immunoglobulin molecules bearing kappa light chains.
- The Anti-Lambda antibodies react with free lambda light chains, as well as intact immunoglobulin molecules bearing lambda light chains.
- The CD56 antigen is expressed on natural killer cells (activated and resting), on CD3+ T cell subsets, and CD56 may be expressed by dysplastic monocytes and neutrophils.
- The CD38 antigen is expressed by plasma cells, B cell precursors, other BM precursors, activated T cells, monocytes, dendritic cells and macrophages. Expression of CD38 helps characterize several hematological malignancies.
- The CD45 antigen recognizes human leukocytes including lymphocytes, monocytes, granulocytes, and eosinophils. Erythrocytes, platelets and non-hematopoietic cells do not express CD45 antigen.
- The CD117 antigen is expressed on myeloid precursors and mast cells in bone marrow. In around one-third of both multiple myeloma cases and patients with monoclonal gammopathy of undetermined significance, myelomatous plasma cells display reactivity for CD117.
- The CD81 antigen is expressed by normal lymphocytes, monocytes and eosinophils, but not by platelets and neutrophils. Most B lymphocytes, at all stages of cellular differentiation, express CD81.
- The CD27 antigen is expressed by lymphocytes identifying maturation stages, as well as by normal plasma cells. The CD27 expression can be altered (lost or diminished) by abnormal plasma cells.
- The CD138 antigen is expressed by mature, bone marrow plasma cells. The expression of CD138 can be diminished by Heparin anticoagulants and by sample age. The CD138 expression might also be altered on abnormal plasma cells.

Accuracy-Trueness

The percentages of normal and abnormal plasma cells have been compared between two panel versions:

- Previous Panel: EuroFlow™ proposed lyophilized alternative reagents including EF reference drop-ins (CD27 BV510 and CD138 BV421).
- New Panel: CYT-MM-MRD8 (Cytognos, S.L.).

A total of 29 EDTA bone marrow specimens (12 normal BM, 11 MM follow-up and 6 MM diagnostic) were included in the study and acquired in BD FACSCanto™ II or BD FACSLyric™ flow cytometers. Data was analyzed with Infinicyt™ software. The following table shows that the results are equivalent in their reactivity in bone marrow specimens in terms of percentage (frequency) of the target plasma cell populations.

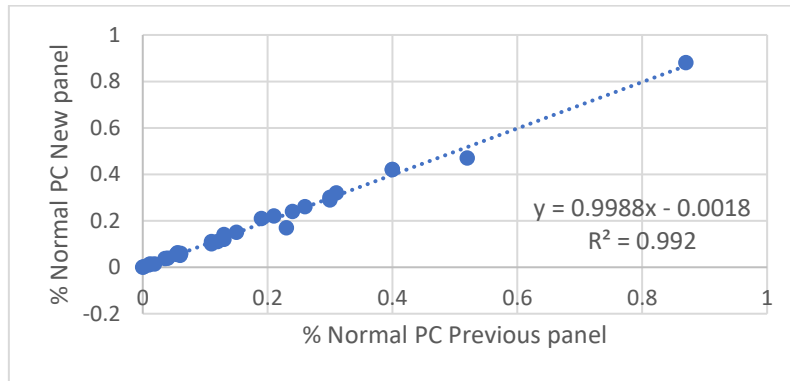


Figure 1: Correlation of normal plasma cells frequencies between both panels.

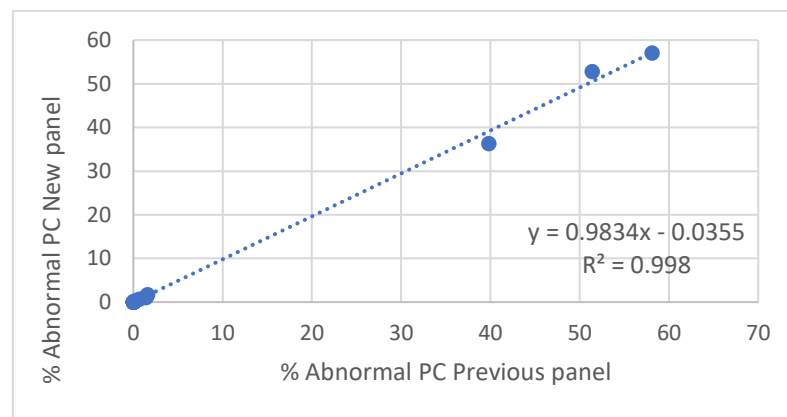


Figure 2: Correlation of abnormal plasma cells frequencies between both panels.

Table 2: Accuracy-Trueness Results for MM-MRD.

Target Populations	Acceptance criteria	Results
Frequency of Normal plasma cells	Coefficient of determination $R^2 > 0.8$	Frequency of normal plasma cells: $R^2 = 0.99$
Frequency of Abnormal plasma cells		Frequency of abnormal plasma cells: $R^2 = 0.99$

Precision (Repeatability and Reproducibility)

For precision studies, two lots of MM-MRD were used. An artificial matrix (composed by BD Multi-Check™ Control, CD-Chex CD117 Plus® and CD-Chex CD103 Plus®) was stained with the two lots in triplicate per run (morning and afternoon) by two operators and acquired on two BD FACSCanto™ II and on two BD FACSLyric™ flow cytometers for five days, respectively.

Using the Infinicyt™ software to gate the populations of interest, results were analysed separately for the BD FACSCanto™ II and BD FACSLyric™ to compare multiple lots, operators and the number of instruments across two runs per day for five days.

The frequency of the populations was determined and % CV calculated across each instrument platform independently (BD FACSCanto™ II or BD FACSLyric™) taking into account reagent lots, replicates, runs and operators.

Table 3: BD FACSCanto™ II precision measured as %CV of frequencies for indicated populations.

Populations	MM-MRD8 precision frequencies (BD FACS Canto™ II)	
	Tube 1	Tube 2
	% CV Frequency	% CV Frequency
B Cells	3.79	3.2
Normal plasma cells	12.61	14.59
Ig Kappa Mature B cells	-	4.03
Ig Lambda Mature B cells	-	3.05
CD56 +TNK cells	11.11	8.55
Monocytes	3.93	4.09
CD138 + cells	9.39	6.63
CD117 + cells	9.64	-

Table 4: BD FACSLytic™ precision measured as %CV of frequencies for indicated populations.

Populations	MM-MRD8 precision frequencies (BD FACS Lyric™)	
	Tube 1	Tube 2
	% CV Frequency	% CV Frequency
B Cells	2.43	3.24
Normal plasma cells	13.21	14.48
Ig Kappa Mature B cells	-	3.31
Ig Lambda Mature B cells	-	3.32
CD56 +TNK cells	11.17	14.72
Monocytes	4.13	4.77
CD138 + cells	9.71	8.37
CD117 + cells	2.42	-

LIMITATIONS

- It is advisable to acquire stained samples as soon as possible to optimize results because prolonged exposure of samples to lytic reagents may cause white cell destruction, targeted population cell loss and nonspecific staining.
- When using whole blood lysing procedures some red blood cells may not lyse, for instance if there are nucleated red blood cells or if abnormal protein concentration and hemoglobinopathies are observed. This may cause misleading results since unlysed red blood cells are counted as leukocytes.
- Results obtained by flow cytometry may be erroneous if the cytometer lasers are misaligned or if gates are incorrectly set.
- Knowledge of antigen normal expression pattern and its relation to other relevant antigens is paramount to carry out an adequate analysis.



QUALITY CONTROL

- To set PMT voltages for target fluorescence channel for BD FACS Canto™ II, use SPHERO™ Rainbow Calibration Particles (SPH-RCP-30-5A); for BD FACS Lyric use CS&T beads (656504 or 656505) for specific instrument setup. Follow instructions on Cytognos' website (<https://www.cytognos.com/products/cyt-mm-mrd8/>) for instrument set-up recommendations.
- Pipettes precision and cytometer calibration should be verified to obtain optimal results.
- In multicolor panels, fluorochromes emit in wavelengths that can show certain spectral overlap which must be corrected by electronic compensation. Optimal compensation levels can be established by analyzing cells from healthy individuals stained with mutually exclusive monoclonal antibodies conjugated with appropriate fluorochromes.
- This product has been manufactured in accordance with standards of production and quality system of the ISO 9001:2015 and ISO 13485:2016 standard.

NOTICE

EU Only: Users should report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local Cytognos representative for any incident or inquiry related to this device.




REFERENCES








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This product is warranted only to conform to the quantity and contents stated on the label. There are no warranties that extend beyond the description on the label of the product. Cytognos' sole liability is limited to either replacement of the product or refund of the purchase price.

EXPLANATION OF SYMBOLS

	Use-by date
	Catalogue number
	Batch code

	Temperature limit
	Keep away from sunlight
	Consult <i>instructions for use</i> or consult <i>electronic instructions for use</i>
	Manufacturer
	In vitro diagnostic medical device
	Contains sufficient for <n> tests
	CE marking; Signifies European technical conformity

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VERSION HISTORY

Version	Date	Modifications
1.0	2022-04-25	Initial release