THESE ARE EXAMPLE INSTRUCTIONS ONLY

ROUND-SPECIFIC INSTRUCTIONS WILL BE PROVIDED AFTER REGISTERING TO A ROUND

Instructions for performing the EuroFlow MM MRD EQA round

Scheme: MM MRD
Year: 2025
Round: II
Start reporting period: 1 September 2025 (00:00 CEST)
End reporting period: 29 September 2025 (23:59 CEST)

Participation in the EuroFlow MM MRD EQA scheme will provide laboratories, which routinely use the EuroFlow Next Generation Flow (NGF) MM MRD antibody panel and relevant EuroFlow SOPs, with an external control for multiparametric flow cytometry detection of MRD in patients with MM after therapy.
***Note**: In this document, links to EuroFlow SOPs are shown in blue and can be accessed after user login on* https://app.euroflow.org/downloads/public.

Case files and patients' data:
You will analyze 3 bone marrow MM MRD cases (2 FCS files per case; one for Tube 1 and one for Tube 2), which were generated by two expert EuroFlow reference centers following the NGF for MM MRD methodology[1] for sample processing, staining, and acquisition.

Case 2025 II #1: Male, 46y, End of induction with Daratumumab-Lenalidomide-Dexamethasone
Case 2025 II #2: Female, 70y, End of induction with Daratumumab-Lenalidomide-Dexamethasone
Case 2025 II #3: Female, 65y, End of induction with Daratumumab-Lenalidomide-Dexamethasone

General instructions:
For full compliance with NGF recommendations, you are advised to analyze the files using the Infinicyt™ software and automated gating & identification (AG&I) analysis tool and database. Nonetheless, alternative analysis software may also be used.

All major cell populations in the samples should be identified. The estimation of limit of detection (LoD) and limit of quantitation (LoQ) will follow previously published recomendations[1,2] i.e., 20 dots and 50 dots, respectively, using total nucleated cells as denominator.

Data analysis:
Analyze each case's files and obtain the following data, as applicable:

- Percentage of cell populations present (out of total cellularity, excluding debris and doublets), namely:

  - Normal plasma cells
  - Aberrant plasma cells
  - B-cell precursors
  - Mature B cells
  - T/NK cells & basophils
  - Eosinophils
  - Neutrophils
  - Monocytes
  - Myeloid precursors
  - Mast cells
  - Nucleated red blood cells

- Percentage of normal and aberrant plasma cells from total plasma cell population (i.e., using total plasma cells as denominator)

- Calculated LoD
- Calculated LoQ

- Immunophenotypic characterization of aberrant plasma cells
  - FSC
  - SSC
  - CD38
  - CD138
  - CD45
  - CD19
  - CD56
  - CD27
  - CD81
  - CD117
  - cylgκ
  - cylgλ

- Overall analysis results and conclusion
  - MRD status (Negative/Positive but not quantifiable/Positive)
  - Signs of hemodilution (Yes/No)
  - The number of analyzed events was adequate according to the recommended standard (Yes/No)
  - A written conclusion of your analysis as for a final report

Data reporting:

- Log in to the ESLHO EQA Portal
- Complete the results form for each case.
- Note that, so long as there is an internet connection, data entries are auto-saved constantly. This is indicated by the "All changes are saved" notification in the top right corner of each results form. This allows you to partially complete the form and return to it later for further

completion (or to make changes to the entered data, if needed) so long as the reporting period is open.

- Note that blue fields are mandatory to complete and that submission of results is only possible when all mandatory fields are filled in.
- After filling in the form, click on "Submit results" (note that you will need to do this for each case separately). You will receive a confirmation in the browser that the results are submitted. Additionally, all contacts linked to the round receive a confirmation email, which includes a pdf file with the submitted results.
- Even after submitting the results, you can still make changes to the data entered in the results form and resubmit it so long as the reporting period is open. This allows you to make any corrections and resubmit, as needed.

Questions/comments:

In case you have any questions or comments, please do not hesitate to contact us at EuroFlow.EQA@eslho.org (please state your name and institution/laboratory in the e-mail).

Recommended references:

1. Flores-Montero J et al., **Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma.** Leukemia. 2017 Oct;31(10):2094-2103.
2. Arroz M et al., **Consensus guidelines on plasma cell myeloma minimal residual disease analysis and reporting.** Cytometry B Clin Cytom. 2016 Jan;90(1):31-9.
3. Rawstron AC et al., **Report of the European Myeloma Network on multiparametric flow cytometry in multiple myeloma and related disorders.** Haematologica. 2008 Mar;93(3):431-8.
4. Flores-Montero J et al., **Immunophenotype of normal vs. myeloma plasma cells: Toward antibody panel specifications for MRD detection in multiple myeloma.** Cytometry B Clin Cytom. 2016 Jan;90(1):61-72.
5. EuroFlow SOP for bulk lysis in MRD panels. Version 1.3 - June 2018