MM MRD EQA certificate

Participation and performance certificate of the EuroFlow MM MRD EQA scheme

Round [YYYY X]

[Laboratory name]

[Organization name]

[City, Country]

Code of the laboratory: A000

The Flow Cytometry Units at the Hematology Department of the University Hospital of Salamanca, and at University of Salamanca (Salamanca, Spain) operate as the lead expert laboratories of the MM MRD scheme, with Dr. Juan Flores-Montero in the role of lead subject-matter expert. In addition, other EuroFlow subject-matter experts provide support with case selection, determination of reference results, data analysis, performance evaluation, and reporting (see p. 12 for a complete list).

This MM MRD EQA certificate, including the round summary report, was authorized by Prof. Dr. Jacques J.M. van Dongen, ESLHO EQA Program Coordinator, and was issued on behalf of ESLHO.

A guide through the certificate

This EQA certificate consists of four parts in which performance results are provided:

1) An overview of your results compared to the reference result regarding the conclusion on MRD. This part includes a performance score. An explanation on how performance is scored can be found on p. 9-10.
2) A summary table including your results, the reference result, and the summary of all participants in the round.
3) An overview of your results compared to the reference result regarding the phenotypic characterization of aberrant plasma cells.
4) The Round summary report, which contains the round's summary statistics and key issues.

Note that in the following tables:

For quantitative parameters, the reference result is defined as the mean value of three subject-matter experts' results. The results from all participants are expressed as the median (min–max) of the values submitted by all participants in this round.

For qualitative parameters, the reference result is defined as the mode value of three subject-matter experts' results. The results from all participants are shown as the percentage of participants selecting either option.

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Case 1

	A000	Reference	All participants	
% Aberrant plasma cells	0.00047	0.0008	0.00107 (0 - 0.014)	
Calculated limit of detection (LOD); %	0.00018	0.0002	0.00019 (0.0001 - 0.0004)	
Acceptable limits for LOD (reference LOD ± 20%)	0.00016 - 0.00024			
Calculated limit of quantitation (LOQ); %	0.00042	0.0005	0.00048 (0.00039 - 0.0009)	
Acceptable limits for LOQ (reference LOQ ± 20%)	0.0004 - 0.0006			
	A000	**Reference**	**MRD Positive/PNQ/Negative % of all participants** *(PNQ = positive but not quantifiable)*	
MRD status	MRD Positive	MRD Positive	86%/2%/12%	
	A000	**Reference**	**Yes % of all participants**	**No % of all participants**
Signs of hemodilution	No	No	54%	46%
Adequate number of events analysed	Yes	Yes	100%	0%

Conclusion of the analysis as for a final report

A000	Reference
Bone marrows compatible with positive MRD	BM sample compatible with MRD positive. A total of 0.0008% of aberrant/clonal plasma cells are detected.

Your performance based on comparison to the reference result across the main parameters LoD, LoQ, the percentage of aberrant plasma cells detected, and the MRD status:

Warning

You have been assigned a Warning performance score for this case because you reported a percentage of aberrant plasma cells that was below the reference LoQ, while the reference percentage of aberrant plasma cells was above the reference LoQ .

Case 1: Complementary results

Relative contributions of major cell populations to the total cellularity, including nucleated red blood cells.			
	A000	Reference	All participants *
% Normal plasma cells	0.0508	0.089	0.0855 (0.0007 - 84)
% B-cell precursors	0.01	0.04	0 (0 - 0.27)
% Mature B-cells	0.57	0.79	0.72517 (0.27 - 0.85)
% T/NK cells & Basophils	8.3	12.26	10.65 (7.4 - 14.5)
% Eosinophils	2	3.23	3.135 (1.29 - 7.5)
% Neutrophils	73	75.2	73.6 (68.2 - 80.0682)
% Monocytes	6.8	5.26	7 (4.6216 - 13.7)
% Myeloid precursors	1.6	1.63	1.6 (0.066 - 2.8)
% Mast cells	0.0003	0.0071	0.0006 (0 - 0.066)
% Nucleated red blood cells	0.7	2.2	2.1 (0.33 - 3.8)
Relative contributions of plasma cell populations to the total plasma cells			
	A000	Reference	All participants**
% normal plasma cells from total plasma cells	99.2	99.11	98.69 (8 - 99.6)
% aberrant plasma cells from total plasma cells	0.8	0.89	1.3 (0.4 - 92)

*All participants who assessed this parameter

** All participants who reported % aberrant plasma cells >0

Immunophenotypic characterization of aberrant plasma cells				
	A000		Reference	
Marker	immunophenotype	% positive cells	immunophenotype	% positive cells
FSC	High		Normal	
SSC	Normal		Normal	
CD38	Normal positive		Dim positive	
CD138	Normal positive		Normal positive	
CD45	Negative		Negative	
CD19	Dim positive		Negative	
CD56	Bright positive		Bright positive	
CD27	Bright positive		Dim positive	
CD81	Negative		Negative	
CD117	Dim positive		Negative	
cyIgK	Negative		Negative	
cyIgL	Negative		Normal positive	

Case 2

	A000	Reference	All participants	
% Aberrant plasma cells	0.00017	0	0 (0 - 0.01456)	
Calculated limit of detection (LOD); %	0.0002	0.00026	0.00023 (0.00019 - 0.0004)	
Acceptable limits for LOD (reference LOD ± 20%)	0.0002 - 0.00032			
Calculated limit of quantitation (LOQ); %	0.00049	0.00059	0.00058 (0.00049 - 0.0009)	
Acceptable limits for LOQ (reference LOQ ± 20%)	0.00047 - 0.00071			
	A000	**Reference**	**MRD Positive/PNQ/Negative % of all participants** *(PNQ = positive but not quantifiable)*	
MRD status	MRD Positive but not quantifiable	MRD Negative	5%/2%/93%	
	A000	**Reference**	**Yes % of all participants**	**No % of all participants**
Signs of hemodilution	No	No	5%	95%
Adequate number of events analysed	Yes	Yes	89%	11%

Conclusion of the analysis as for a final report

A000	Reference
Bone marrow compatible with positive but not quantifiable MRD.	BM sample compatible with MRD negative. No abnormal plasma cells are detected above the LOD of 0.00026%.

Your performance based on comparison to the reference result across the main parameters LoD, LoQ, the percentage of aberrant plasma cells detected, and the MRD status:

Critical

You have been assigned a Critical performance score for this case because you were unable to correctly determine the MRD status (Negative, Positive but not quantifiable, or Positive) based on your reported percentage of aberrant plasma cells and your reported pLoD and pLoQ values. Additionally, you did not define an accurate pLoD and/or pLoQ. Failure to accurately calculate them would be considered a significant mistake.

Case 2: Complementary results

Relative contributions of major cell populations to the total cellularity, including nucleated red blood cells.			
	A000	Reference	All participants*
% Normal plasma cells	0.5682	0.76	0.735 (0.4933 - 100)
% B-cell precursors	2.01	3.16	3 (0.29 - 4)
% Mature B-cells	3.25	4.46	3.275 (2.4 - 6.5)
% T/NK cells & Basophils	24	31.16	30.8 (24.2 - 44.26)
% Eosinophils	0.65	2.16	2.1 (0.47 - 7)
% Neutrophils	38.6	41.16	43.9 (29.6 - 49.77)
% Monocytes	9.4	9.13	9.4 (5.42 - 11.395)
% Myeloid precursors	1.2	1.7	1.6 (0.1795 - 2.93732)
% Mast cells	0.14	0.11	0.13 (0.028 - 0.15)
% Nucleated red blood cells	6.1	7.4	5.6 (0.99 - 10)
Relative contributions of plasma cell populations to the total plasma cells			
	A000	Reference	All participants**
% normal plasma cells from total plasma cells	99.96		99.35 (97.3904 - 99.97)
% aberrant plasma cells from total plasma cells	0.04		0.65 (0.03 - 2.6096)

*All participants who assessed this parameter

** All participants who reported % aberrant plasma cells >0

Immunophenotypic characterization of aberrant plasma cells				
	A000		Reference	
Marker	immunophenotype	% positive cells	immunophenotype	% positive cells
FSC	High			
SSC	Low			
CD38	Normal positive			
CD138	Bright positive			
CD45	Dim positive			
CD19	Negative			
CD56	Negative			
CD27	Negative			
CD81	Negative			
CD117	Negative			
cyIgK	Normal positive			
cyIgL	Negative			

Case 3

	A000	Reference	All participants	
% Aberrant plasma cells	0	0.018	0.017 (0 - 0.034)	
Calculated limit of detection (LOD); %	0.00041	0.0002	0.0002 (0.0001 - 0.00045)	
Acceptable limits for LOD (reference LOD ± 20%)	0.00016 - 0.00024			
Calculated limit of quantitation (LOQ); %	0.00019	0.00051	0.0005 (0.00018 - 0.0009)	
Acceptable limits for LOQ (reference LOQ ± 20%)	0.0004 - 0.00062			
	A000	**Reference**	**MRD Positive/PNQ/Negative % of all participants** *(PNQ = positive but not quantifiable)*	
MRD status	MRD Negative	MRD Positive	91%/0%/9%	
	A000	**Reference**	**Yes % of all participants**	**No % of all participants**
Signs of hemodilution	No	No	2%	98%
Adequate number of events analysed	Yes	Yes	96%	4%

Conclusion of the analysis as for a final report

A000	Reference
Bone marrow compatible with negative MRD.	BM sample compatible with MRD positive. A total of 0.018% of aberrant/clonal plasma cells are detected.

Your performance based on comparison to the reference result across the main parameters LoD, LoQ, the percentage of aberrant plasma cells detected, and the MRD status:

Critical

You have been assigned a Critical performance score for this case because you reported a percentage of aberrant plasma cells that was below the reference LoD (i.e., MRD Negative), while the reference conclusion was MRD Positive. Additionally, you did not define an accurate pLoD and/or pLoQ. Given that the rest of the MRD interpretation is based on these two parameters, failure to accurately calculate them would be considered a significant mistake.

Case 3: Complementary results

Relative contributions of major cell populations to the total cellularity, including nucleated red cells.			
	A000	Reference	All participants*
% Normal plasma cells	0.2568	0.38	0.39 (0.22 - 0.91)
% B-cell precursors	0.14	0.25	0.23 (0.026 - 0.28)
% Mature B-cells	0.66	1	1 (0.55 - 1.57)
% T/NK cells & Basophils	3.8	6.5	6 (3.1 - 6.75)
% Eosinophils	3.65	1.2	1.2 (0.52 - 8.6)
% Neutrophils	79.3	79	79.22 (71.8245 - 83.72)
% Monocytes	4.48	4.13	4.1 (3.4 - 5.7)
% Myeloid precursors	2.91	2.3	2.2 (0.15 - 3.69474)
% Mast cells	0.01658	0.021	0.021 (0.0041 - 0.03)
% Nucleated red blood cells	4.62	6.16	6.06505 (0.17 - 8.4)
Relative contributions of plasma cell populations to the total plasma cells			
	A000	Reference	All participants**
% normal plasma cells from total plasma cells		95.53	95.8 (91 - 98.2)
% aberrant plasma cells from total plasma cells		4.47	4.15 (1.8 - 9)

*All participants who assessed this parameter

** All participants who reported % aberrant plasma cells >0

Immunophenotypic characterization of aberrant plasma cells				
	A000		Reference	
Marker	immunophenotype	% positive cells	immunophenotype	% positive cells
FSC			High	
SSC			Normal	
CD38			Dim positive	
CD138			Dim positive	
CD45			Dim positive	
CD19			Bright positive	
CD56			Negative	
CD27			Dim positive	
CD81			Dim positive	
CD117			Negative	
cyIgK			Normal positive	
cyIgL			Normal positive	

General

In the EuroFlow MM MRD EQA scheme, three MM cases (2 FCS files per case) from patient bone marrow samples generated following the EuroFlow Next Generation Flow for MM MRD methodology (Flores-Montero et al. 2017) are provided to the participant. The MM MRD scheme is suitable for laboratories seeking to evaluate their performance in the analysis and interpretation of FCS files that were acquired using this methodology. Participating in this scheme will provide laboratories that routinely use the EuroFlow MM MRD antibody panel and relevant EuroFlow standard operating procedures (SOPs) with an external control for multiparametric flow cytometry detection of MRD in patients with MM after therapy.

Confidentiality

Your raw data, performance results, and individual report are strictly confidential between your laboratory, ESLHO, and the Service Provider(s). Results may, however, be shared with other authorized parties for the purpose of quality improvement (e.g., accreditation bodies). Furthermore, under no circumstances may laboratories share this EQA certificate, including the Round summary report, with other laboratories not participating in the EQA scheme.

Data analysis and performance

Participants report their quantitative and qualitative results of the main and complementary parameters:

Main parameters:

- Limit of Detection (LoD) (%)
- Limit of Quantitation (LoQ) (%)
- Percentage of aberrant plasma cells out of total cellularity (%)
- MRD status (“MRD Negative”, “MRD Positive but not quantifiable”, or “MRD Positive”)

Complementary parameters:

- Percentage of normal plasma cells out of total cellularity (%)
- Signs of hemodilution (“Yes” or ”No”)
- The number of analyzed events was adequate according to the recommended standard (“Yes” or ”No”)
- A qualitative description of the aberrant plasma cell phenotype for:
  - FSC and SSC as either “Low”, “Normal”, or “High”
  - CD38, CD138, CD45, CD19, CD56, CD27, CD81, CD117, cIg kappa, cIg lambda as either: “Negative”, “Dim positive”, “Normal positive”, “Bright positive”, “Positive and heterogenous” or “Bimodal”
  - Percentage of positive cells for those markers that show a "Positive and heterogenous" or "Bimodal" expression (%)
- If assessed, the percentage of other cell populations out of total cellularity present in the sample (%) for populations: B-cell precursors, mature B cells, T/NK cells & basophils, eosinophils, neutrophils, monocytes, myeloid precursors, mast cells, and nucleated red blood cells.
- Percentage of normal and aberrant plasma cells from total plasma cell population (i.e., using total plasma cells as denominator) (%)
- Interpretation for the clinician as for a final report

Participant performance is assessed based on the three main parameters (LoD, LoQ, and the percentage of aberrant plasma cells detected in each case), as well as the qualitative conclusion derived from these parameters (i.e., MRD status), categorized as “MRD Negative”, “MRD Positive but not quantifiable”, or “MRD Positive”.

Participant performance for complementary parameters is not scored. Only a side-by-side comparison with the reference results is provided for didactic purposes.

Performance is assessed in three steps:

1) The first step focuses on whether the participant defined an accurate LoD and LoQ (pLOD and pLOQ) as compared to the reference LoD (rLoD) and LoQ (rLoQ). The acceptable limits are as follows:

- **pLoD is within ±20% of rLoD (rounded to 5 decimal places using directional truncation: lower bound rounded down, upper bound rounded up)**
- **pLoQ is within ±20% of rLoQ (rounded to 5 decimal places using directional truncation: lower bound rounded down, upper bound rounded up)**

Given that the rest of the interpretation is based on these parameters, failure to accurately calculate them would be considered a significant mistake. As a result, it would prevent the participant from achieving the highest performance score for that case (i.e., “Satisfactory”).

2) Next, the second step assesses whether the participant correctly determined the MRD status based on the relationship between the percentage of aberrant plasma cells (p%) detected and the calculated pLoD and pLoQ. The classification criteria are as follows:

- **MRD Negative: p% below pLoD**
- **MRD Positive but not quantifiable: p% at or above pLoD and below pLoQ**
- **MRD Positive: p% at or above pLoQ**

In case the two parameters do not align as shown above, the participant is considered to have incorrectly determined the MRD status, which would be considered a critical mistake. As such, the participant will be assigned a “Critical” performance score for the case and the third step will not be conducted.

3) In case the two parameters in the second assessment align, the third step involves comparing the participant’s p% with the reference results (r%, rLoD, or rLoQ) for each case. Performance scoring is applied based on the conditions specified in Table 1. A descriptive outcome is provided as a qualitative reference of how the participant vs. the reference conclusions compare.

Table 1. Performance scoring system

A) Step 1 and Step 2 were performed correctly (participant defined an accurate pLoD and pLoQ and correctly determined the MRD status based on the reported percentage of aberant plasma cells.)			
Reference MRD status	**Participant's p% compared to reference values**	**Performance score**	**Descriptive outcome**

Reference MRD status	Participant's p% compared to reference values	Performance score	Descriptive outcome

B) Step 2 was performed correctly (participant correctly determined the MRD status based on the reported percentage of aberant plasma cells) but Step 1 was not correct (participant failed to define an accurate pLoD and pLoQ).			
Reference MRD status	**Participant's p% compared to reference values**	**Performance score**	**Descriptive outcome**

Reference MRD status	Participant's p% compared to reference values	Performance score	Descriptive outcome

Reference MRD status	Participant's p% compared to reference values	Performance score	Descriptive outcome

Reference MRD status	Participant's p% compared to reference values	Performance score	Descriptive outcome

C) Step 2 was not correct (participant failed to correctly determine the MRD status based on the reported percentage of aberant plasma cells.)

D) Both Step 1 and Step 2 were not correct (participant failed to define an accurate pLoD and pLoQ as well as to correctly determine the MRD status based on the reported percentage of aberant plasma cells.)

Task division

The following persons are involved in the design and operation of the MM MRD EQA scheme:			
ESLHO			
Name	**Organization/Institute**	**Role**	**Tasks**
Prof. Dr. Jacques J. M. van Dongen	ESLHO, Zutphen, NL	EQA Program Coordinator	Final responsibility over EuroFlow EQA program; review and authorization of the MM MRD EQA certificate
Evelien Rijkers	ESLHO, Zutphen, NL	EQA Officer (lead)	Overall responsible for organization and operation of the MM MRD scheme by ESLHO
Dr. Bart Lubbers	ESLHO, Zutphen, NL	EQA Officer Deputy EQA Program Coordinator	Supports in the organization and operation of the MM MRD scheme; back-up for review and authorization of the MM MRD EQA certificate
Lead expert laboratory			
Name	**Organization/Institute**	**Role**	**Tasks**
Dr. Juan Flores-Montero	Flow Cytometry Units, Hematology Department of the University Hospital of Salamanca and University of Salamanca, Salamanca, Spain	Lead subject-matter expert	Pre-round: Coordinates case selection and determines the reference values. Supports preparation of the rounds. Post-round: Provides expert input on/review of the results and the summary. Responds to participants´ inquiries about the technical aspects of the scheme, and participant results. Provides targeted help to participants.
Additional expert laboratories			
Name	**Organization/Institute**	**Role**	**Tasks**
Dr. Naděžda Brdičková	Charles University, Prague, CZ	Subject-matter expert	Pre-round: Supports preparation of the rounds. Post-round: Cleaning and analysis of the submitted results, preparation of the EQA certificate, support in performance evaluation and reporting.
Dr. Paula Fernandez	Kantonsspital, Aarau, Switzerland	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Dr. Leire Burgos Rodríguez	University of Navarra, Pamplona, ES	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results
Dr. Joana Caetano	Champalimaud Foundation, Lisbon, Portugal	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results

Prof. Dr. Elaine S. Costa	Federal University of Rio de Janeiro, Rio de Janeiro, Brasil	Subject-matter expert	Pre-round: Case selection, expert analysis, input for determination of reference results

For more information or in case you have questions about the EuroFlow MM MRD EQA scheme, or other EuroFlow EQA schemes, please contact EuroFlow.EQA@eslho.org.

Round summary report

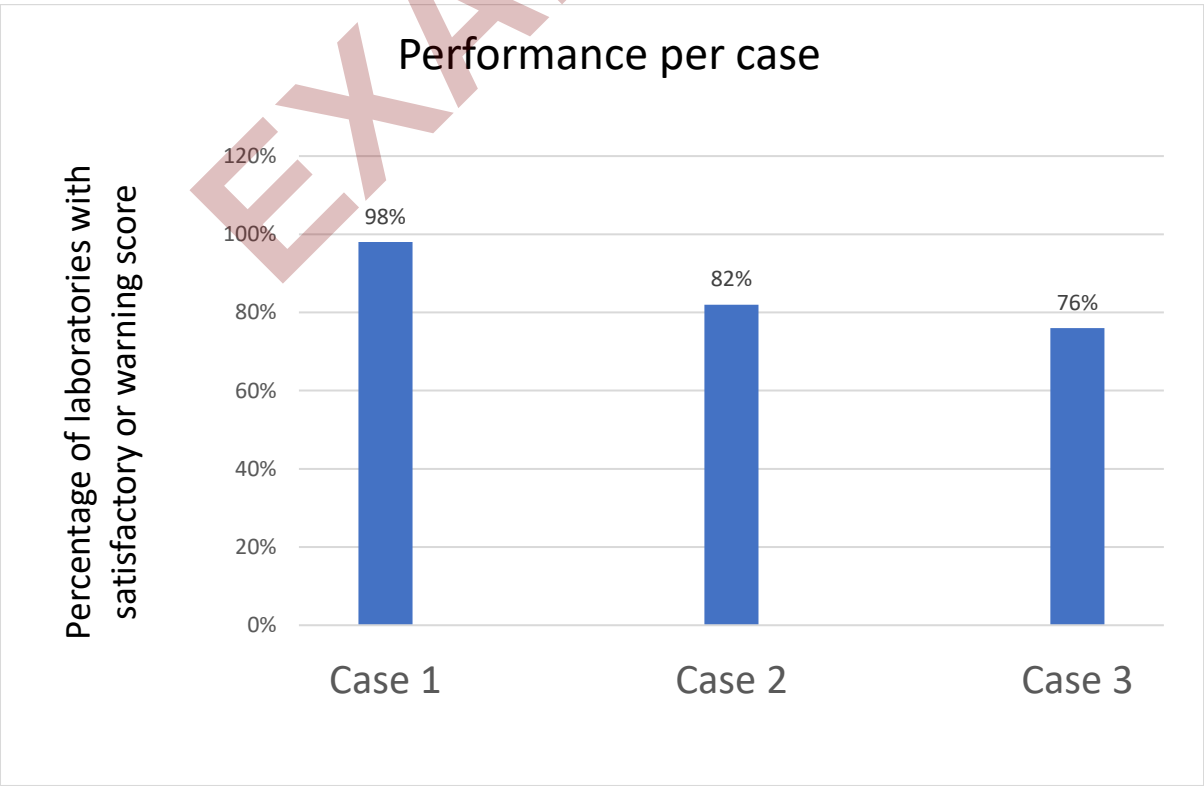
<The round summary report offers an expert analysis of the round's results, highlighting key findings, commonly observed issues, frequent mistakes, and important reminders. Designed as both a practical and educational resource, this report provides participants with valuable expert insights into areas that may require attention and improvement. By identifying what went wrong and outlining points to focus on in future rounds, the report supports continuous learning and helps strengthen and improve diagnostic practice within your laboratory.>

Thank you for participating in round …

In total…

Based on this assessment …

One particular observation in this round …



Authorization

This EuroFlow MM MRD EQA certificate is authorized by …

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Date Signature

Resources & Literature

www.euroflow.org (SOP protocols)

1. Flores-Montero J et al., **Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma**. Leukemia. 2017.