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Standardization of flow cytometric minimal residual disease assessment in international clinical trials - a feasibility study from the European Myeloma Network

by Davine Hofste op Bruinink, Stefania Oliva, Lucie Rihova, Alexander Schmitz, Milena Gilestro, Jeroen te Marvelde, Romana Kralova, Helle Høholt, Annemiek Broijl, Hans Erik Johnsen, Roman Hajek, Mario Boccadoro, Pieter Sonneveld, Paola Omedè, Vincent H.J. van der Velden, and European Myeloma Network

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Title: Standardization of flow cytometric minimal residual disease assessment in international clinical trials - a feasibility study from the European Myeloma Network

Running title: Standardization of MFC MRD assessment in MM trials

Authors

Davine Hofste op Bruinink[1,2]*, Stefania Oliva[3]*, Lucie Rihova[4], Alexander Schmitz[5], Milena Gilestro[3], Jeroen te Marvelde[2], Romana Kralova[4], Helle Høholt[5], Annemiek Broijl[1], Hans Erik Johnsen[5†], Roman Hajek[6], Mario Boccadoro[3], Pieter Sonneveld[1], Paola Omedè[3#] and Vincent H.J. van der Velden[2#], on behalf of the European Myeloma Network[7]

Affiliations

[1]*Department of Hematology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands*

[2]*Laboratory Medical Immunology, Department of Immunology, Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands*

[3]*Myeloma Unit, Division of Hematology, University of Torino, Torino, Italy*

[4]*Department of Hematology, University Hospital Brno, Brno, Czech Republic*

[5]*Department of Hematology, Clinical Cancer Research Center, Aalborg University Hospital, Aalborg, Denmark*

[6]*Department of Hemato-oncology, University Hospital Ostrava and Faculty of Medicine, Ostrava University, Ostrava, Czech Republic*

[7]*https://www.myeloma-europe.org*

*[#]These authors contributed equally: DHoB, SO; PO, VvdV.

[†]Deceased

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Corresponding authors

Davine Hofste op Bruinink (d.hofsteopbruinink@erasmusmc.nl) and Vincent H.J. van der Velden (v.h.j.vandervelden@erasmusmc.nl).

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To the editor:

For many decades, international collaborative efforts have driven therapeutic advances in multiple myeloma (MM). The establishment of uniform response criteria by the International Myeloma Working Group (IMWG) has been pivotal for this progress, as adherence to strict definitions warrants data comparability between trials. An essential prerequisite for the use of uniform criteria is the application of standardized methods. Of particular interest herein is the assessment of minimal residual disease (MRD) by multiparametric flow cytometry (MFC). This has been incorporated into the IMWG response criteria since 2011 to enable better risk stratification of a growing number of patients reaching a complete remission and has the promise to be used both as a surrogate marker for overall and progression-free survival and to inform treatment decisions[1, 2]. However, in contrast to most routine diagnostic tests for response assessment in MM, this assay has until recently suffered from large interlaboratory variations in terms of sample processing and data acquisition, resulting in highly heterogeneous sensitivities[3]. To enable uniform and sensitive MFC MRD assessment between laboratories, EuroFlow has developed standardized operating procedures (SOPs)[4-6]. Their next generation flow (NGF) method has been incorporated as the golden standard for MFC MRD measurements in the latest IMWG response criteria, which is expected to greatly improve data validity and comparability[7].

Even though the establishment of standardized protocols has been an important step towards achieving uniform MFC MRD assessment in MM, their usefulness highly depends on a successful implementation in a wide range of laboratories. This is of particular relevance for international clinical trials that depend on a collaborative effort of multiple reference laboratories for timely MRD assessment, irrespective of geographical location of sampling. Yet, it remains largely unknown whether fully standardized multi-laboratory MM MFC MRD assessment can be achieved in such a setting[8]. To investigate this, a novel QA program has been established in 2016 within the framework of the European Myeloma Network (EMN): the EMN MRD QA program. This program aims to assess the validity and comparability of MFC MRD measurements within and between EMN trials by distributing fresh MM bone marrow (BM) and peripheral blood (PB) samples and complements existing QA programs led by EuroFlow using PB samples from healthy donors[9] or raw data files from

MM MFC MRD measurements. Data obtained within the EMN MRD QA program show that it is feasible to fully standardize MFC MRD assessment between laboratories, resulting in a high concordance over the entire range of detectable MRD levels. Participation in QA programs is essential to warrant complete interlaboratory standardization without compromising on data quality, as minor protocol deviations were commonly observed at initial implementation.

From 2016 to 2019, MFC MRD results from 20 MM patients have been compared within the EMN MRD QA program among four EMN reference laboratories willing to commit to EuroFlow protocols in the context of the EMN02/HO95 MM trial: Aalborg University Hospital, Denmark (laboratory 1), University Hospital Brno, Czech Republic (laboratory 2), Erasmus MC Rotterdam, the Netherlands (laboratory 3, EuroFlow member) and University of Torino, Italy (laboratory 4)[10, 11]. In total, four QA rounds were organized, each comprising five different fresh samples from MM patients with variable levels of disease burden and received treatment (Figure 1A). Samples were collected at Erasmus MC Rotterdam, the Netherlands and Ospedale Molinette di Torino, Italy, at random days throughout the year. Immediately after collection, samples were equally divided and shipped by overnight express courier to the participating laboratories. Samples from distributing hospitals were kept at room temperature for 24 hours to ensure similar sample processing dates between laboratories. Using standardized forms, MRD results were collected centrally by one person, who kept these confidential until the end of each QA round, after which results were shared and discussed.

Timely sample processing is an essential prerequisite for a high validity of MFC MRD results, as MM cells have a limited capacity to survive outside of the BM. Hence, the IMWG recommends processing of MFC MRD samples within 24-48 hours. Considering all 67 samples from QA rounds 1-4, our data show that two laboratories were able to process 20/20 (100%) received samples within this recommended timeframe. Laboratory 1 processed 6/7 (86%) and laboratory 4 18/20 (90%) samples within 48 hours after sampling (Figure 1B).

Throughout QA rounds 1-4, laboratory 3 strictly adhered to EuroFlow SOPs, which was considered the reference for all other participating laboratories. In QA rounds 1-2, second-generation flow protocols from EuroFlow were applied. QA round 1 was followed by a workshop to further standardize

protocols and gating strategies, which resulted in the use of significantly more comparable SOPs between laboratories in QA round 2 (Supplementary Table 1-2). A minimal number of 20 monoclonal plasma cells (mPCs) was required for MRD positivity[12]. Despite complete standardization of protocols not being possible in laboratory 2 and 4 due to ongoing consumable contracts and local unavailability of certain reagents, MFC MRD results were highly concordant in QA rounds 1-2 at every level of residual disease. All participating laboratories reported the same MRD result in 9/10 (90%) samples (Figure 2A).

The ability to uniformly quantify MRD irrespective of daratumumab treatment status was tested in seven BM samples that were distributed in QA rounds 3-4. Here, the EuroFlow NGF pipeline was implemented, which contains a multi-epitope antibody against CD38 in its staining panel that circumvents epitope blocking by daratumumab[6]. Of note, at this stage all participating reference laboratories had committed to fully standardized protocols in terms of data collection, instrument setup, performance checks, sample preparation, sample staining, data acquisition and data analysis (Supplementary Table 1-2), resulting in a second series of highly concordant MFC MRD results and 10/10 (100%) samples with a uniformly classified MRD result (Figure 2A).

To compare interlaboratory test sensitivities in MRD negative samples, the formula for limit of detection (LOD) was used: 20 / number of acquired leukocytes * 100. This showed a median LOD of 5.4×10^{-6} in the 34 MRD negative samples from QA rounds 1-4. Laboratory 3 reached a LOD <0.001% in 10/10 (100%) MRD negative assays, whereas the other laboratories achieved a LOD <0.001% in 50-80% of MRD negative assays. Overall, in all except one assay a LOD <0.01% was reached.

Recent reports indicate that the majority of newly diagnosed MM (NDMM) patients have detectable circulating tumor cells (CTCs) before treatment start when the highly sensitive NGF protocols are used[13]. As mPC infiltration is typically low in both NDMM PB CTC and MRD BM samples, this raises the question whether NDMM PB CTC samples could also be used for MM MRD QA purposes. The feasibility to do so was assessed in QA round 4. CTCs were uniformly detected in 2/2 (100%) NDMM PB samples, both at highly comparable levels between 0.001% and 0.01%. This indicates that PB

samples from NDMM patients may be a less invasive alternative to MRD BM samples to assess interlaboratory standardization of MM MRD protocols.

To test the interlaboratory concordance of the detected mPC immunophenotypes (IPs), laboratories were asked to report staining intensities as positive, dim or negative. 10/20 (50%) samples were classified as MRD positive and generally showed a high similarity between laboratories for markers that are essential for mPC gating: CD38, CD138, CD45, CD19, CD56, CyIgK and CyIgL (Figure 2B). Yet, the reported expression of other informative markers (i.e. CD27, CD81 and CD117) showed more variability. Even though this did not affect mPC quantification, it underscores the importance of using strict definitions in terms of data analysis to ensure reproducibility of mPC IP data.

Finally, MFC MRD assessment has the advantage over molecular MRD techniques to also generate information on the cellular composition of non-MM populations, which could be used to infer BM sample quality. To this end, the EMN suggested in its consensus from 2008 to always state polyclonal plasma cell (pPC) levels in the final MRD report[14]. To test the concordance of this reference population between laboratories, information on pPC levels was collected from all samples in QA rounds 2-4 (Figure 2C). According to expectation, PB samples had a lower median pPC level than BM samples. Moreover, the interlaboratory concordance of reported pPC levels was generally good. Yet, it was inferior to that of reported MRD levels, suggesting that pPC levels are more susceptible to interlaboratory variations in sample processing and data analysis than mPCs.

In conclusion, our data indicate that full standardization of interlaboratory MM MFC MRD assessment is feasible, resulting in highly concordant MRD data. Moreover, QA programs using fresh material from MM patients are a straightforward and effective way to monitor and improve MFC MRD data quality within clinical trials. This is of particular relevance for studies that depend on reference laboratories with no or limited prior experience with the EuroFlow protocols, as these are not always fully adhered to from the beginning. Therefore, we strongly recommend the incorporation of both the annual EuroFlow QA rounds and trial group-specific QA rounds with fresh MM samples in future clinical trial designs to ensure further advancement of the field in terms of standardized MFC MRD response assessment.

Ethics declarations

Conflict of interest

The authors declare the following potential conflicts of interest: SO, Honoraria: Celgene, Janssen, Amgen, Adaptive Biotechnologies. AB, Honoraria/Advisory board: Celgene, Janssen, Amgen, Takeda. PS: Research support: Janssen, Celgene, Amgen. Advisory board: Janssen, Celgene, Amgen. DHoB, LR, AS, MG, JtM, RK, HH, HEJ, RH, MB, PO, VvdV: no conflicts of interest.

Ethical approval and informed consent

This study was approved by the Medical Ethical Committees of Erasmus MC Rotterdam, the Netherlands and A.O.U. Città della Salute e della Scienza di Torino, Italy. Written informed consent was obtained from all participating patients, in accordance with the Declaration of Helsinki.

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Figure legends

Figure 1: Sample characteristics and logistics for QA rounds 1-4 of the EMN MRD QA program. A total of 20 fresh MM samples were used in QA rounds 1-4, resulting in 67 MFC MRD assessments performed in four participating laboratories in Europe.

A: In total, 17 BM and three PB samples were collected from MM patients with variable disease burden, with six patients receiving daratumumab treatment at the time of sampling. Sample volumes ranged from 2-6 mL, whereas sample WBC counts ranged from $<5 \times 10^9$/L to $>25 \times 10^9$/L. Response status was determined according to the IMWG 2016 criteria[7].

B: From March 15, 2016 to December 17, 2019, MM samples were collected and distributed from hospitals in Rotterdam, the Netherlands and Torino, Italy. In QA rounds 1-2, second-generation flow protocols (EuroFlow) were used by all laboratories, whereas NGF protocols (EuroFlow) were used in QA rounds 3-4. All laboratories participated in the full EMN MRD QA program, except for laboratory 1. In 2018 and 2019, this laboratory did not serve as a reference laboratory for any EMN trials requiring the use of NGF protocols and therefore decided to not join QA rounds 3-4. In general, laboratories were able to process 86-100% of received samples within the IMWG recommended timeframe of 24-48 hours after sampling.

Abbreviations: **CR**: complete response | **MM**: multiple myeloma | **NDMM**: newly diagnosed multiple myeloma | **PD**: progressive disease | **PR**: partial response | **sCR**: stringent complete response | **TBSSA**: time between sampling and sample arrival | **TBSSP**: time between sampling and sample processing | **VGPR**: very good partial response | **WBC**: white blood cell

Figure 2: Concordance of MRD levels, mPC IPs and pPC levels between EMN MFC laboratories.

MRD levels were highly concordant between laboratories, irrespective of disease burden, disease stage, treatment status, sample type and QA round.

A: A total of 10/20 (50%) samples were MRD positive, which could be confirmed by all laboratories in 9/10 (90%) cases, using a cutoff of ≥20 mPCs for MRD positivity. MRD negative results were concordant between laboratories in 10/10 (100%) cases. In MRD negative assays, a LOD <0.001% was reached in 11/16 (69%) samples in QA round 1-2, versus 14/18 (78%) of samples in QA round 3-4. In contrast, a LOQ <0.001% was reached in 4/16 (25%) of MRD negative assays in QA round 1-2, versus 9/18 (50%) of MRD negative assays in QA round 3-4. MRD positive samples showed a high degree of concordance between laboratories at every level of (residual) disease, ranging from 0.001-0.01% to 1-10%.

B: Qualitative expression of essential markers for mPC gating (i.e. CD38, CD138, CD45, CD19, CD56, CyIgK, CyIgL) showed a high degree of concordance between laboratories, whereas other informative markers (i.e. CD27, CD117, CD81) showed higher variability, indicating that strict uniformization of protocols is essential to ensure IP data reproducibility.

C: The level of pPCs is commonly used as a surrogate marker for BM sample quality and generally showed a good concordance between laboratories. Yet, the concordance is inferior to that of mPC levels, suggesting that pPC levels are more susceptible to interlaboratory variations in sample processing and data analysis strategies than mPC levels.

Abbreviations: **IP**: immunophenotype | **LOD**: lower limit of detection = 20 / total number of leukocytes | **LOQ**: lower limit of quantitation = 50 / total number of leukocytes[15] | **MRD**: minimal residual disease

Figure 1

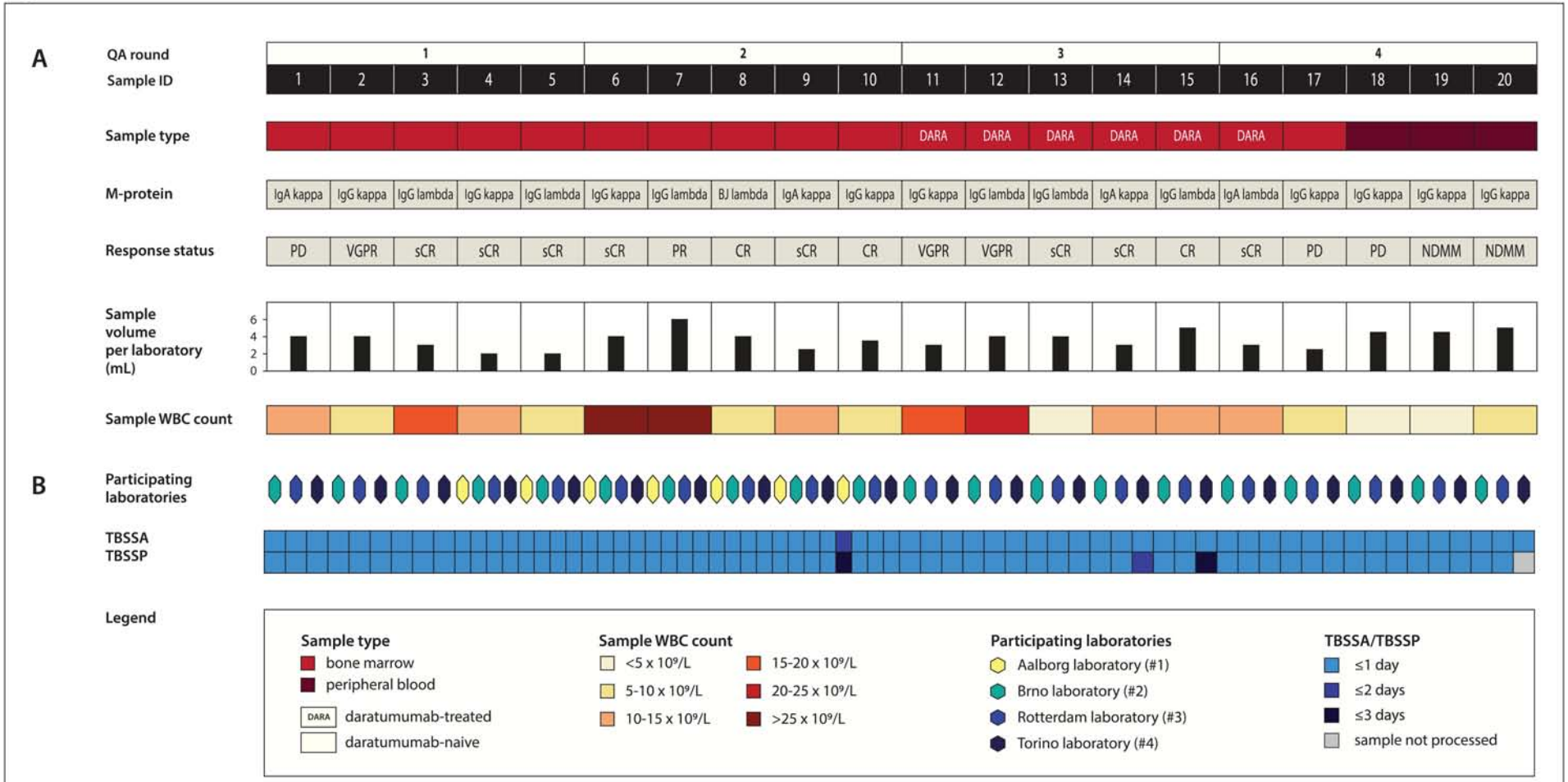
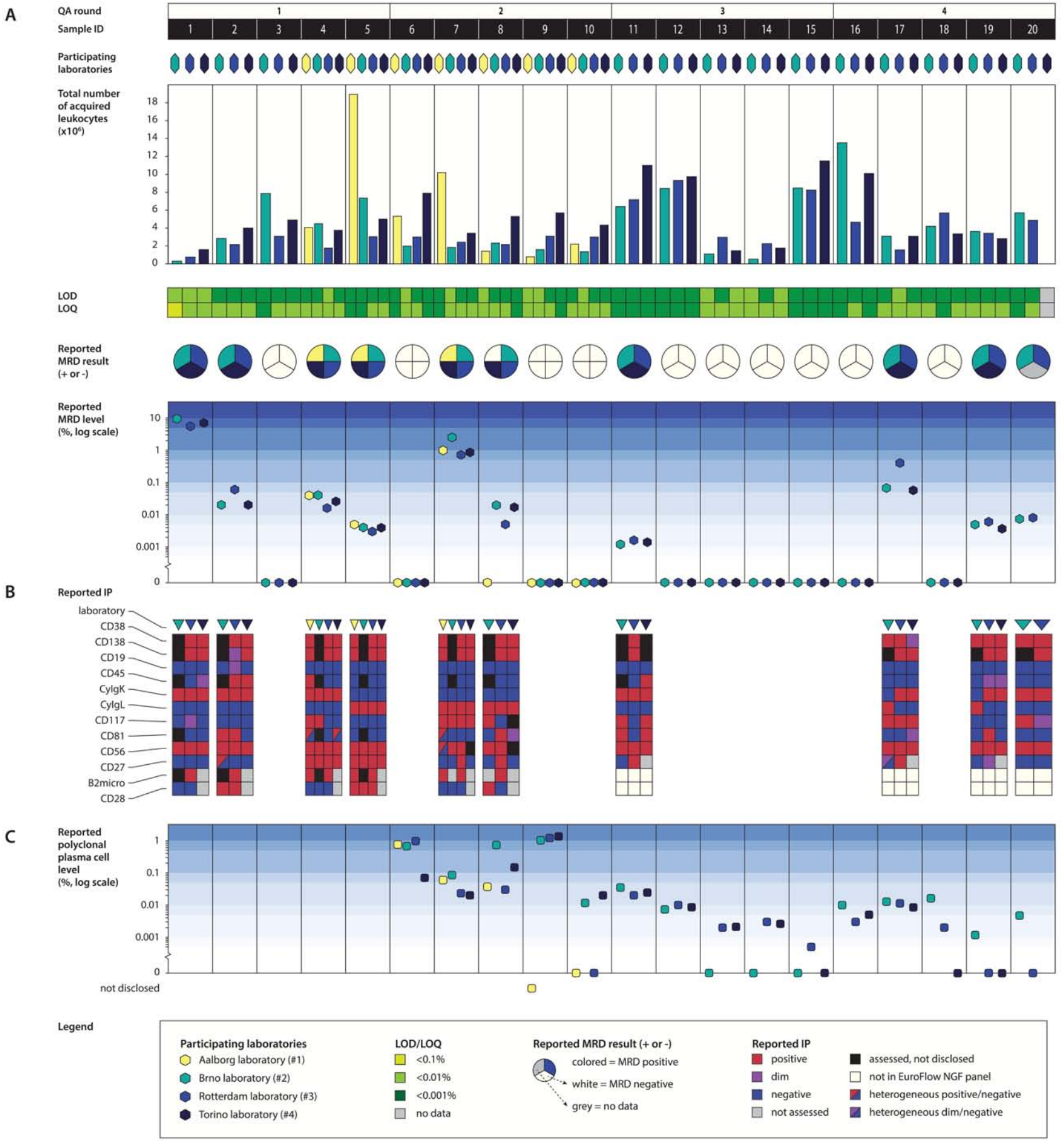

A
QA round
1
2
3
4
Sample ID
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Sample type
DARA DARA DARA DARA DARA DARA
M-protein
IgA kappa | IgG kappa | IgG lambda | IgG kappa | IgG lambda | IgG kappa | IgG lambda | BJ lambda | IgA kappa | IgG kappa | IgG kappa | IgG lambda | IgG lambda | IgA kappa | IgG lambda | IgA lambda | IgG kappa | IgG kappa | IgG kappa | IgG kappa
Response status
PD | VGPR | sCR | sCR | sCR | sCR | PR | CR | sCR | CR | VGPR | VGPR | sCR | sCR | CR | sCR | PD | PD | NDMM | NDMM
Sample volume per laboratory (mL)
6
4
2
0
Sample WBC count
B
Participating laboratories
TBSSA
TBSSP
Legend
Sample type
bone marrow
peripheral blood
DARA daratumumab-treated
daratumumab-naive
Sample WBC count
<5 x 10⁹/L
5-10 x 10⁹/L
10-15 x 10⁹/L
15-20 x 10⁹/L
20-25 x 10⁹/L
>25 x 10⁹/L
Participating laboratories
Aalborg laboratory (#1)
Brno laboratory (#2)
Rotterdam laboratory (#3)
Torino laboratory (#4)
TBSSA/TBSSP
≤1 day
≤2 days
≤3 days
sample not processed

Figure 2


A
QA round
1
2
3
4
Sample ID
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Participating laboratories
Total number of acquired leukocytes (x10⁶)
0 2 4 6 8 10 12 14 16 18
LOD
LOQ
Reported MRD result (+ or -)
Reported MRD level (%, log scale)
10 1 0.1 0.01 0.001 0
B
Reported IP
laboratory
CD38
CD138
CD19
CD45
CyIgK
CyIgL
CD117
CD81
CD56
CD27
B2micro
CD28
C
Reported polyclonal plasma cell level (%, log scale)
1 0.1 0.01 0.001 0
not disclosed
Legend
Participating laboratories
Aalborg laboratory (#1)
Brno laboratory (#2)
Rotterdam laboratory (#3)
Torino laboratory (#4)
LOD/LOQ
<0.1%
<0.01%
<0.001%
no data
Reported MRD result (+ or -)
colored = MRD positive
white = MRD negative
grey = no data
Reported IP
positive
dim
negative
not assessed
assessed, not disclosed
not in EuroFlow NGF panel
heterogeneous positive/negative
heterogeneous dim/negative

Supplementary Table 1: Used Methods

Topic	Subtopic	QA round 1				QA round 2				QA round 3 & 4		
		Aalborg	Brno	Rotterdam	Torino	Aalborg	Brno	Rotterdam	Torino	Brno	Rotterdam	Torino
Sampling	Material used for MRD analysis at response evaluation	BM & PB	BM	BM	BM	BM				BM		
	Tube used for sampling	BM: heparin, PB: EDTA	EDTA	heparin or EDTA	heparin	heparin or EDTA				heparin or EDTA		
Data collection	Information collected for sample identification											
	EMN patient number	yes				yes				yes		
	Patient year of birth	yes										
	Patient sex	yes	yes	yes	no							
	Patient hospital	yes										
	Disease stage	no	yes	yes	no							
	Sample source	yes										
	Date of sample collection	yes	yes	yes	no							
	Information collected in laboratory											
	Date of sample processing	yes				yes				yes		
	Sample volume	yes	no	yes	no	yes	no	yes	no	no	yes	yes
	Sample leukocyte count	no	no	yes	yes	no	no	yes	yes	no	yes	yes
	Presence of coagulation in sample	yes				yes				yes		
Machine	Flowcytometer	FACSCanto™ II (BD) & FACSAria™ II (BD)	FACSCanto™ II (BD)	FACSCanto™ II (BD)	Navios (Beckman Coulter)	FACSCanto™ II (BD) & FACSAria™ II (BD)	FACSCanto™ II (BD)	FACSCanto™ II (BD)	Navios (Beckman Coulter)	FACSCanto™ II (BD) / FACSLyric™ (BD)		Navios (Beckman Coulter)
	Instrument setup	according to EuroFlowTM Cytometer Setup Standard Operating Procedures				according to EuroFlowTM Cytometer Setup Standard Operating Procedures				according to EuroFlowTM Cytometer Setup Standard Operating Procedures		
Performance checks	CS&T beads	daily	weekly	daily	daily	daily				daily		
	Rainbow beads	daily	daily	daily	not used	daily				daily		
	Compensation beads	after every service event of the machine				after every service event of the machine				after every service event of the machine		
Sample preparation	Timing	≤48 hours after sampling	≤36 hours after sampling	≤36 hours after sampling	≤48 hours after sampling	≤36 hours after sampling				≤36 hours after sampling		
	Sample volume used for staining	2 mL	step 1: 60 uL step 2: ≤2 mL, depending on %PC in step 1	2 mL	volume corresponding to $20x10^6$ leukocytes	2 mL			volume corresponding to $20x10^6$ leukocytes	whole sample	volume corresponding to $20x10^6$ leukocytes	volume corresponding to $20x10^6$ leukocytes
	MNC fraction separation	no				no				no		
	Bulk lysis	tube lysis	only if low PC infiltration	yes	yes	yes				yes		
	Erythrocyte lysis solution	EasyLyse (Dako)	NH_4Cl	NH_4Cl	NH_4Cl	NH_4Cl			Bulklysis (Cytognos)	NH_4Cl	NH_4Cl	Bulklysis (Cytognos)
	Fixation/permeabilisation buffer	Cytofix/Cytoperm™ (BD)	Intraprep (Beckman Coulter)	Fix & Perm (An der Grub)	Fix & Perm (Nordic MUbio)	Fix & Perm (An der Grub)			Fix & Perm (Nordic MUbio)	Fix & Perm (Nordic MUbio)	Fix & Perm (An der Grub)	Fix & Perm (Nordic MUbio)
Data acquisition	Number of data acquisition steps	two	two	one	one	one				one		
	Definition of events to be acquired	step 1: ungated; step 2: CD38+/CD19+, CD38+/CD19-, CD38-CD19+ events	ungated	ungated	ungated	ungated				ungated		
	Doublets	not recorded	recorded	recorded	recorded	recorded				recorded		
	Debris	not recorded				not recorded				not recorded		
	Target number of events to be acquired	step 1: $>1x10^5$ events per tube; step 2: all CD38+/CD19+, CD38+/CD19-, CD38-CD19+ events in both tubes	2-$10x10^6$ events in step 2	≥$2x10^6$ leukocytes or ≥$1x10^4$ plasma cells	2-$4x10^6$ leukocytes	≥$2x10^6$ leukocytes per tube or ≥$1x10^4$ plasma cells				≥$5x10^6$ leukocytes per tube or ≥$1x10^4$ plasma cells		
Data analysis	Analysis program	Infinicyt (Cytognos)	Infinicyt (Cytognos)	Infinicyt (Cytognos)	Kaluza (Beckman Coulter)	Infinicyt (Cytognos)	Infinicyt (Cytognos)	Infinicyt (Cytognos)	Kaluza (Beckman Coulter)	Infinicyt (Cytognos)		
	Gating strategy	plasma cell gating on CD38/CD45 and CD138, subsequent gating based on aberrancies	plasma cell gating on CD38/CD138, subsequent gating based on aberrancies	plasma cell gating on CD38/CD45 and CD138, subsequent gating based on aberrancies	plasma cell gating on CD38/CD45 and CD138, subsequent gating based on aberrancies	plasma cell gating on CD38/CD45 and CD138, subsequent gating based on aberrancies				plasma cell gating on CD38/CD45 and CD138, subsequent gating based on aberrancies		
	Diagnostic sample used for comparison	mostly	yes	no	yes	not necessarily				not necessarily		
	Cut-off for MRD positivity	≥25 mPCs	≥25 mPCs	≥10 mPCs	≥20 mPCs	≥20 mPCs				≥20 mPCs		
	Denominator in MRD equation	calculated total number of cells	total number of CD45+ leukocytes, including PCs	total number of leukocytes	total number of leukocytes	total number of leukocytes				total number of leukocytes		
	Merging of data from tube 1 and 2	percentage of MRD = (n mPCs tube 1 + n mPCs tube 2) / total number of leukocytes				percentage of MRD = (n mPCs tube 1 + n mPCs tube 2) / total number of leukocytes				percentage of MRD = (n mPCs tube 1 + n mPCs tube 2) / total number of leukocytes		
	Report of polyclonal plasma cell percentage on final report	yes	yes	no	yes	yes				yes		

Supplementary Table 2: Used Antibody Panels

QA round 1 - PCD tubes (Second-Generation Flow panel, EuroFlow)

Marker	Characteristic	Aalborg	Brno	Rotterdam	Torino
CD138	Tube number	1 + 2			
	Fluorochrome	HV500-C	PacO	PacO	PerCP-Cy5.5
	Clone	MI15	B-A38	B-A38	B-A38
	Company	BD Biosciences	Exbio	Exbio	Beckman Coulter
	EuroFlow compatible	alternative	reference	reference	no
CD38	Tube number	1 + 2			
	Fluorochrome	FITC	FITC	FITC + Pure	PB
	Clone	LD38	LD38	LD38	LSI98-4-3
	Company	Cytognos	Cytognos	Cytognos	Beckman Coulter
	EuroFlow compatible	no	no	reference	no
CD45	Tube number	1 + 2			
	Fluorochrome	PacB	PacB	PacB	KO
	Clone	T29/33	T29/33	T29/33	J.33
	Company	Dako	Dako	Dako	Beckman Coulter
	EuroFlow compatible	reference	reference	reference	no
CD19	Tube number	1 + 2			
	Fluorochrome	PE-Cy7	PE-Cy7	PE-Cy7	PE-Cy7
	Clone	J3-119	J3-119	J3-119	J3-119
	Company	Beckman Coulter	Beckman Coulter	Beckman Coulter	Beckman Coulter
	EuroFlow compatible	reference	reference	reference	reference
CD81	Tube number	1			
	Fluorochrome	APC-H7	APC-H7	APC-H7	FITC
	Clone	JS-81	JS-81	JS-81	JS64
	Company	BD Biosciences	BD Biosciences	BD Biosciences	Beckman Coulter
	EuroFlow compatible	reference	reference	reference	no
CD27	Tube number	1			
	Fluorochrome	PerCP-Cy5.5	PerCP-Cy5.5	PerCP-Cy5.5	PE
	Clone	L128	L128	L128	1A4CD27
	Company	BD Biosciences	BD Biosciences	BD Biosciences	Beckman Coulter
	EuroFlow compatible	reference	reference	reference	no
CD28	Tube number	1			not used
	Fluorochrome	PE	PE	PE	
	Clone	L293	L293	L293	
	Company	BD Biosciences	BD Biosciences	BD Biosciences	
	EuroFlow compatible	reference	reference	reference	
CD117	Tube number	1			2
	Fluorochrome	APC	APC	APC	APC-AF750
	Clone	104D2	104D2	104D2	104D2D1
	Company	BD Biosciences	Exbio	BD Biosciences	Beckman Coulter
	EuroFlow compatible	reference	no	reference	no
CD20	Tube number	not used			1
	Fluorochrome				APC
	Clone				B9-E9
	Company				Beckman Coulter
	EuroFlow compatible				no
CD56	Tube number	2			
	Fluorochrome	PE	PE	PE	APC
	Clone	C5.9	LT56	C5.9	N901
	Company	Cytognos	Exbio	Cytognos	Beckman Coulter
	EuroFlow compatible	reference	no	reference	no
CyIgK	Tube number	2			
	Fluorochrome	APC	APC	APC	PE
	Clone	polyclonal	polyclonal	polyclonal	polyclonal
	Company	Dako	Dako	Dako	Dako
	EuroFlow compatible	reference	reference	reference	no
CyIgL	Tube number	2			
	Fluorochrome	APC-C750	APC-H7	APC-C750	FITC
	Clone	polyclonal	1-155-2	polyclonal	polyclonal
	Company	Cytognos	BD Biosciences	Cytognos	Dako
	EuroFlow compatible	reference	alternative	reference	no
B2micro	Tube number	2			not used
	Fluorochrome	PerCP-Cy5.5	PerCP-Cy5.5	PerCP-Cy5.5 + Pure	
	Clone	Tü99	Tü99	Tü99	
	Company	BD Biosciences	BD Biosciences	BD Biosciences	
	EuroFlow compatible	no	no	reference	

QA round 2 - PCD tubes (Second-Generation Flow panel, EuroFlow)

Marker	Characteristic	Aalborg	Brno	Rotterdam	Torino
CD138	Tube number	1 + 2			
	Fluorochrome	HV500-C	PacO	PacO	PerCP-Cy5.5
	Clone	MI15	B-A38	B-A38	B-A38
	Company	BD Biosciences	Exbio	Exbio	Beckman Coulter
	EuroFlow compatible	alternative	reference	reference	no
CD38	Tube number	1 + 2			
	Fluorochrome	FITC	FITC	FITC + Pure	PB
	Clone	LD38	LD38	LD38	LSI98-4-3
	Company	Cytognos	Cytognos	Cytognos	Beckman Coulter
	EuroFlow compatible	no	no	reference	no
CD45	Tube number	1 + 2			
	Fluorochrome	PacB	PacB	PacB	KO
	Clone	T29/33	T29/33	T29/33	J.33
	Company	Dako	Dako	Dako	Beckman Coulter
	EuroFlow compatible	reference	reference	reference	no
CD19	Tube number	1 + 2			
	Fluorochrome	PE-Cy7	PE-Cy7	PE-Cy7	PE-Cy7
	Clone	J3-119	J3-119	J3-119	J3-119
	Company	Beckman Coulter	Beckman Coulter	Beckman Coulter	Beckman Coulter
	EuroFlow compatible	reference	reference	reference	reference
CD81	Tube number	1			
	Fluorochrome	APC-H7	APC-H7	APC-H7	FITC
	Clone	JS-81	JS-81	JS-81	JS64
	Company	BD Biosciences	BD Biosciences	BD Biosciences	Beckman Coulter
	EuroFlow compatible	reference	reference	reference	no
CD27	Tube number	1	1 + 2		1
	Fluorochrome	PerCP-Cy5.5	PerCP-Cy5.5	PerCP-Cy5.5	PE
	Clone	L128	L128	L128	1A4CD27
	Company	BD Biosciences	BD Biosciences	BD Biosciences	Beckman Coulter
	EuroFlow compatible	reference	reference	reference	no
CD28	Tube number	1			not used
	Fluorochrome	PE	PE	PE	
	Clone	L293	L293	L293	
	Company	BD Biosciences	BD Biosciences	BD Biosciences	
	EuroFlow compatible	reference	reference	reference	
CD117	Tube number	1			2
	Fluorochrome	APC	APC	APC	APC-AF750
	Clone	104D2	104D2	104D2	104D2D1
	Company	BD Biosciences	Exbio	BD Biosciences	Beckman Coulter
	EuroFlow compatible	reference	no	reference	no
CD20	Tube number	not used			1
	Fluorochrome				APC
	Clone				B9-E9
	Company				Beckman Coulter
	EuroFlow compatible				no
CD56	Tube number	2			
	Fluorochrome	PE	PE	PE	APC
	Clone	C5.9	C5.9	C5.9	N901
	Company	Cytognos	Cytognos	Cytognos	Beckman Coulter
	EuroFlow compatible	reference	reference	reference	no
CyIgK	Tube number	2			
	Fluorochrome	APC	APC	APC	PE
	Clone	polyclonal	polyclonal	polyclonal	polyclonal
	Company	Dako	Dako	Dako	Dako
	EuroFlow compatible	reference	reference	reference	no
CyIgL	Tube number	2			
	Fluorochrome	APC-C750	APC-H7	APC-C750	FITC
	Clone	polyclonal	1-155-2	polyclonal	polyclonal
	Company	Cytognos	BD Biosciences	Cytognos	Dako
	EuroFlow compatible	reference	alternative	reference	no
B2micro	Tube number	2	not used	2	not used
	Fluorochrome	PerCP-Cy5.5		PerCP-Cy5.5 + Pure	
	Clone	Tü99		Tü99	
	Company	BD Biosciences		BD Biosciences	
	EuroFlow compatible	no		reference	

QA round 3 & 4 - MM MRD tubes (Next Generation Flow panel, EuroFlow)

Marker	Characteristic	Brno	Rotterdam	Torino
CD138	Tube number	1 + 2		
	Fluorochrome	BV421	BV421	BV421
	Clone	MI15	MI15	MI15
	Company	BD Biosciences	BD Biosciences	BioLegend
	EuroFlow compatible	reference	reference	no
CD38	Tube number	1 + 2		
	Fluorochrome	FITC	FITC	FITC
	Clone	multi-epitope	multi-epitope	multi-epitope
	Company	Cytognos	Cytognos	Cytognos
	EuroFlow compatible	reference	reference	reference
CD45	Tube number	1 + 2		
	Fluorochrome	PerCP-Cy5.5	PerCP-Cy5.5	PerCP-Cy5.5
	Clone	HI30	HI30	E01
	Company	BioLegend	BioLegend	Cytognos
	EuroFlow compatible	reference	reference	alternative (part of MM-MRD kit)
CD19	Tube number	1 + 2		
	Fluorochrome	PE-Cy7	PE-Cy7	PE-Cy7
	Clone	J3-119	J3-119	19-1
	Company	Beckman Coulter	Beckman Coulter	Cytognos
	EuroFlow compatible	reference	reference	alternative (part of MM-MRD kit)
CD81	Tube number	1		
	Fluorochrome	APC-C750	APC-C750	APC-C750
	Clone	M38	M38	M38
	Company	Cytognos	Cytognos	Cytognos
	EuroFlow compatible	reference	reference	reference
CD27	Tube number	1 + 2		
	Fluorochrome	BV510	BV510	BV510
	Clone	O323	O323	O323
	Company	BioLegend	BioLegend	BioLegend
	EuroFlow compatible	reference	reference	reference
CD28	Tube number	not used		
	Fluorochrome			
	Clone			
	Company			
	EuroFlow compatible			
CD117	Tube number	1		
	Fluorochrome	APC	APC	APC
	Clone	104D2	104D2	104D2
	Company	BD Biosciences	BD Biosciences	BD Biosciences
	EuroFlow compatible	reference	reference	reference
CD20	Tube number	not used		
	Fluorochrome			
	Clone			
	Company			
	EuroFlow compatible			
CD56	Tube number	1 + 2		
	Fluorochrome	PE	PE	PE
	Clone	C5.9	C5.9	C5.9
	Company	Cytognos	Cytognos	Cytognos
	EuroFlow compatible	reference	reference	reference
CyIgK	Tube number	2		
	Fluorochrome	APC	APC	APC
	Clone	polyclonal	polyclonal	polyclonal
	Company	Dako	Dako	Dako
	EuroFlow compatible	reference	reference	reference
CyIgL	Tube number	2		
	Fluorochrome	APC-C750	APC-C750	APC-C750
	Clone	polyclonal	polyclonal	polyclonal
	Company	Cytognos	Cytognos	Cytognos
	EuroFlow compatible	reference	reference	reference
B2micro	Tube number	not used		
	Fluorochrome			
	Clone			
	Company			
	EuroFlow compatible			