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

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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			