

Casuïstiek en valkuilen van flowcytometrie in MDS



Experimenteel

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Valkuilen



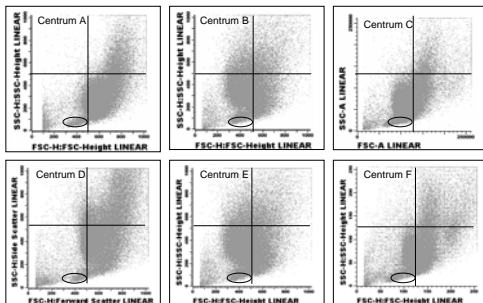
- Experimenteel
 - Instrument Setup
 - Scatter
 - Fluorescentie
 - Procedure
 - Antistoffen
 - Fluorochromen
 - Clonen
 - Fluorescentiepatronen
- Data interpretatie



Instrument Setup



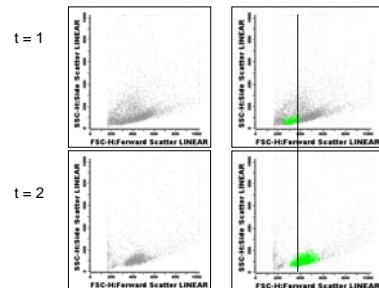
- Scatterpatronen
 - 1 BM monster
 - 6 centra



Instrument Setup



- Scatter
 - 1 centrum
 - 2 monsters zelfde patient, 2 tijdstippen



Lymfocyt



Instrument Setup

- Treshold

te laag te hoog correct

- Debris zoveel mogelijk excluderen
- Leukocyten plus kernhoudend rood zoveel mogelijk includeren

Instrument Setup

- Fluorescentie
 - PMT instelling
 - Sterk signaal
 - Lage achtergrond
 - Optimaal scheidend vermogen

te hoog te laag correct

Valkuilen

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Procedure

- Stain-Lyse-Wash
- Buis per buis lysis

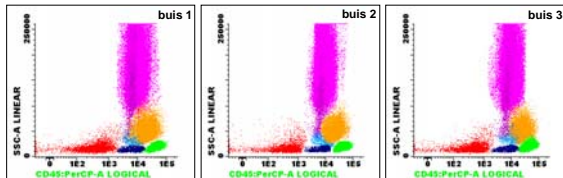
buis 1 buis 2 buis 3

Buis tot buisvariaties door:

- Kans op het meepipetteren van een stolsel is groter
- Ongelijke erylysis
- Wegvangen van moabs door niet gelyseerd rood (bv CD235a)
- Geen constant aantal leukocyten per buis dus detectielimiet ongelijk

Procedure

- Lyse-Stain-Wash
- Bulklysis



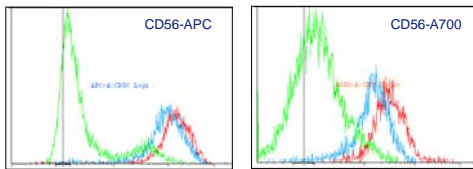
Nagenoeg geen buis tot buisverschillen

Valkuilen

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Antistoffen

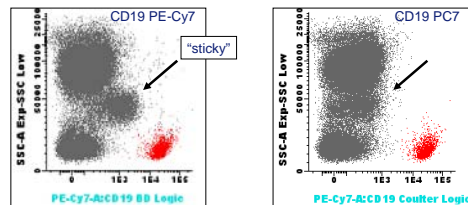
- Fluorochromen



- Voor expressie van CD56 op blasten is een voldoende sterke fluorescentie nodig

Antistoffen

- Fluorochromen



- Lage achtergrondfluorescentie vergemakkelijkt gating en interpretatie

Antistoffen

- Clonen

CD15-NA FITC, MCS-1 Monoclonal Antibody
Non-Agglutinating CD15, FITC Labeled

Cat No: T1680
Lot: N/A

W: 1mg

CLONE: MCS-1 Isotype: IgG3 Applications: Flow Cytometry Species Reactivity: Human

CONCENTRATION: Product provided/boxed for use in flow cytometry when used as indicated below.


COMMENTS: This CD15 antibody is an IgG3 antibody as compared to other CD15 antibodies available which are IgM's. These IgM's cause the cells labeled with them to agglutinate in the cytometer and read all incorrect, producing lower numbers of cells to be recorded. Our CD15 antibody does not cause the cells to agglutinate, yielding much more accurate results.

FORMULATION: Provided as 0.2µm sterile filtered solution in 0.1 M NaOxymine, pH 7.4, 0.15M NaCl, 30 % glycerol with 0.05% sodium azide.

Storage Conditions: Product should be stored at 4-8°C. DO NOT FREEZE.

STABILITY: Reagents are stable for the period shown on the vial label when stored properly.

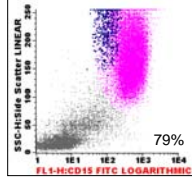
USE:
DISMC: Add 10 µl of MAB107 PMAC in 100 µl PBS. Mix gently and incubate for 15 minutes at 2° to 8°C. Wash twice with PBS and analyzer or fix with 0.5% v/v of paraformaldehyde in PBS and analyze.
WBC/BLOOD: Add 10 µl of MAB107 µl of whole blood. Mix gently and incubate for 15 minutes at room temperature (+37°C). Lyse the whole blood. Wash once with PBS and analyzer or fix with 0.5% v/v of paraformaldehyde in PBS and analyze. See instrument manufacturer's instructions for Lysed Whole Blood and immunofluorescence analysis with a flow cytometer or microscope.



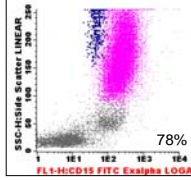
Antistoffen

- Clonen


Clone: MMA
Isotype: IgM



Clone: MCS1
Isotype: IgG3



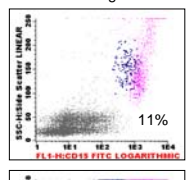
Labeling: CD15-CD36-CD45-CD14



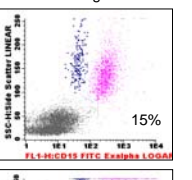
Antistoffen

- Clonen

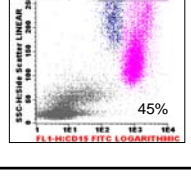
MMA-IgM

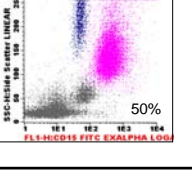



MCS1-IgG3



PB2








Antistoffen

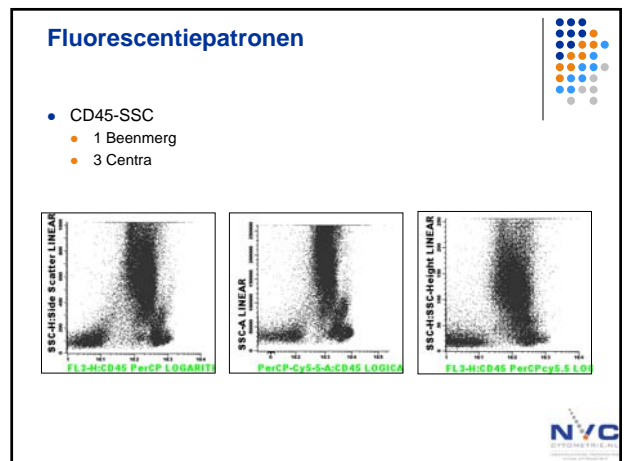
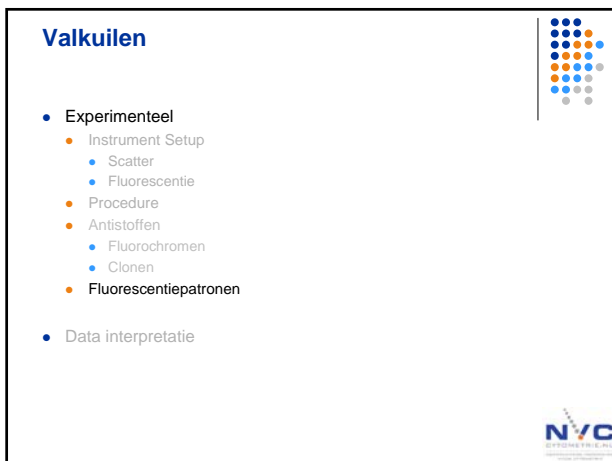
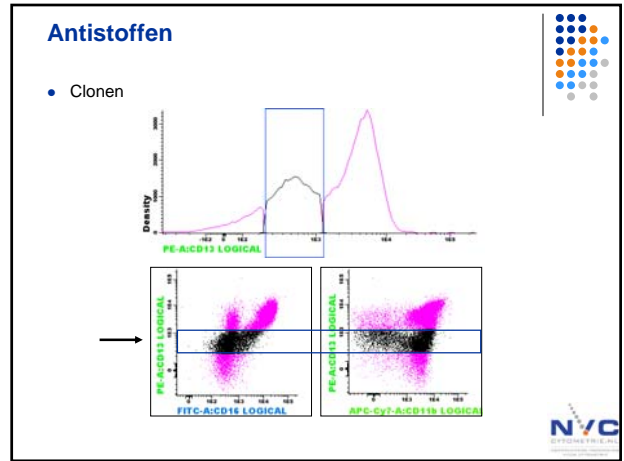
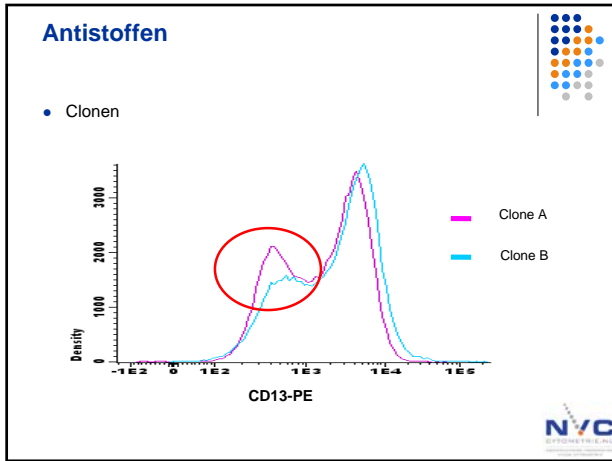
- Clonen
 - Isotype CD15-FITC

			CD15-FITC BD	CD15-FITC Exalpha	<-> 5%	Kleuren Mono's aan?	
			clone:MMA Isotype:IgM	clone:MCS-1 Isotype:IgG3	=	MMA(IgM) MCS-1(IgG3)	
1	B.	CML	LWBM	54,39	54,28	=	nee ja
2	U.	GB	LWBM	90,69	88,49	=	nee ja
3	L.	AML	LWBM	93,29	93,13	=	ntb ntb
4	V.	NHL	LWBM	77,86	79,05	=	deels ja
5	Jo.	MDS	LWBM	67,25	66,62	=	deels ja
6	Ja.	MDS Sq.	LWBM	57,34	55,07	=	deels ja
1	N.	GB	LWPB	79,56	77,92	+	nee ja
2	D.	wipB Fup	LWPB	45,35	50,05	+	deels ja
3	K.	AML Fup	LWPB	11,12	14,51	+	deels nee
4	Kr.	ITP	LWPB	42,39	43,70	=	deels nee

Conclusies:

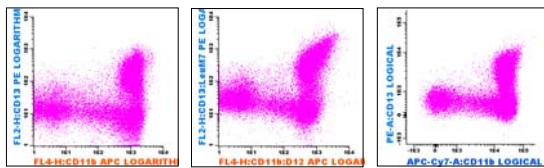
- De geteste BM geven procentueel geen verschil in CD15 aankleuring
- In 50% van de geteste PB geeft de het IgG3 moab een significant hoger % CD15 positieve events





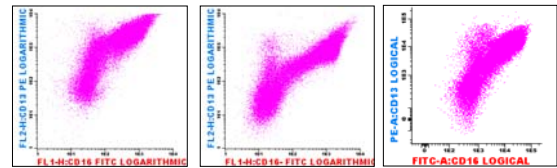
Fluorescentiepatronen

- CD11b-CD13
 - 1 Beenmerg
 - 3 Centra



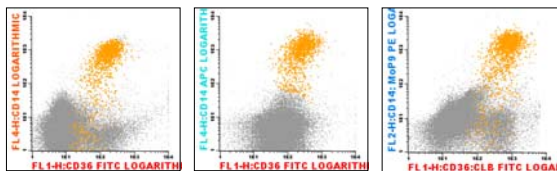
Fluorescentiepatronen

- CD16-CD13
 - 1 Beenmerg
 - 3 Centra



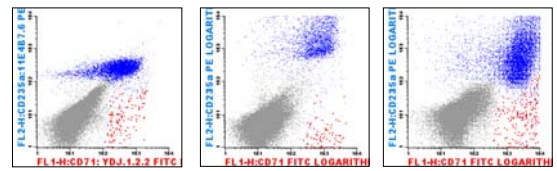
Fluorescentiepatronen

- 36-14
 - 1 Beenmerg
 - 3 Centra



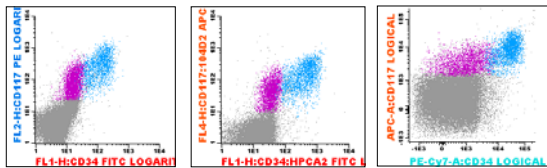
Fluorescentiepatronen

- CD71-CD235a
 - 1 Beenmerg
 - 3 Centra



Fluorescentiepatronen

- CD34-CD117
 - 1 Beenmerg
 - 3 Centra



Conclusies

- Experimenteel
 - Instrument Setup
 - Correct m.b.t. scatter, treshold en fluorescentie
 - Continuïteit waarborgen
 - Procedure
 - Celsuspensie verkrijgen door bulklysis (zonder fixatief)
 - Antistoffen
 - Fluorochroomkeuze afhankelijk van het doel
 - Reactiviteit verschillende clonen testen
 - Fluorescentiepatronen
 - Afhangelijk van apparatuur, setup, procedure en antistoffen

Vergelijkingen in de tijd binnen één centrum maar ook tussen centra:
Richtlijnen voor instrument setup, procedures en antistoffen noodzakelijk.

- Data interpretatie

Conclusies

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