

NovoCyte clinical evaluation

IQProducts, Groningen

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Agenda

- **Antibodies test (singles, duals, triples)**
- **Isotypes control**
- **FMH QuikQuant (*Fetal Maternal Hemorrhage*)**
- **Fetal Cell Count kit**
- **HITAlert (*Heparin Induced Thrombocytopenia*)**
- **Conclusions**

Materials and methods

- **ACEA NovoCyte 3000 (488, 642, 405 nm lasers), 13 colors**
- **Competitor IVD cytometer (488, 633, 405 nm lasers), 8 colors**
- **IQProducts antibodies and kits**



Agenda

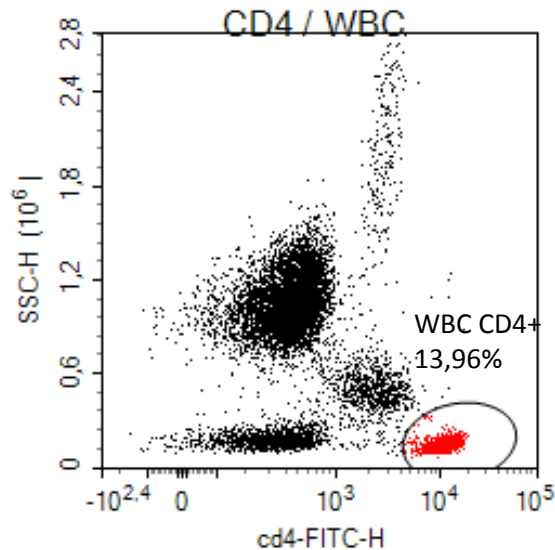
- **Antibodies test (singles, duals, triples)**
- Isotypes control
- FMH QuikQuant (*Fetal Maternal Hemorrhage*)
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Antibodies test

Single	Double	Triple
CD4-FITC	CD4+CD19	CD4+CD8+CD3
CD8-PE	CD4+CD8	CD3+CD16+CD56
CD19-PE	CD3+CD19	
CD3-APC	CD3+CD45	
CD45-APC		
CD45-CyQ		
CD45-IQP-Qdot 655		

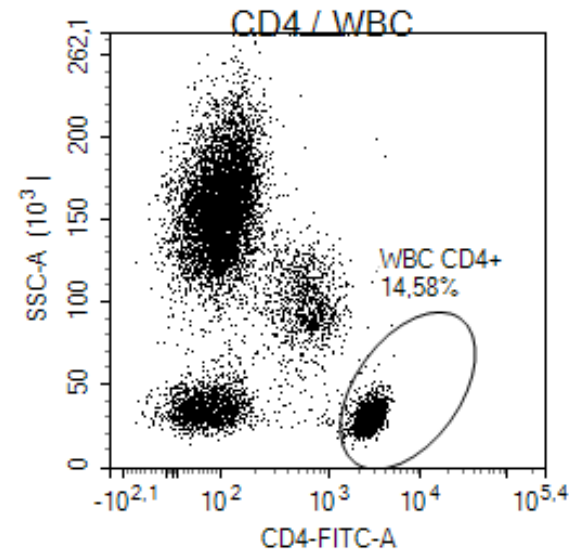
Single- CD4

NovoCyte



Gate	Count	% WBC	Mean X	Mean Y	CVX	CVY
WBC	10 408	100,00 %	2 121	783 995	176,43 %	59,50 %
WBC CD4+	1 453	13,96 %	10 931	140 723	22,03 %	22,82 %

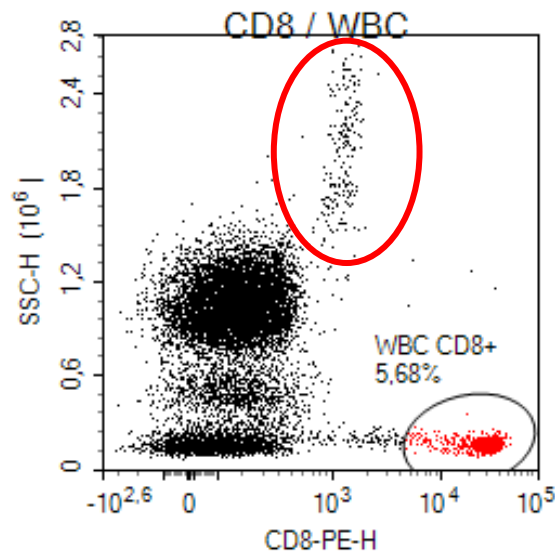
Competitor



Gate	Count	% WBC	Mean X	Mean Y	CVX	CVY
WBC	9 982	100,00 %	539	116 695	173,75 %	53,52 %
WBC CD4+	1 455	14,58 %	2 683	29 833	20,37 %	22,45 %

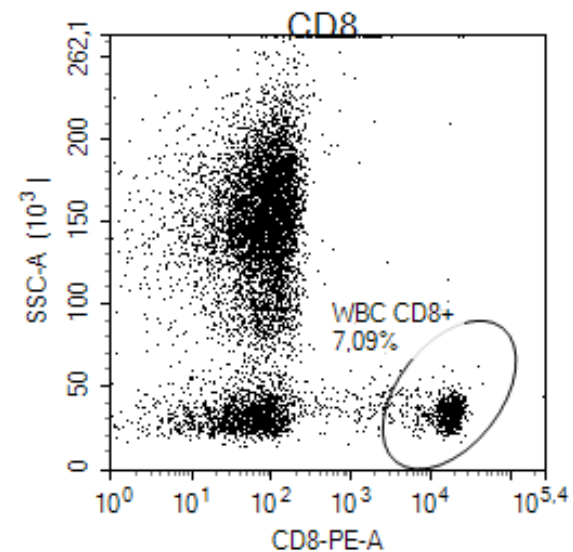
Single- CD8

NovoCyte



Gate	Count	% WBC	Mean X	Mean Y	CVX	CVY
WBC	11 427	100,00 %	1 833	786 675	500,29 %	57,86 %
WBC CD8+	649	5,68 %	27 656	159 801	34,58 %	20,43 %

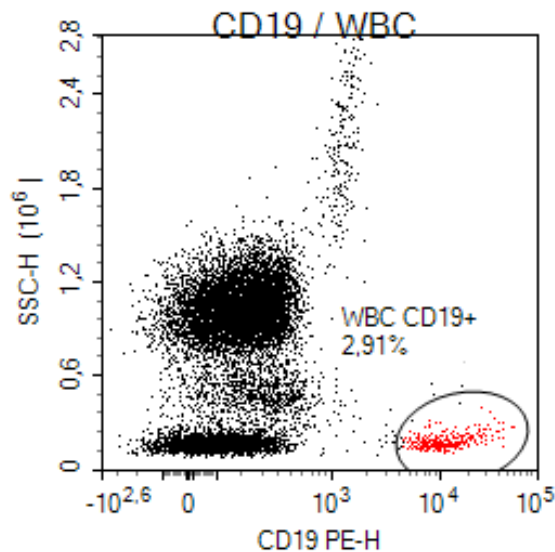
Competitor



Gate	Count	% All	Mean X	Mean Y	CVX	CVY
All	10 000	100,00 %	1 341	115 216	398,83 %	54,97 %
WBC CD8+	709	7,09 %	17 151	33 328	34,06 %	20,64 %

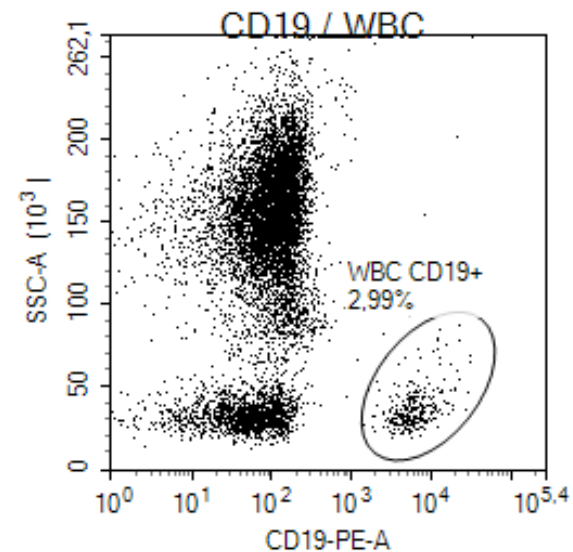
Single- CD19

NovoCyte



Gate	Count	% WBC	Mean X	Mean Y	CVX	CVY
WBC	10 855	100,00 %	2 092	776 811	7 371,26 %	58,21 %
WBC CD19+	316	2,91 %	12 943	177 549	67,68 %	26,10 %

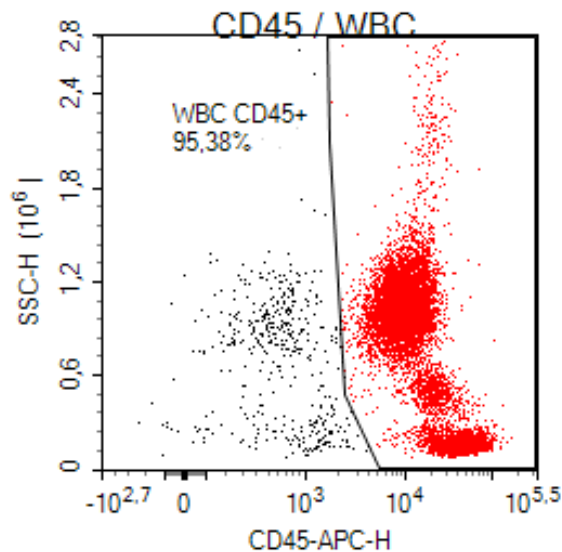
Competitor



Gate	Count	% WBC	Mean X	Mean Y	CVX	CVY
WBC	9 976	100,00 %	331	123 725	467,37 %	50,97 %
WBC CD19+	298	2,99 %	7 342	37 041	64,88 %	32,56 %

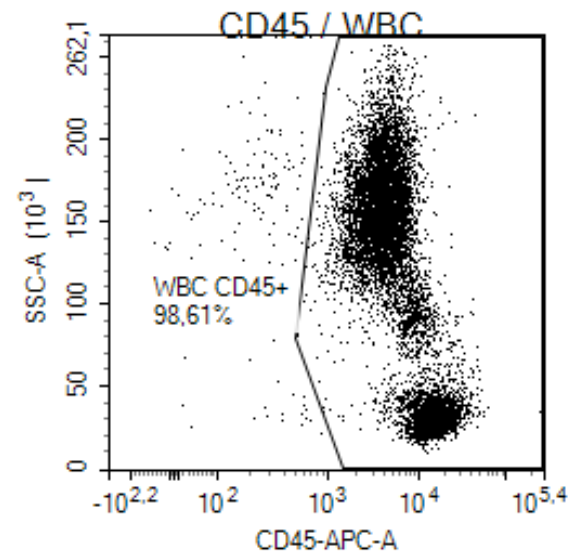
Single- CD45

NovoCyte



Gate	Count	% WBC	Mean X	Mean Y	CV X	CV Y
WBC	9 887	100,00 %	18 860	778 420	89,28 %	58,70 %
WBC CD45+	9 430	95,38 %	19 710	778 709	85,07 %	58,20 %

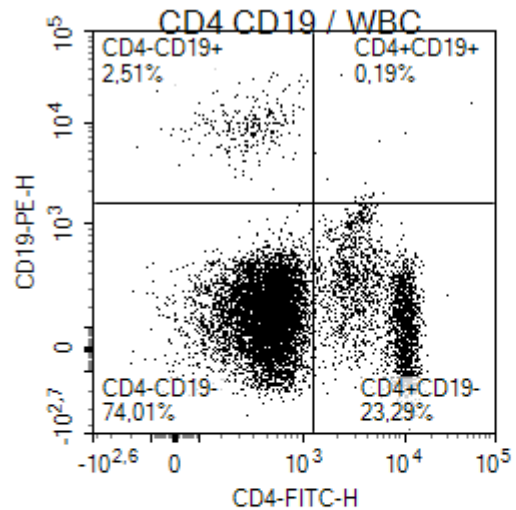
Competitor



Gate	Count	% WBC	Mean X	Mean Y	CV X	CV Y
WBC	9 987	100,00 %	7 572	123 902	90,80 %	51,99 %
WBC CD45+	9 848	98,61 %	7 675	123 459	89,49 %	52,16 %

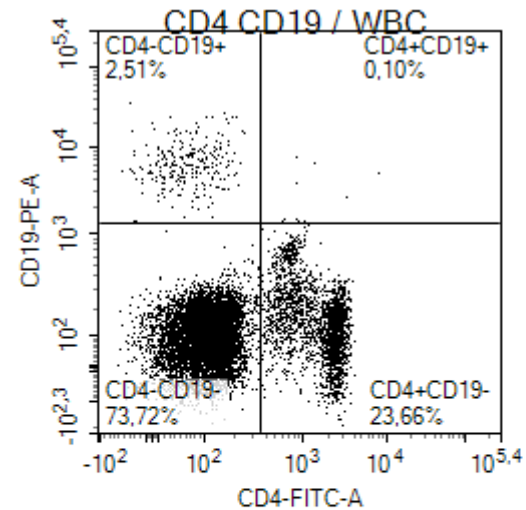
Double- CD4+CD19

NovoCyte



Gate	Count	% WBC	Mean X	Mean Y	CV X	CV Y
WBC	9 845	100,00 %	2 102	470	164,80 %	440,93 %
CD4-CD19+	247	2,51 %	395	10 566	54,64 %	58,72 %
CD4+CD19+	19	0,19 %	7 003	8 586	172,78 %	196,55 %
CD4-CD19-	7 286	74,01 %	544	178	39,34 %	94,03 %
CD4+CD19-	2 293	23,29 %	7 194	241	55,67 %	129,94 %

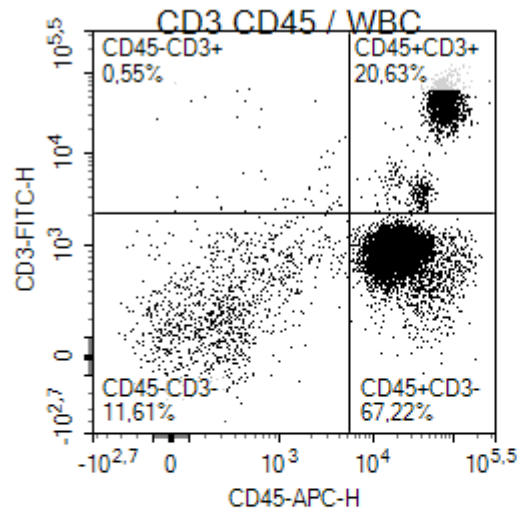
Competitor



Gate	Count	% WBC	Mean X	Mean Y	CV X	CV Y
WBC	9 956	100,00 %	501	304	165,00 %	451,58 %
CD4-CD19+	250	2,51 %	71	7 307	82,10 %	66,68 %
CD4+CD19+	10	0,10 %	1 833	3 184	125,75 %	73,37 %
CD4-CD19-	7 340	73,72 %	113	103	51,98 %	83,68 %
CD4+CD19-	2 356	23,66 %	1 753	176	50,93 %	106,71 %

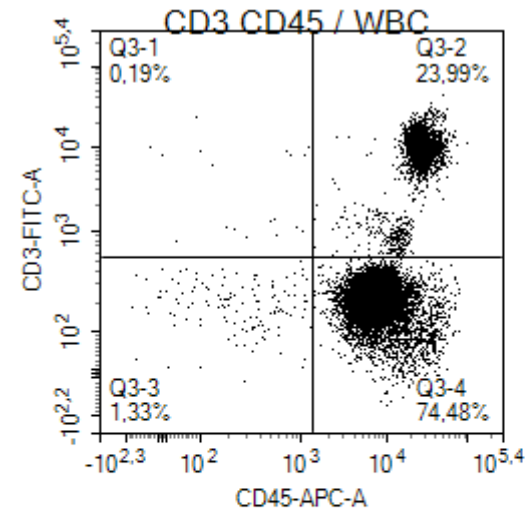
Double- CD3+CD45

NovoCyte



Gate	Count	% WBC	Mean X	Mean Y	CV X	CV Y
WBC	9 115	100,00 %	31 796	8 670	94,47 %	206,38 %
CD45-CD3+	50	0,55 %	1 754	13 257	81,07 %	139,18 %
CD45+CD3+	1 880	20,63 %	71 844	38 590	36,36 %	52,53 %
CD45-CD3-	1 058	11,61 %	508	366	146,77 %	106,61 %
CD45+CD3-	6 127	67,22 %	25 156	886	81,50 %	31,06 %

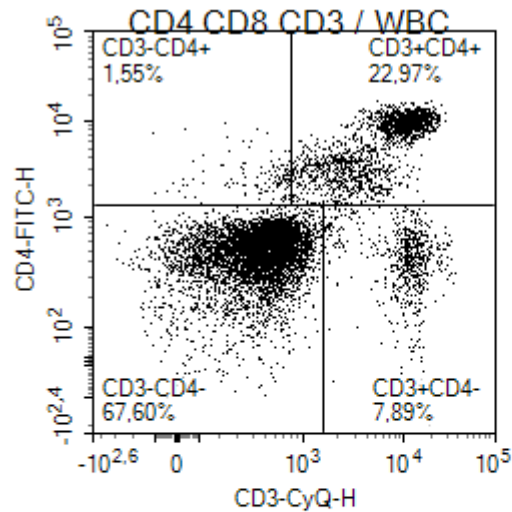
Competitor



Gate	Count	% WBC	Mean X	Mean Y	CV X	CV Y
WBC	9 965	100,00 %	13 302	2 463	81,65 %	188,50 %
Q3-1	19	0,19 %	509	5 289	86,84 %	109,54 %
Q3-2	2 391	23,99 %	26 198	9 545	35,68 %	50,63 %
Q3-3	133	1,33 %	392	204	92,21 %	49,00 %
Q3-4	7 422	74,48 %	9 411	215	80,98 %	39,48 %

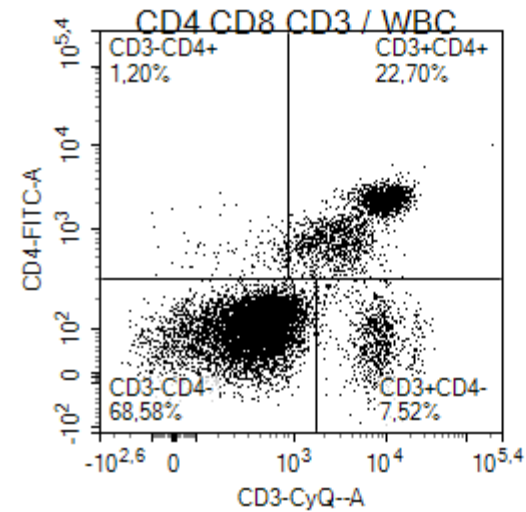
Triple- CD4+CD8+CD3

NovoCyte



Gate	Count	% WBC	Mean X	Mean Y	CV X	CV Y
WBC	8 280	100,00 %	3 207	2 075	169,30 %	161,17 %
CD3-CD4+	128	1,55 %	493	2 723	49,42 %	53,22 %
CD3+CD4+	1 902	22,97 %	8 504	7 216	74,66 %	51,47 %
CD3-CD4-	5 597	67,60 %	481	499	58,62 %	40,93 %
CD3+CD4-	653	7,89 %	11 682	487	52,14 %	55,01 %

Competitor



Gate	Count	% WBC	Mean X	Mean Y	CV X	CV Y
WBC	9 978	100,00 %	2 831	471	177,60 %	167,91 %
CD3-CD4+	120	1,20 %	492	678	58,34 %	54,34 %
CD3+CD4+	2 265	22,70 %	7 930	1 702	82,92 %	51,33 %
CD3-CD4-	6 843	68,58 %	484	102	60,10 %	51,85 %
CD3+CD4-	750	7,52 %	9 220	86	63,69 %	84,15 %

Agenda

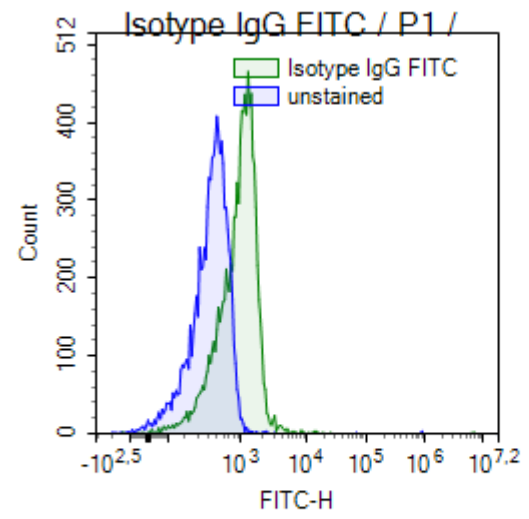
- Antibodies test (singles, duals, triples)
- **Isotypes control**
- FMH QuikQuant (*Fetal Maternal Hemorrhage*)
- Fetal Cell Count kit
- HITAlert (*Heparin Induced Thrombocytopenia*)
- Conclusions

Isotypes control

Single	Double	Triple
IgG-FITC		CD45+CD16+CD3
IgG2A-PE		CD45+CD3+HLA-DR
IgG2B-FITC		CD45RO+CD3+CD4
IgM-FITC		

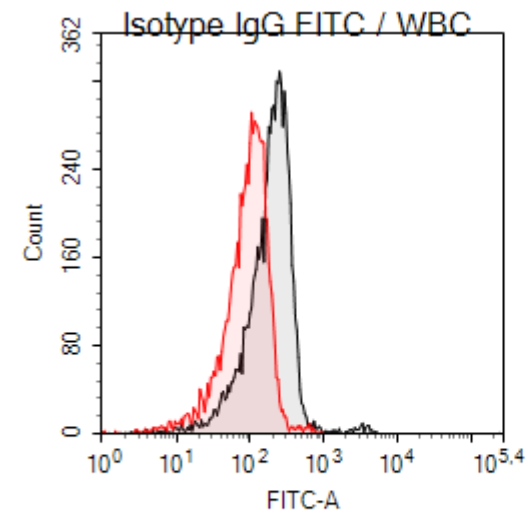
Single (igG-FITC)

NovoCyte



#	Sample	Gate	Count	% WBC	Mean X	CVX
1	Isotype IgG FITC	WBC	9 904	100,00 %	6 958	2 597,96 %
2	unstained	WBC*	9 063	100,00 %	896	2 095,30 %

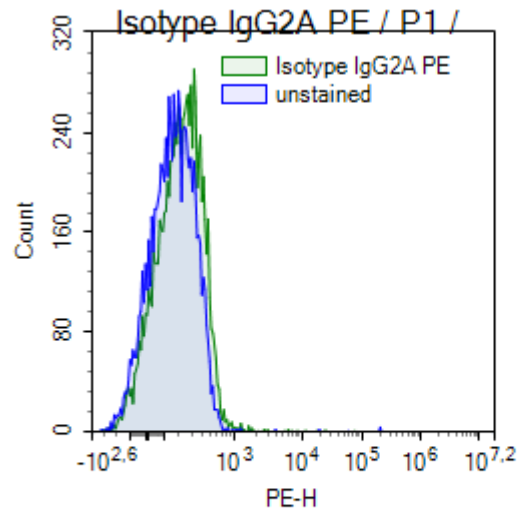
Competitor



#	Sample	Gate	Count	% WBC	Mean X	CVX
1	Isotype IgG FITC	WBC	8 749	100,00 %	278	1 012,62 %
2	Isotype 20160712 blanco	WBC*	8 230	100,00 %	106	76,14 %

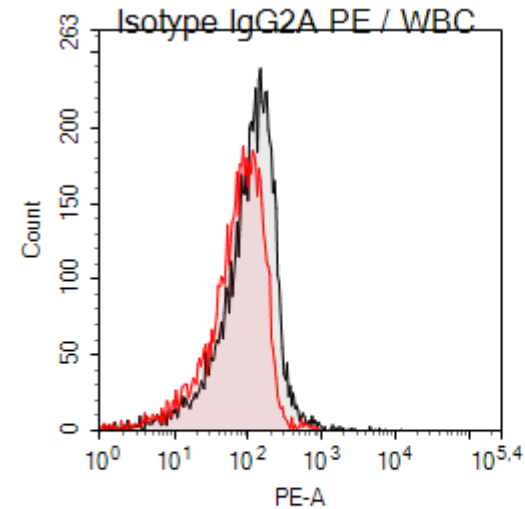
Single (igG2A-PE)

NovoCyte



#	Sample	Gate	Count	% WBC	Mean X	CV X
1	Isotype IgG2A PE	WBC	9 384	100,00 %	252	379,53 %
2	unstained	WBC*	9 063	100,00 %	274	1 543,53 %

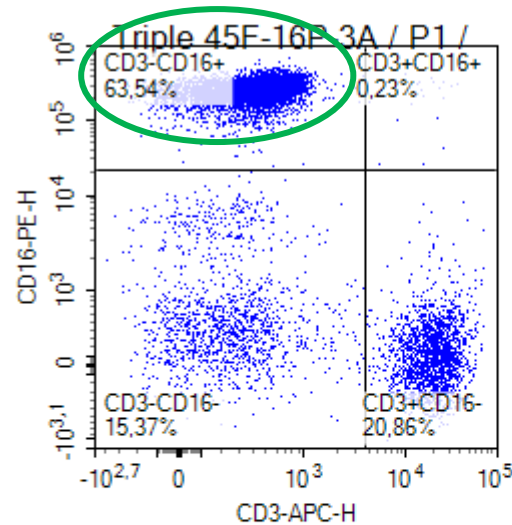
Competitor



#	Sample	Gate	Count	% WBC	Mean X	CV X
1	Isotype IgG2A PE	WBC	8 815	100,00 %	122	210,96 %
2	Isotype 20160712 blanco	WBC*	8 230	100,00 %	70	126,25 %

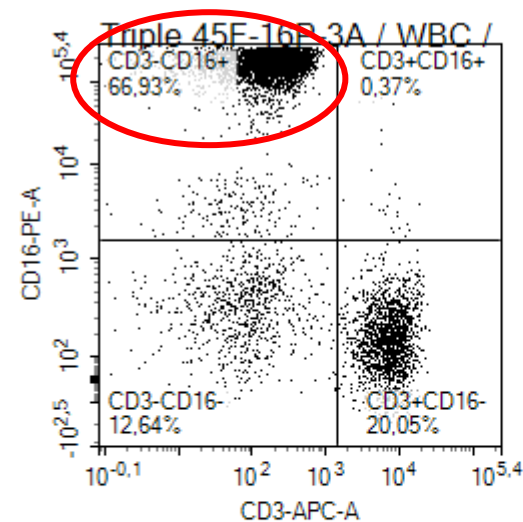
Triple (45F-16P-3A)

NovoCyte



Gate	Count	% CD45+	Mean X	Mean Y	CVX	CVY
CD45+	9 153	100,00 %	4 829	163 807	205,49 %	84,52 %
CD3-CD16+	5 816	63,54 %	425	256 626	59,58 %	30,93 %
CD3+CD16+	21	0,23 %	16 736	226 892	55,52 %	51,89 %
CD3-CD16-	1 407	15,37 %	321	1 247	151,42 %	176,17 %
CD3+CD16-	1 909	20,86 %	21 435	142	50,98 %	366,60 %

Competitor



Gate	Count	% CD45+	Mean X	Mean Y	CVX	CVY
CD45+	8 537	100,00 %	1 686	110 720	217,56 %	83,28 %
CD3-CD16+	5 714	66,93 %	72	165 109	801,47 %	36,99 %
CD3+CD16+	32	0,37 %	7 998	31 879	44,50 %	185,06 %
CD3-CD16-	1 079	12,64 %	88	399	221,31 %	68,44 %
CD3+CD16-	1 712	20,05 %	7 961	196	49,08 %	94,35 %

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- Antibodies test (singles, duals, triples)
- Isotypes control
- **Fetal Cell Count Kit**
- FMH QuikQuant (*Fetal Maternal Hemorrhage*)
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Fetal Cell Count Kit

- The assay is based on a combination of two antibodies. One is directed against HbF, while the second is specific for carbonic anhydrase (CA), an enzyme present in adult in late pregnancy stage.



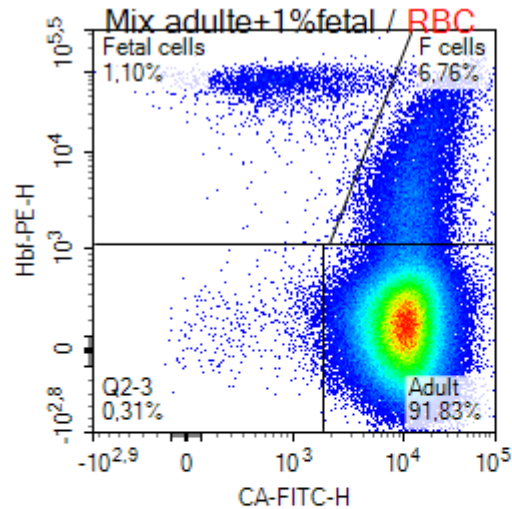
Fetal Cell Count Kit

- **Unique quantification of Fetomaternal Hemorrhage by flow cytometry**
- **Complete assay for routine diagnosis of Fetomaternal Hemorrhage**
- **Detection as low as 0.02% fetal cells in maternal blood**
- **Detection of fetal haemoglobin (HbF) and Carbonic Anhydrase (CA)**



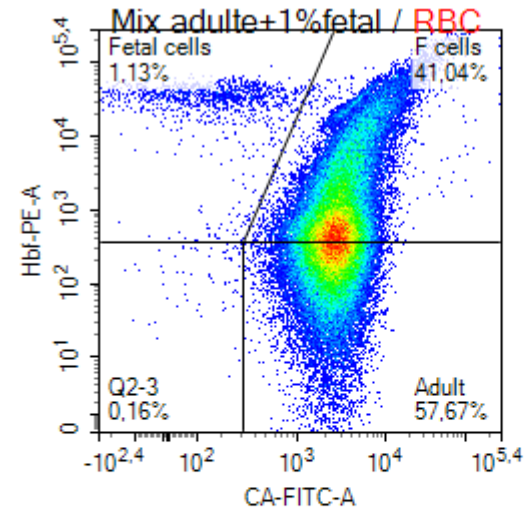
Mix adult+1%fetal

NovoCyte



Gate	Count	% RBC	Mean X	Mean Y	CVX	CVY
RBC	211 103	100,00 %	12 456	1 608	554,50 %	639,69 %
Fetal cells	2 316	1,10 %	1 294	67 994	103,35 %	41,87 %
F cells	14 270	6,76 %	23 567	10 467	1 120,82 %	236,04 %
Q2-3	657	0,31 %	1 008	203	52,53 %	111,24 %
Adult	193 860	91,83 %	11 810	167	58,43 %	143,72 %

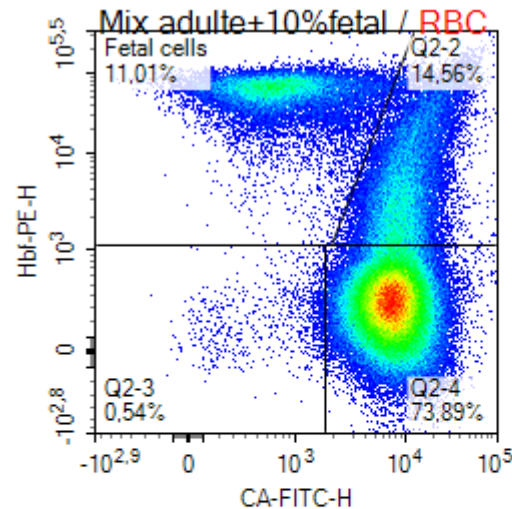
Competitor



Gate	Count	% RBC	Mean X	Mean Y	CVX	CVY
RBC	99 772	100,00 %	3 208	2 623	128,96 %	399,77 %
Fetal cells	1 131	1,13 %	216	33 327	121,29 %	41,52 %
F cells	40 950	41,04 %	4 169	5 472	146,60 %	270,23 %
Q2-3	157	0,16 %	139	-61	99,34 %	-420,62 %
Adult	57 534	57,67 %	2 591	-1	52,50 %	-18 429,01 %

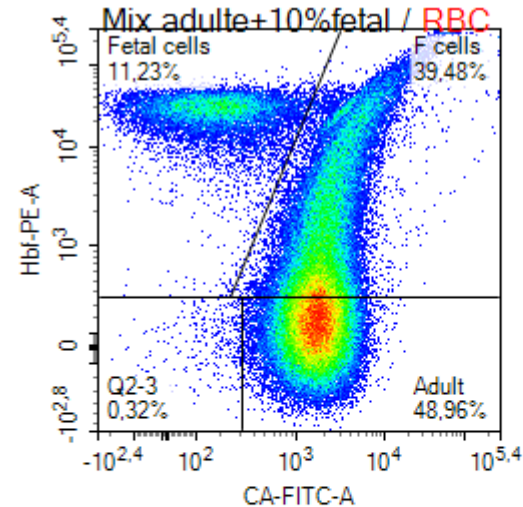
Mix adult+10%fetal

NovoCyte



Gate	Count	% RBC	Mean X	Mean Y	CVX	CVY
RBC	169 822	100,00 %	8 700	8 493	675,47 %	254,63 %
Fetal cells	18 700	11,01 %	1 171	58 086	102,73 %	41,25 %
Q2-2	24 722	14,56 %	16 005	12 708	957,65 %	186,17 %
Q2-3	920	0,54 %	992	265	55,65 %	89,03 %
Q2-4	125 480	73,89 %	8 440	333	60,28 %	71,24 %

Competitor



Gate	Count	% RBC	Mean X	Mean Y	CVX	CVY
RBC	99 499	100,00 %	2 291	6 366	213,91 %	252,81 %
Fetal cells	11 177	11,23 %	234	27 145	108,10 %	40,00 %
F cells	39 284	39,48 %	3 472	8 287	216,68 %	255,08 %
Q2-3	319	0,32 %	176	44	65,08 %	390,14 %
Adult	48 719	48,96 %	1 825	92	56,39 %	180,50 %

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- **FMH QuikQuant (*Fetal Maternal Hemorrhage*)**
- *HITAlert (Heparin Induced Thrombocytopenia)*
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FMH QuikQuant

- **Detection and quantification of fetal red blood cells (RBCs) in maternal blood samples is essential for obstetrical management.**
- **Measurement of fetal RBCs is critical as the extent of Fetomaternal Hemorrhage (FMH), the transplacental passage of fetal RBCs into the maternal circulation, has consequences for further treatment of mother and child.**



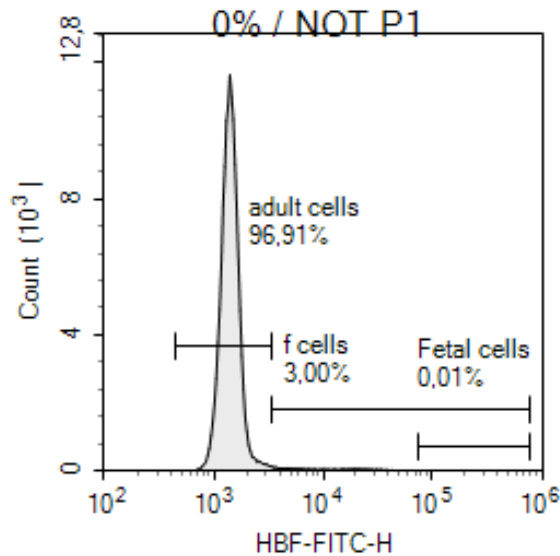
FMH QuikQuant

- The FMH QuikQuant uses an anti-Hemoglobin F monoclonal antibody and Propidium Iodide reagent.
- Rapid assay for Fetomaternal Hemorrhage Quantification
- Detection of fetal hemoglobin (HbF) of as low as 0,06% fetal cells in maternal blood.



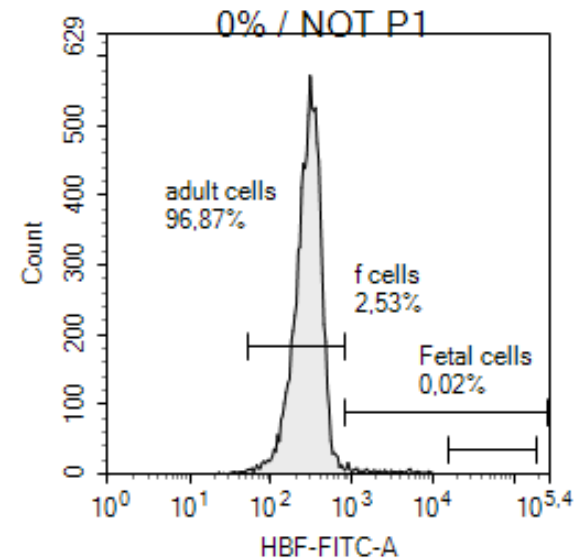
0%fetal cells

NovoCyte



Gate	Count	% NOT P1	Mean X	CV X
NOT P1	139 655	100,00 %	1 873	229,50 %
Fetal cells	17	0,01 %	126 711	110,56 %
f cells	4 184	3,00 %	15 165	109,97 %
adult cells	135 339	96,91 %	1 457	21,34 %

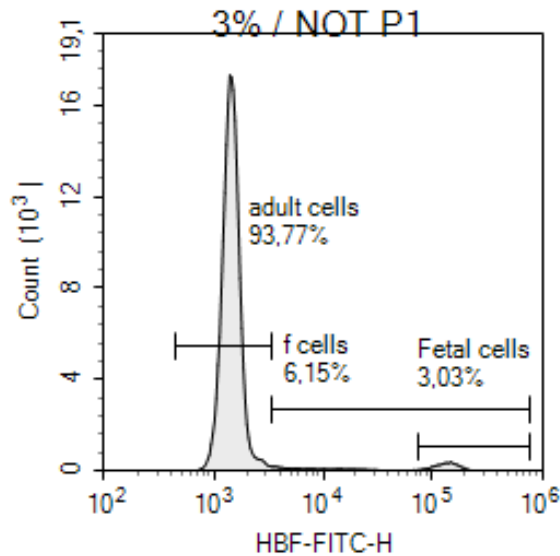
Competitor



Gate	Count	% NOT P1	Mean X	CV X
NOT P1	9 680	100,00 %	389	294,08 %
Fetal cells 2	2	0,02 %	54 969	100,93 %
f cells	245	2,53 %	3 581	178,59 %
adult cells	9 377	96,87 %	308	35,66 %

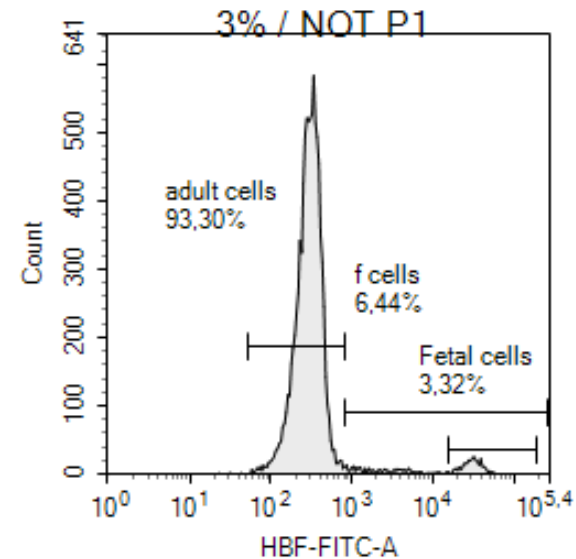
3%fetal cells

NovoCyte



Gate	Count	% NOT P1	Mean X	CVX
NOT P1	218 092	100,00 %	6 362	441,21 %
Fetal cells	6 612	3,03 %	146 590	31,52 %
f cells	13 422	6,15 %	79 899	92,71 %
adult cells	204 500	93,77 %	1 495	21,10 %

Competitor



Gate	Count	% NOT P1	Mean X	CVX
NOT P1	9 662	100,00 %	1 488	410,88 %
Fetal cells	321	3,32 %	32 747	29,92 %
f cells	622	6,44 %	18 562	88,37 %
adult cells	9 015	93,30 %	314	34,01 %

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- **HIT Alert (*Heparin Induced Thrombocytopenia*)**
- Conclusions

HIT Alert

- Heparin-induced thrombocytopenia (HIT) is an acute, immune-mediated process that may result in life-threatening thrombosis.
- HIT is caused by platelet-activating, heparin-dependent antibodies, who form complexes of heparin together with either platelet factor-4 (PF-4) (most of the clinical cases), interleukin-8 (IL-8) or neutrophil-activating peptide 2 (NAP-2).



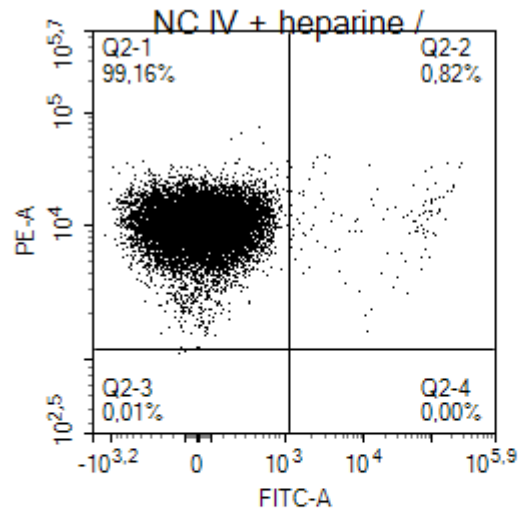
HIT Alert

- Detection of heparin complexes specific antibodies
- Reactivity independent of PF4
- Kit also detects IL-8/heparin and NAP-2/heparin complex antibodies



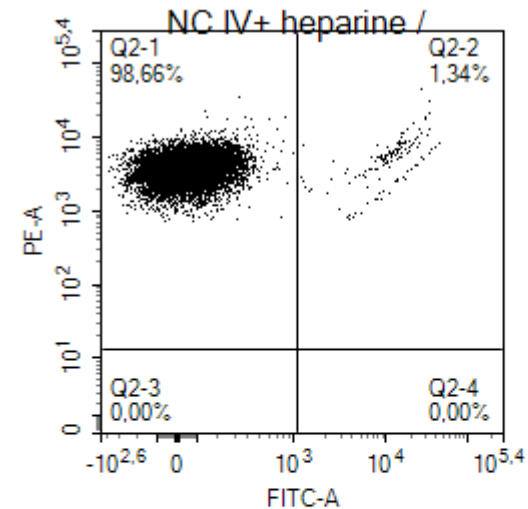
Negative control

NovoCyte



Gate	Count	% PE+	Mean X	Mean Y	CVX	CVY
PE+	14 312	100,00 %	431	10 696	1 654,03 %	40,74 %
Q2-1	14 192	99,16 %	17	10 673	1 708,20 %	40,17 %
Q2-2	118	0,82 %	50 297	13 679	120,62 %	66,47 %
Q2-3	2	0,01 %	-119	1 113	-24,29 %	0,16 %
Q2-4	0	0,00 %	0	0	0,00 %	0,00 %

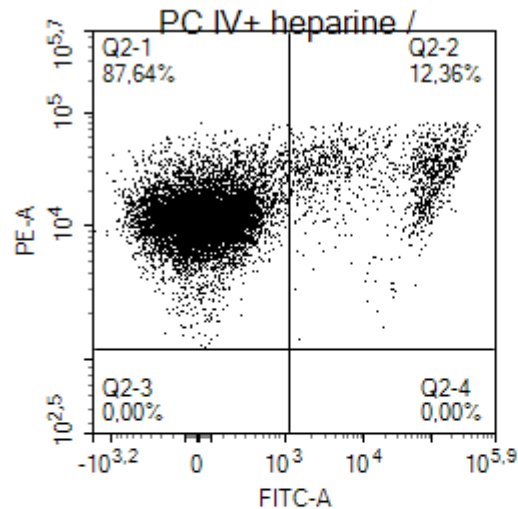
Competitor



Gate	Count	% PE+	Mean X	Mean Y	CVX	CVY
PE+	10 127	100,00 %	239	3 958	786,60 %	44,35 %
Q2-1	9 991	98,66 %	56	3 927	233,52 %	41,49 %
Q2-2	136	1,34 %	13 723	6 258	64,63 %	86,54 %
Q2-3	0	0,00 %	0	0	0,00 %	0,00 %
Q2-4	0	0,00 %	0	0	0,00 %	0,00 %

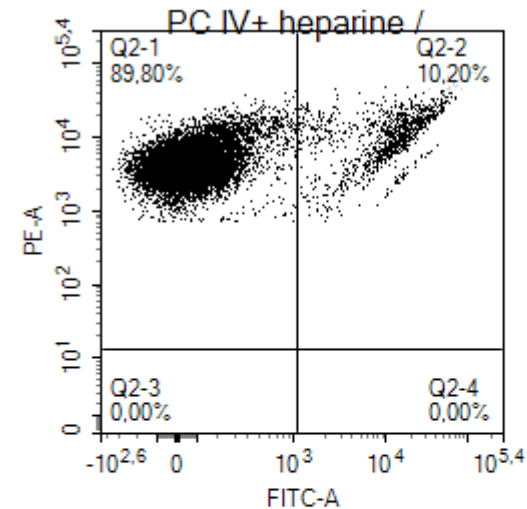
Positive control

NovoCyte



Gate	Count	% PE+	Mean X	Mean Y	CVX	CVY
PE+	9 828	100,00 %	8 561	15 965	421,78 %	72,36 %
Q2-1	8 613	87,64 %	35	13 545	1 121,01 %	59,55 %
Q2-2	1 215	12,36 %	69 001	33 120	115,77 %	50,76 %
Q2-3	0	0,00 %	0	0	0,00 %	0,00 %
Q2-4	0	0,00 %	0	0	0,00 %	0,00 %

Competitor



Gate	Count	% PE+	Mean X	Mean Y	CVX	CVY
PE+	11 156	100,00 %	1 563	5 861	388,59 %	85,59 %
Q2-1	10 018	89,80 %	84	5 051	202,74 %	66,13 %
Q2-2	1 138	10,20 %	14 585	12 989	90,07 %	73,81 %
Q2-3	0	0,00 %	0	0	0,00 %	0,00 %
Q2-4	0	0,00 %	0	0	0,00 %	0,00 %

Agenda

- Antibodies test (singles, duals, triples)
- Isotypes control
- Fetal Cell Count Kit
- FMH QuikQuant (*Fetal Maternal Hemorrhage*)
- HITAlert (*Heparin Induced Thrombocytopenia*)
- **Conclusions**

Conclusions

- Globally, the reagents and the kits performed equally well on both devices.
- NovoCyte did not require PMT's adjustment, the device is able to read and save the whole data (Eosinophils detection). In some case, the signal of the competitor cytometer is saturated, requiring PMT's gain adjustment.
- The combination of IQProducts reagents and NovoCyte flow cytometer, is a reliable solution for the diagnostic market.

References

- <http://www.accela.eu/acea-biosciences/novocyte>
- <http://www.iqproducts.nl/>

THANK YOU FOR YOUR ATTENTION

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