La cytométrie en flux dans la routine du laboratoire d'Hématologie : implantation de l'Hematoflow

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CNBH Toulouse 2012

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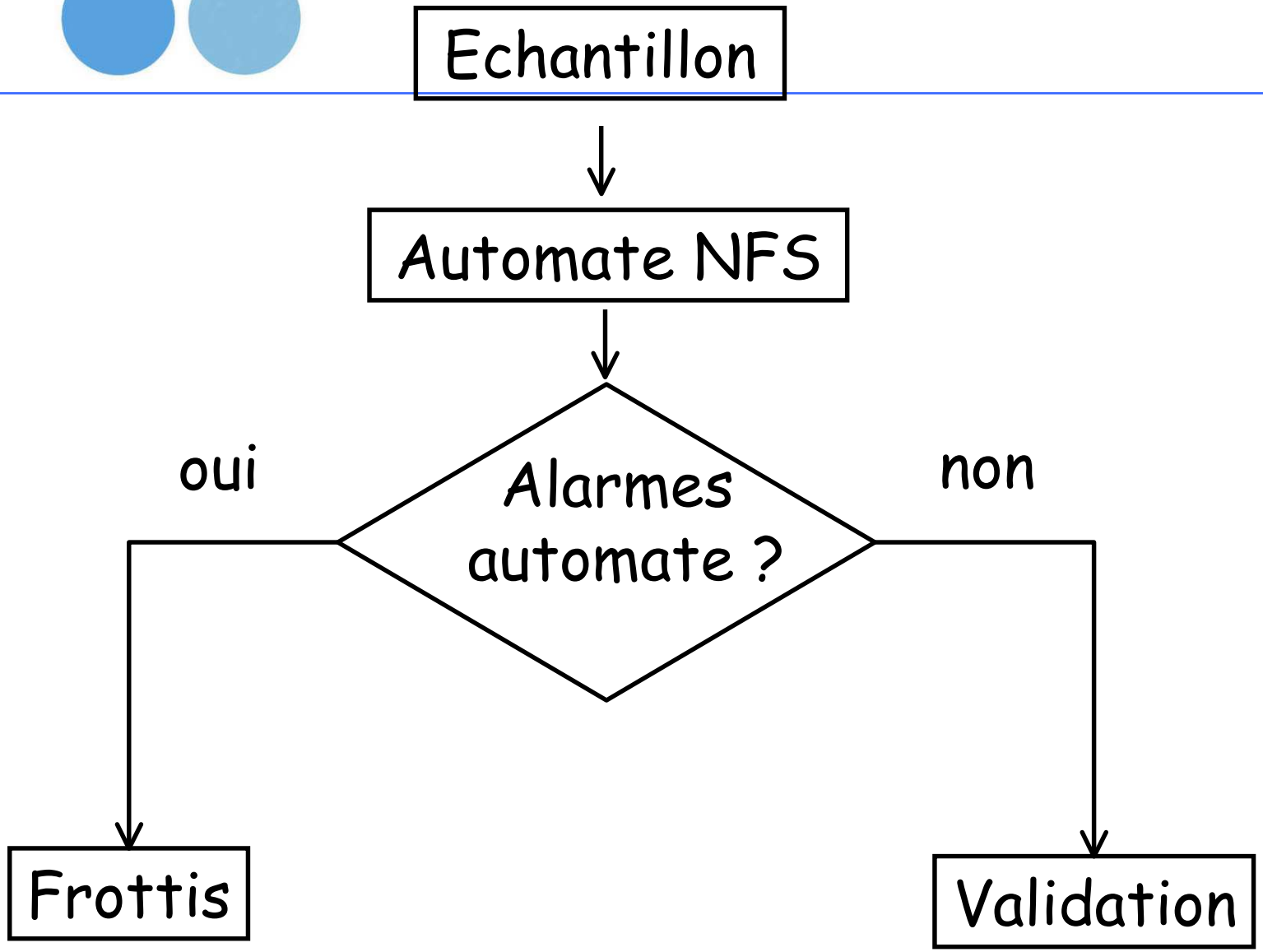

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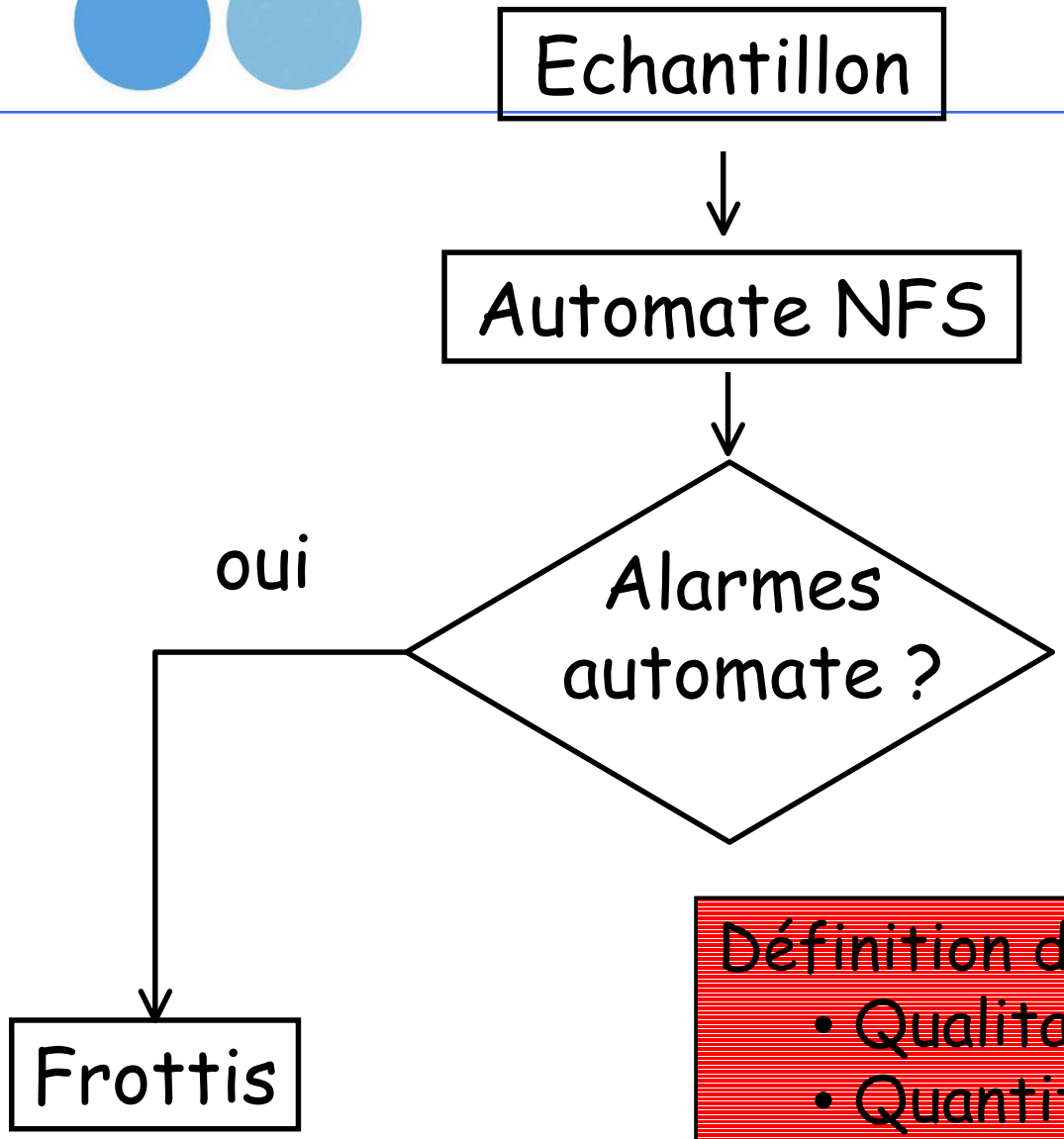

Définition des alarmes
- Qualitatives
- Quantitatives
- Détermination des seuils

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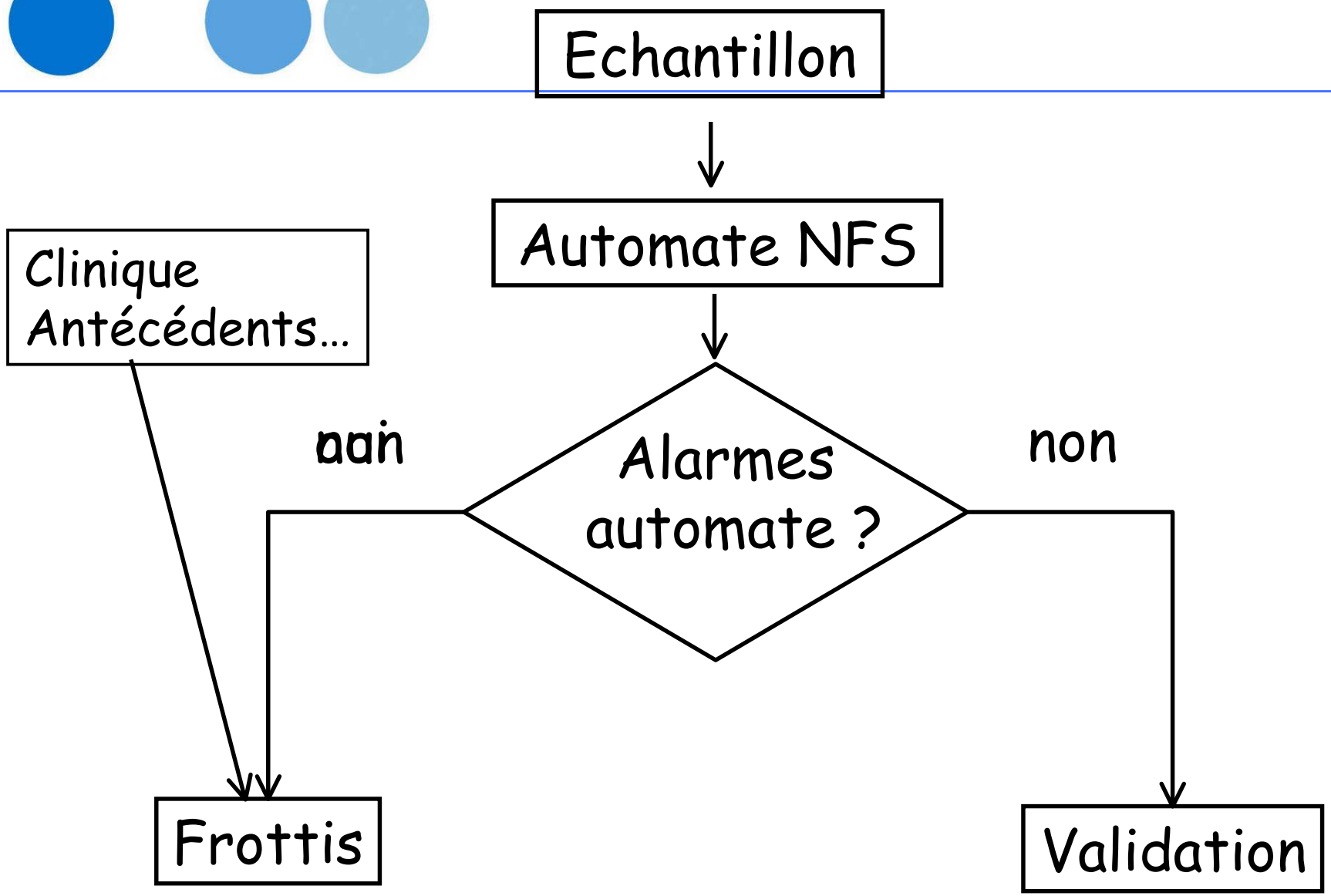

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In Fine,

L'automate de NFS

- Compte les éléments figurés du sang
- Identifie ET compte
  les sous populations leucocytaires normales
- Alarme les sous populations anormales

Rôle de screening

- Excellent compteur
- Identification plus ou moins bonne

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Limitations de la morphologie dans la formule sanguine en routine

- Mauvais choix dans la zone de comptage sur lame
- Erreurs liés à l'observateur
- Mauvaise efficacité (méthode consommatrice de temps)
- Erreurs statistiques sur le comptage des cellules

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Une deuxième vérité…
Table de Rümke

a	n=100	n=200	n=500	n=1.000	n=10.000
0	0 – 3.6	0 – 1.8	0 – 0.7	0 – 0.4	0 – 0.1
1	0.0 – 5.4	0.1 – 3.6	0.3 – 2.3	0.5 – 1.8	0.8 – 1.3
5	1.6 – 11.3	2.4 – 9.0	3.3 – 7.3	3.7 – 6.5	4.5 – 5.5
10	4.9 – 17.6	6.2 – 15.0	7.5 – 13.0	8.2 – 12.0	9.4 – 10.7
15	8.6 – 23.5	10.4 – 20.7	12.0 – 18.4	12.8 – 17.4	14.3 – 15.8
20	12.7 – 29.2	14.7 – 26.2	16.6 – 23.8	17.6 – 22.6	19.2 – 20.8
30	21.2 – 40.0	23.7 – 36.9	26.0 – 34.2	27.2 – 32.9	29.1 – 31.0
40	30.3 – 50.3	33.2 – 47.1	35.7 – 44.4	36.9 – 43.1	39.0 – 41.0
50	39.8 – 60.2	42.9 – 57.1	45.5 – 54.5	46.9 – 53.1	49.0 – 51.0
70	60.0 – 78.8	63.1 – 76.3	65.8 – 74.0	67.1 – 72.8	69.0 – 70.9
80	70.8 – 87.3	73.8 – 85.3	76.2 – 83.4	77.4 – 82.4	79.2 – 80.8
90	82.4 – 95.1	85.0 – 93.8	87.0 – 92.5	88.0 – 91.8	89.3 – 90.6
100	96.4 – 100	98.2 – 100	99.3 – 100	99.6 – 100	99.9 – 100

Fig. 1: Rümke Table – shows the statistical inaccuracy which results when counting out a defined number (n). Thus, the number of e.g. band cells can vary in a slide between 4.9 and 17.6 when 100 cells are counted out and 10 band cells are actually present. (Variation of the number due to subjective assessment is not taken into account here.)

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Conséquences I

L'examen du frottis est réalisé de façon très disparate dans les laboratoires

- étalement manuel ou automatisé
- microscope + compte sur 100 cellules
- microscope + screening
- microscope automatisé (100-120 cellules) avec ou sans reconnaissance automatisée
- Examen réalisé par les techniciens, par les seuls biologistes…

→ **Accréditation ?**

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Conséquences II

Quel est le rôle exact du frottis dans un laboratoire ?

Méthode de screening ?
Méthode de référence ?
Les deux ?
…

Introduction de la formule sanguine immunologique

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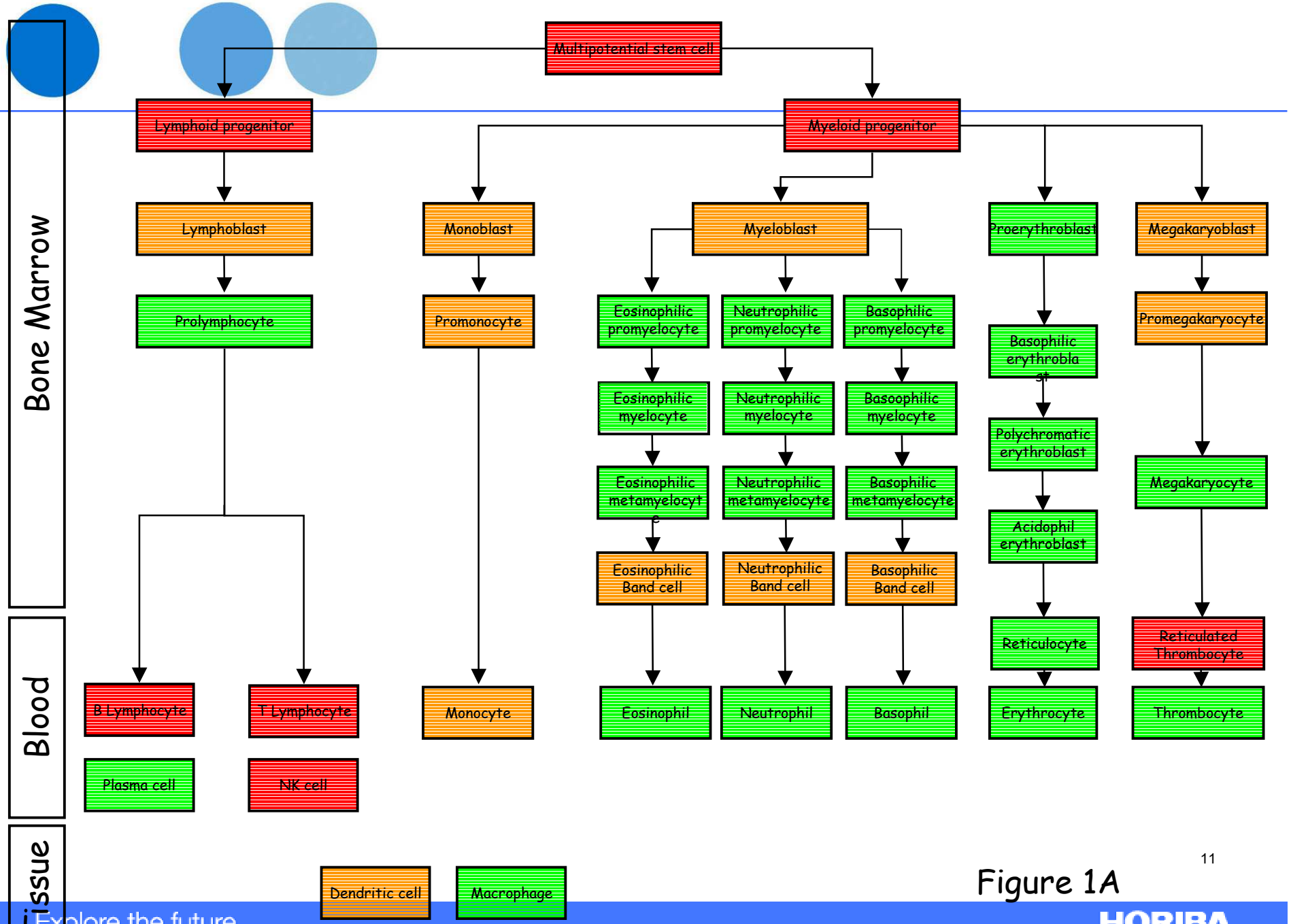

Figure 1A

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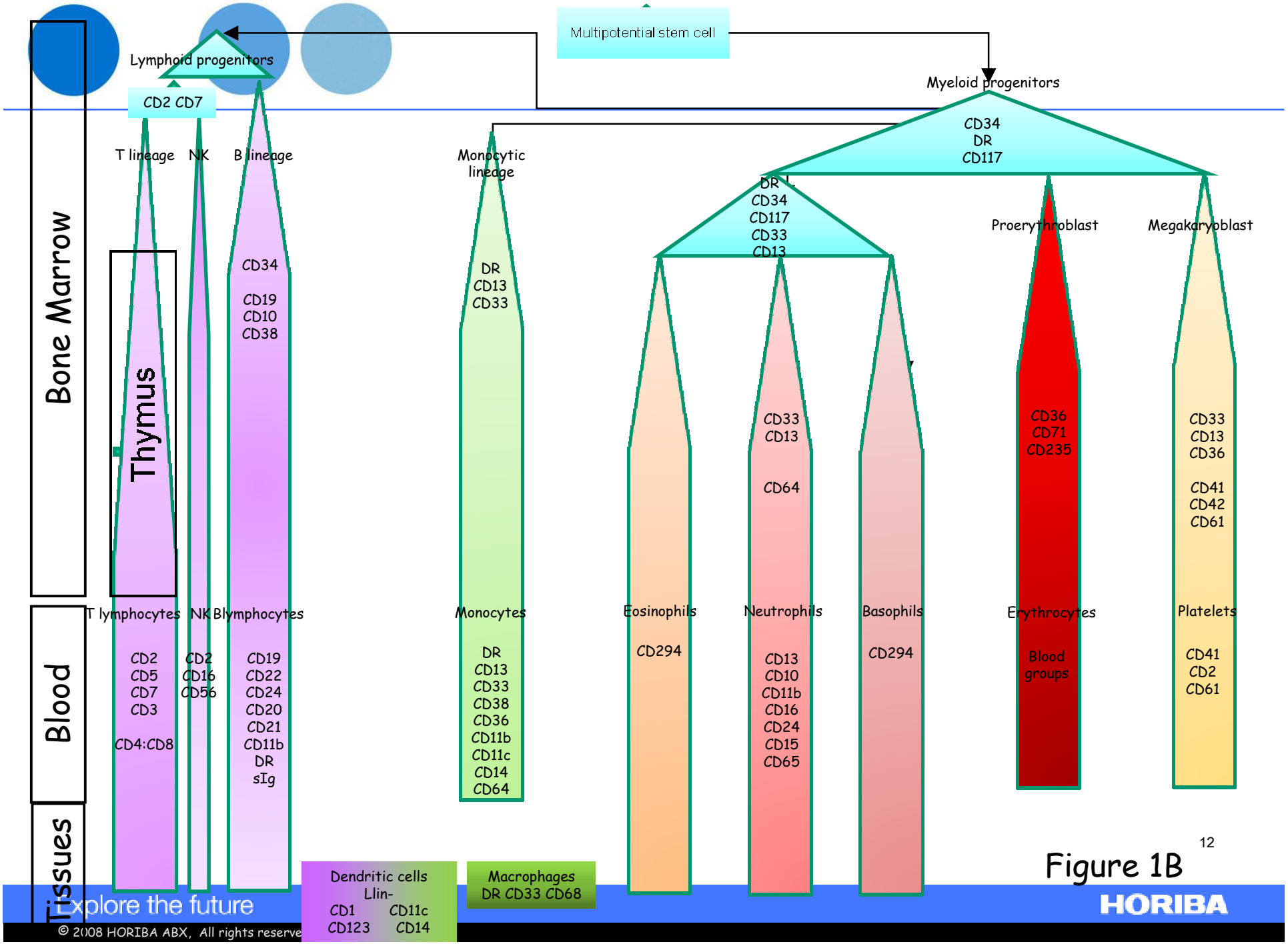
Multipotential stem cell
Lymphoid progenitors
CD2 CD7
Myeloid progenitors
CD34
DR
CD117
T lineage
NK
B lineage
Monocytic lineage
DR
CD34
CD117
CD33
CD13
Proerythroblast
Megakaryoblast
Bone Marrow
Thymus
CD34
CD19
CD10
CD38
DR
CD13
CD33
CD33
CD13
CD64
CD36
CD71
CD235
CD33
CD13
CD36
CD41
CD42
CD61
Blood
T lymphocytes
NK
B lymphocytes
Monocytes
Eosinophils
Neutrophils
Basophils
Erythrocytes
Platelets
CD2
CD5
CD7
CD3
CD4:CD8
CD2
CD16
CD56
CD19
CD22
CD24
CD20
CD21
CD11b
DR
sIg
DR
CD13
CD33
CD38
CD36
CD11b
CD11c
CD14
CD64
CD294
CD13
CD10
CD11b
CD16
CD24
CD15
CD65
CD294
Blood groups
CD41
CD2
CD61
Tissues
Dendritic cells
Llin-
CD1
CD11c
CD123
CD14
Macrophages
DR CD33 CD68
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Figure 1B

Cytometry PART A
Journal of the International Society for Analytical Cytology

"6 Markers/5 Colors" Extended White Blood Cell Differential by Flow Cytometry

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Abstract

Electronic white blood cell (WBC) differential by standard cytology (hematology analyzer and visual inspection of blood smears) is limited to five types and identification of abnormal cells is only qualitative, often problematic, poorly reproducible, and labour costing. We present our results on WBC differential by flow cytometry (FCM) with a 6 markers, 5 colors CD36-FITC/CD2-PE+CRTH2-PE/CD19-ECD/CD16-Cy5/CD45-Cy7 combination, on 379 subjects, with detection of 12 different circulating cell types, among them 11 were quantified. Detection of quantitative abnormalities of whole leucocytes, neutrophils, eosinophils, basophils, monocytes, or lymphocytes was comparable by FCM and by standard cytology in terms of sensitivity and specificity. FCM was better than standard cytology in detection and quantification of circulating blast cells or immature granulocytes, with a first lineage orientation in the former case. All cases of lymphocytosis, with lineage assignment, were detected by FCM. FCM identified a group of patients with excess of CD16pos monocytes as those having an inflammatory syndrome. WBC differential by FCM is at least as reliable as by standard cytology. FCM superiority consists in identification and systematic quantification of parameters that cannot be assessed by standard cytology such as lineage orientation of blast cells or lymphocytes, and expression of markers of interest such as CD16 on inflammatory monocytes. © 2007 International Society for Analytical Cytology

ORIGINAL ARTICLE

Cytometry PART A
Journal of the International Society for Advancement of Cytometry

Refining the White Blood Cell Differential: The First Flow Cytometry Routine Application

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• **Abstract**

A Complete Blood Count performed by an automated hematology analyzer frequently needs a microscopic slide review. This step is time consuming and requires experienced personnel. Recently, several teams have proposed and validated convenient combinations of monoclonal antibodies for an extended white blood cell (WBC) differential by flow cytometry. The aim of this study was to evaluate the usefulness of this approach in the routine workflow of a hematology laboratory. We compared a workflow chain comprised of a robotic blood preparation system (for antibody labeling), a flow cytometer, and data management software to the standard manual review of a blood film and evaluated the diagnostic quality, the turnaround time, and the labor needed for the two different approaches. The study on 1,973 samples was organized, firstly, to determine analytic thresholds and these settings were then validated. The flow cytometric data management software automatically validated 52% of the samples without significant numbers of false negatives. Of the remaining specimens, an operator validated a further 33% of the samples and 15% needed a manual microscopic review. These results were obtained in a mean timeline similar to the traditional microscopic manual review. Our study demonstrates, for the first time, the efficiency of a flow cytometer integrated into a WBC differential workflow in a routine hematology laboratory. © 2010 International Society for Advancement of Cytometry

Hematoflow : en pratique

Ligne automatisée : HematoFlow ®

Morphologie

Analyseur d'hématologie

REMIS

Cytodiff®

FC 500

r

)

Réactif CytoDiff™

- Dec 2009
  - IVD-CE
  - 500 tests, 5 flacons
  - PN: A84341
  - 6 anticorps / 5 couleurs
    - CD36 FITC, CD2+CD294 PE + CD19 ECD + CD16 PC5+ CD45 PC7

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Le tube CytoDiff™ : les anticorps

1 tube 6 anticorps 13 populations identifiées

- CD36FITC : utilisé en combinaison avec le CD45, CD16 et SS pour les monocytes
- CD2+CD294(CRTH2)PE : utilisé en combinaison avec le CD45, CD16 et SS. Marqueur des éosinophiles, basophiles, T activés, T et NK
- CD19ECD : utilisé en combinaison avec le CD45 et SS. Marqueur des B et B précurseurs.
- CD16PC5 : utilisé en combinaison avec le CD45 et le SS. Marqueur des granulocytes Matures et Immatures, et des T Cytotoxiques et Monos ProI
- CD45PC7 : utilisé en combinaison avec le paramètre SS pour définir les leucocytes, et servant de **back Gating** dans la stratégie d'analyse pour les précurseurs hématopoïétiques.

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Objectif de l'Hematoflow®

- Repérer dans un espace à 7 dimensions (FS/SS + 5 couleurs) les sous –populations normales et pathologiques

- Représenter cet espace dans une stratégie d'affichage d'histogrammes biparamétriques

- Identifier chaque sous-population par une stratégie de gating automatique et spécifique

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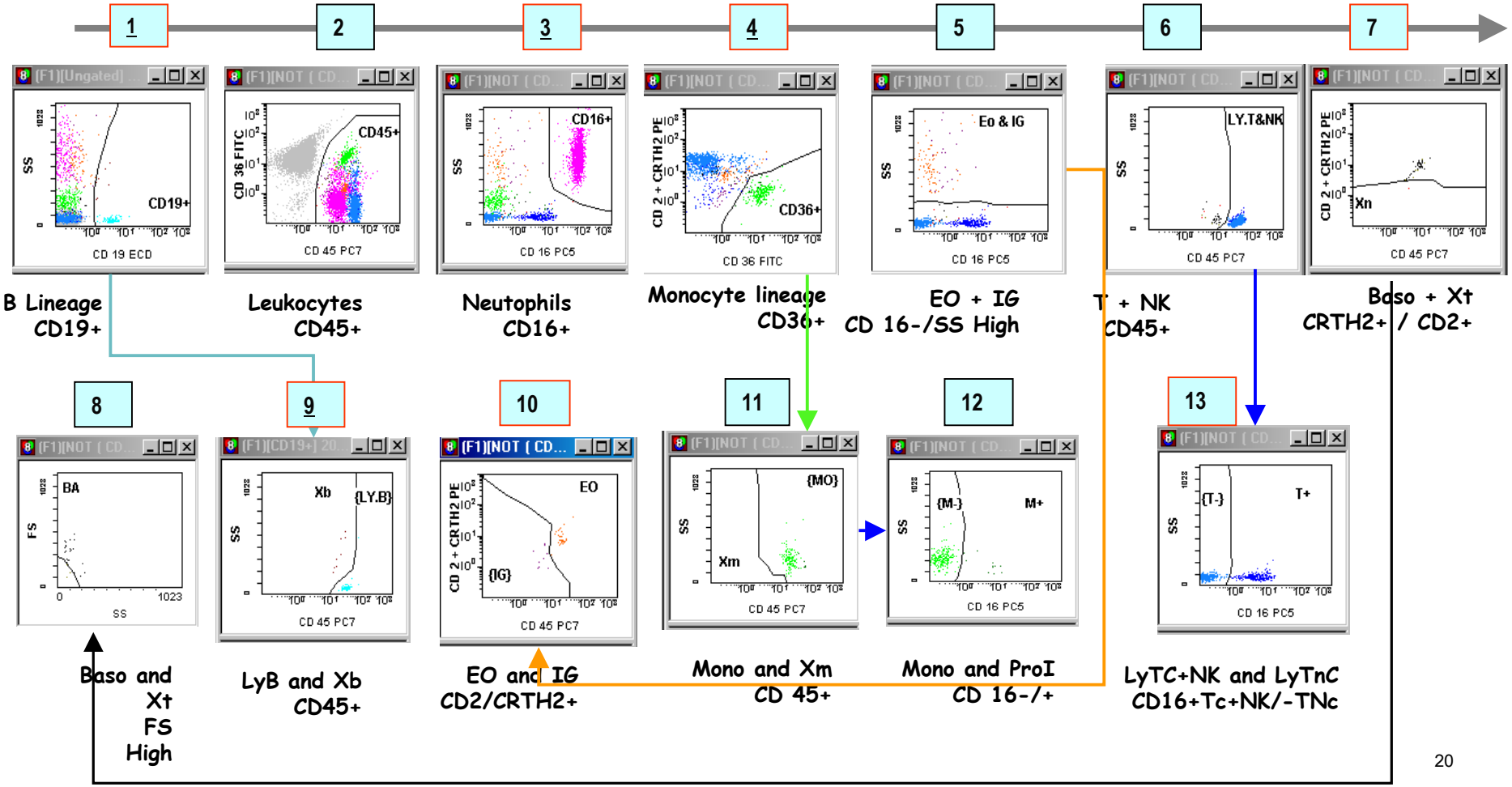
Gating Automatisé



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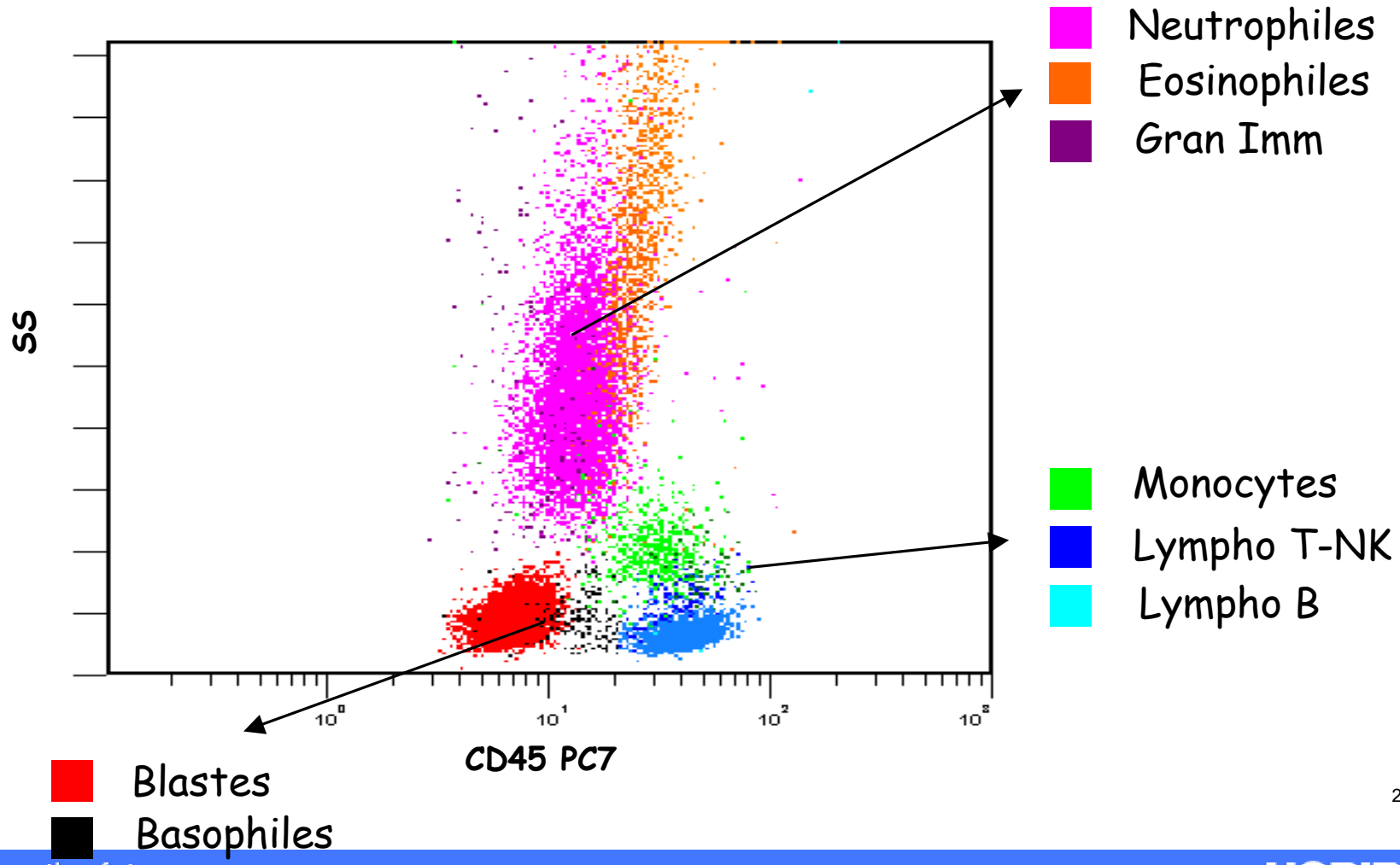
Le tube CytoDiff™ : les populations identifiées



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Color	Name	% Gated	Number
	Lymph.B	2.61	520
	T-	5.68	1134
	T+	2.99	596
	M-	11.60	2313
	M+	0.92	184
	Lymph.T&NK	8.67	1730
	Lymph.Total	11.28	2250
	Mono.Total	12.52	2497
	Ibnn Gran	14.28	2848
	Eosino.Total	5.70	1136
	Neutro.Mature	54.47	10864
	Neutro.Total	68.75	13712
	Xb	0.08	15
	Xa	0.02	3
	Xm	0.05	9
	Xn	0.60	120
	Baso.Total	1.02	203
	Total Diff	100.00	19945

Equivalent morpho

Sous pop immuno

Sous pop patho

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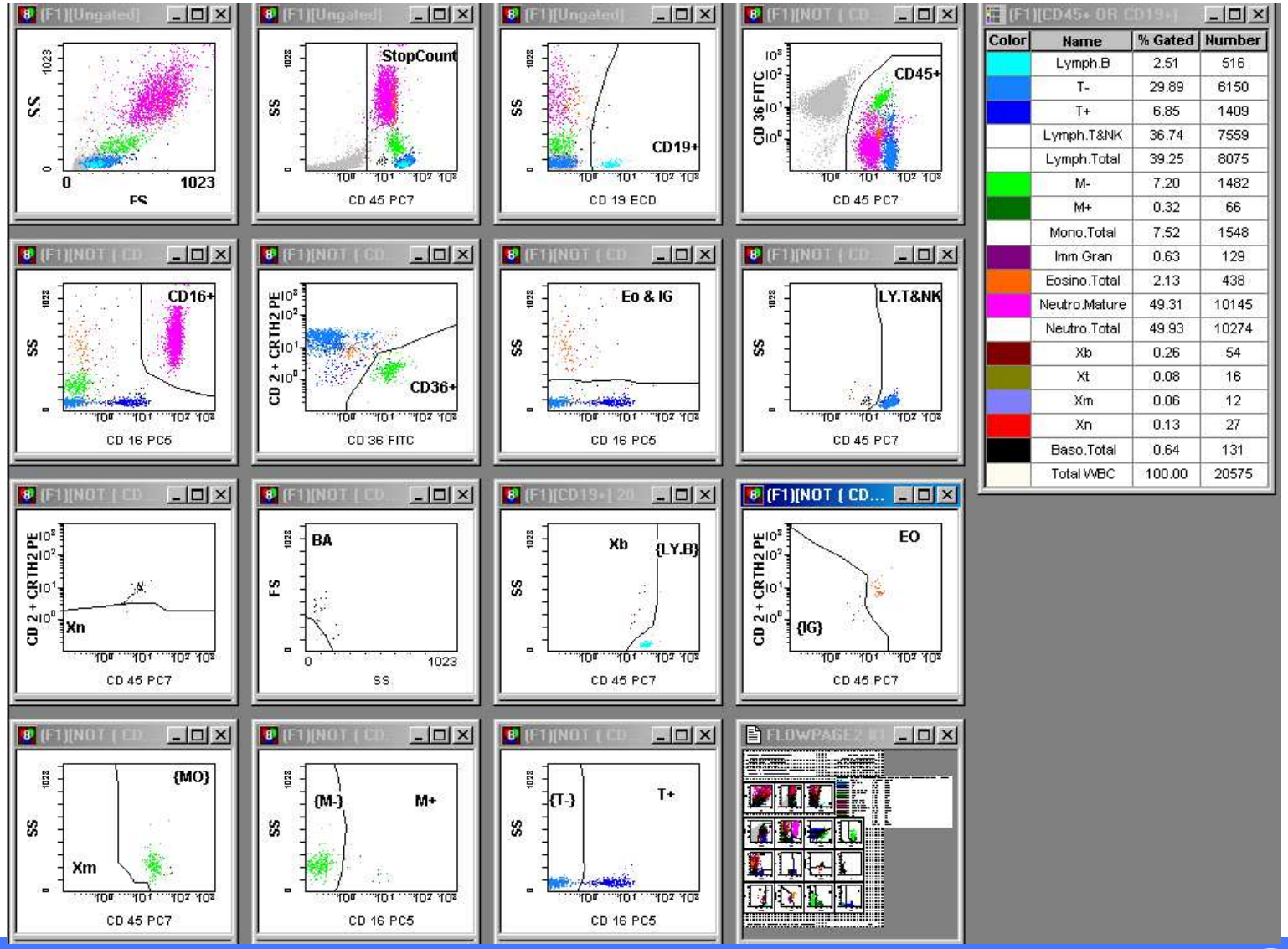
Exemple : Sang normal



(F1)[CD45+ OR CD19+]

Color	Name	% Gated	Number
	Lymph.B	2.51	516
	T-	29.89	6150
	T+	6.85	1409
	Lymph.T&NK	36.74	7559
	Lymph.Total	39.25	8075
	M-	7.20	1482
	M+	0.32	66
	Mono.Total	7.52	1548
	Imm Gran	0.63	129
	Eosino.Total	2.13	438
	Neutro.Mature	49.31	10145
	Neutro.Total	49.93	10274
	Xb	0.26	54
	Xt	0.08	16
	Xm	0.06	12
	Xn	0.13	27
	Baso.Total	0.64	131
	Total WBC	100.00	20575

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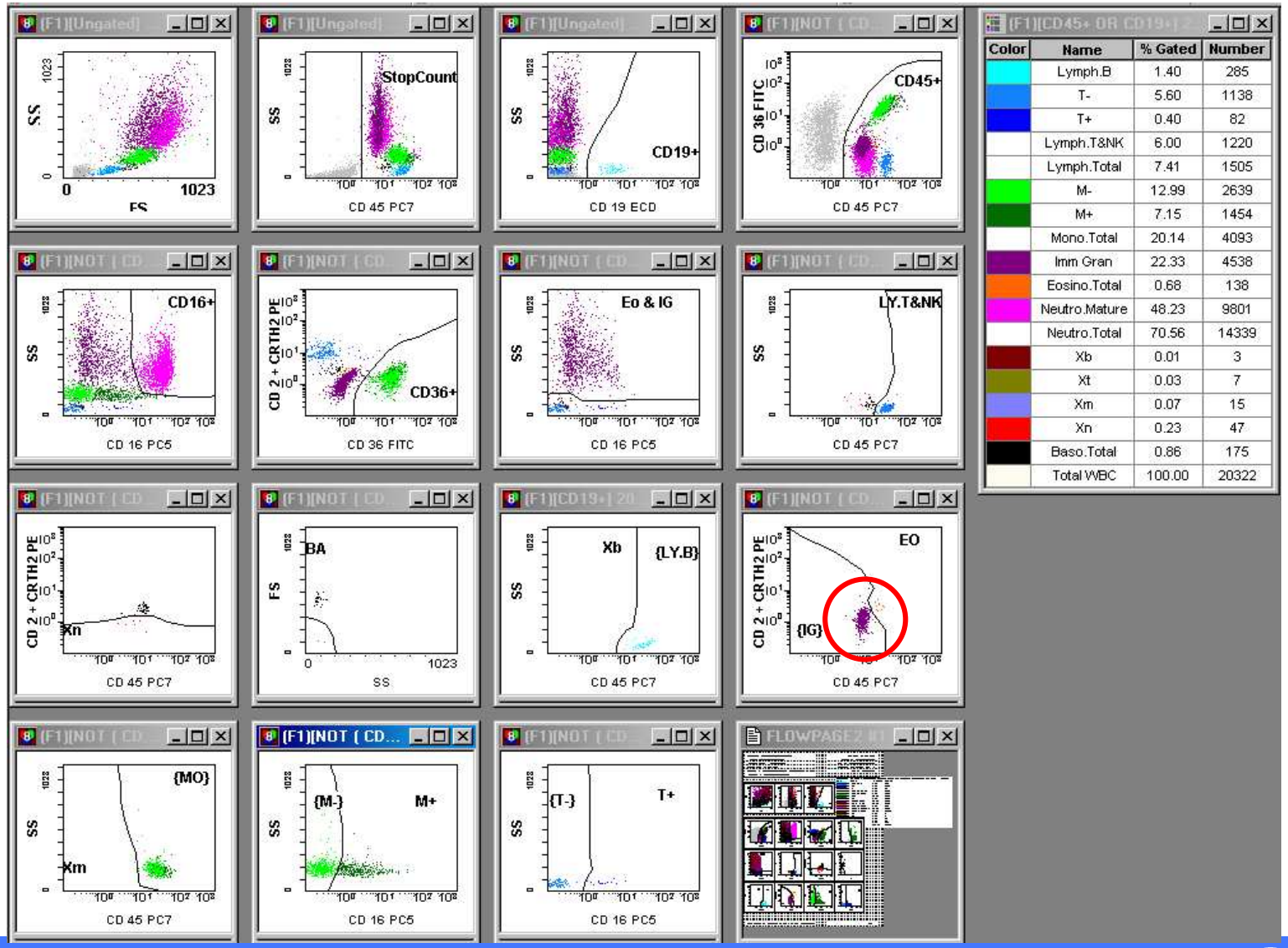
Exemple : Immatures



Color	Name	% Gated	Number
	Lymph.B	1.40	285
	T-	5.60	1138
	T+	0.40	82
	Lymph.T&NK	6.00	1220
	Lymph.Total	7.41	1505
	M-	12.99	2639
	M+	7.15	1454
	Mono.Total	20.14	4093
	Imm Gran	22.33	4538
	Eosino.Total	0.68	138
	Neutro.Mature	48.23	9801
	Neutro.Total	70.56	14339
	Xb	0.01	3
	Xt	0.03	7
	Xm	0.07	15
	Xn	0.23	47
	Baso.Total	0.86	175
	Total WBC	100.00	20322

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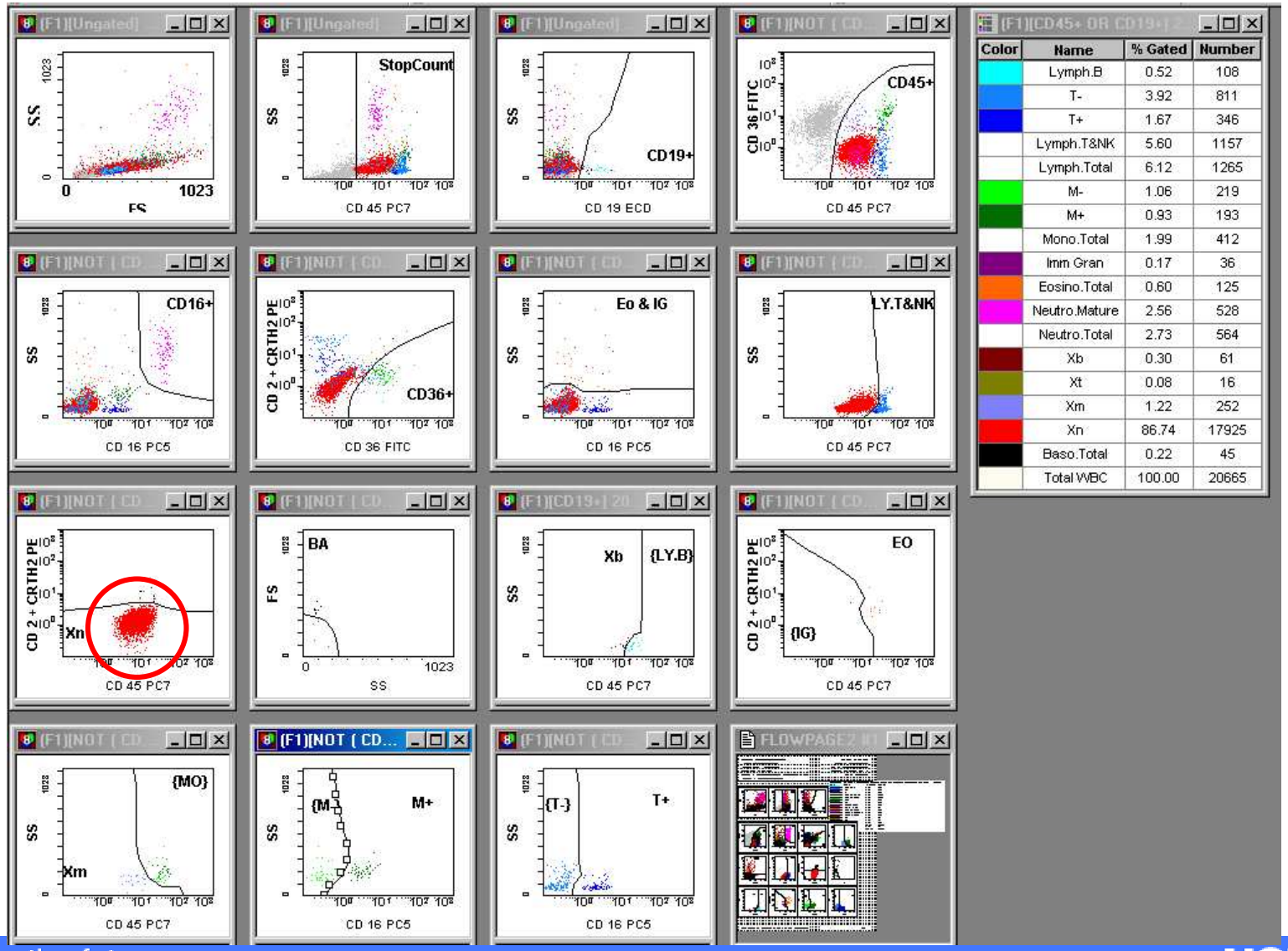
Exemple : Myéloblastes



Color	Name	% Gated	Number
	Lymph.B	0.52	108
	T-	3.92	811
	T+	1.67	346
	Lymph.T&NK	5.60	1157
	Lymph.Total	6.12	1265
	M-	1.06	219
	M+	0.93	193
	Mono.Total	1.99	412
	Imm Gran	0.17	36
	Eosino.Total	0.60	125
	Neutro.Mature	2.56	528
	Neutro.Total	2.73	564
	Xb	0.30	61
	Xt	0.08	16
	Xm	1.22	252
	Xn	86.74	17925
	Baso.Total	0.22	45
	Total WBC	100.00	20665

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Hematoflow : stratégie d'utilisation

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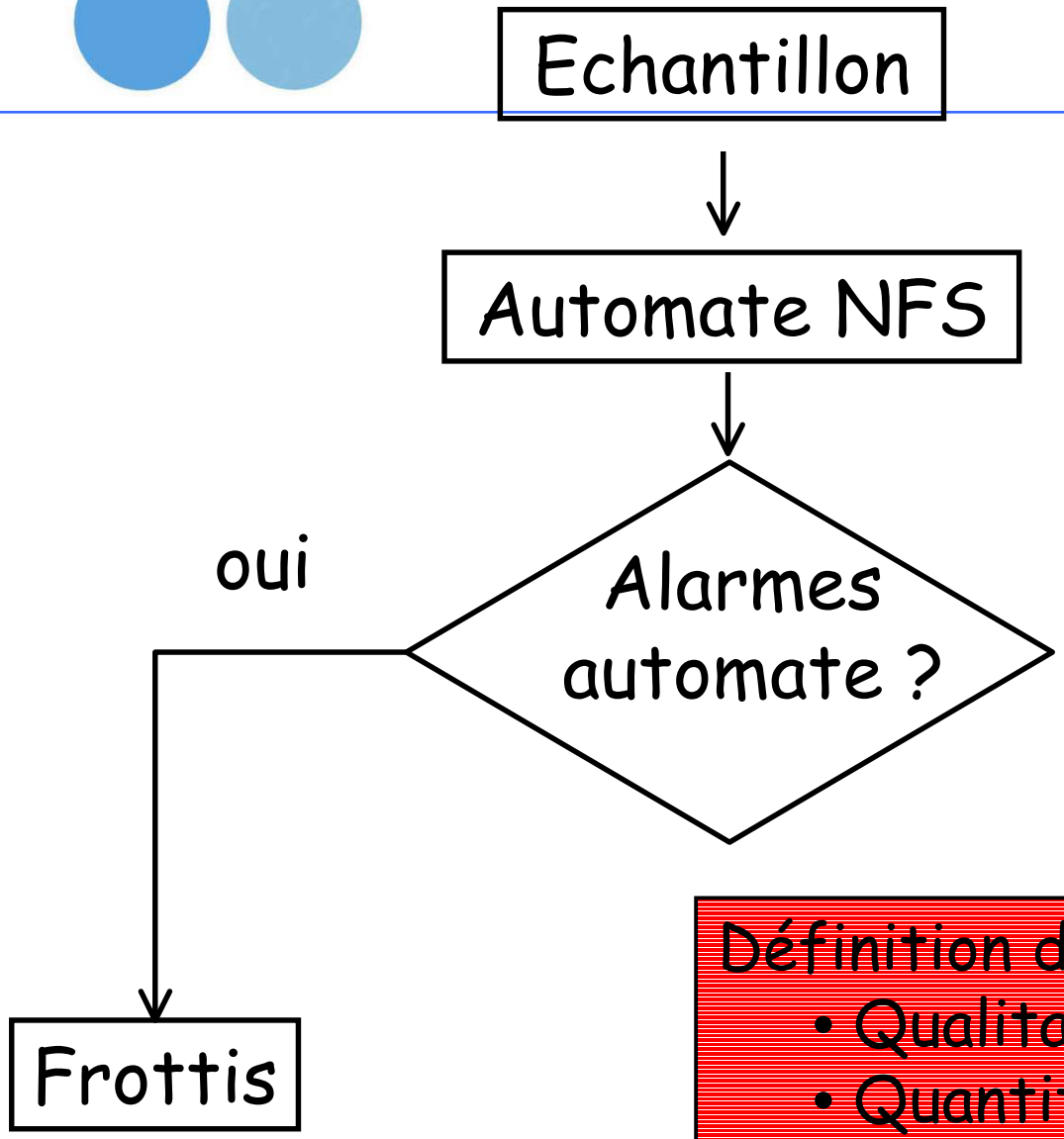

Définition des alarmes
- Qualitatives
- Quantitatives
- Détermination des seuils

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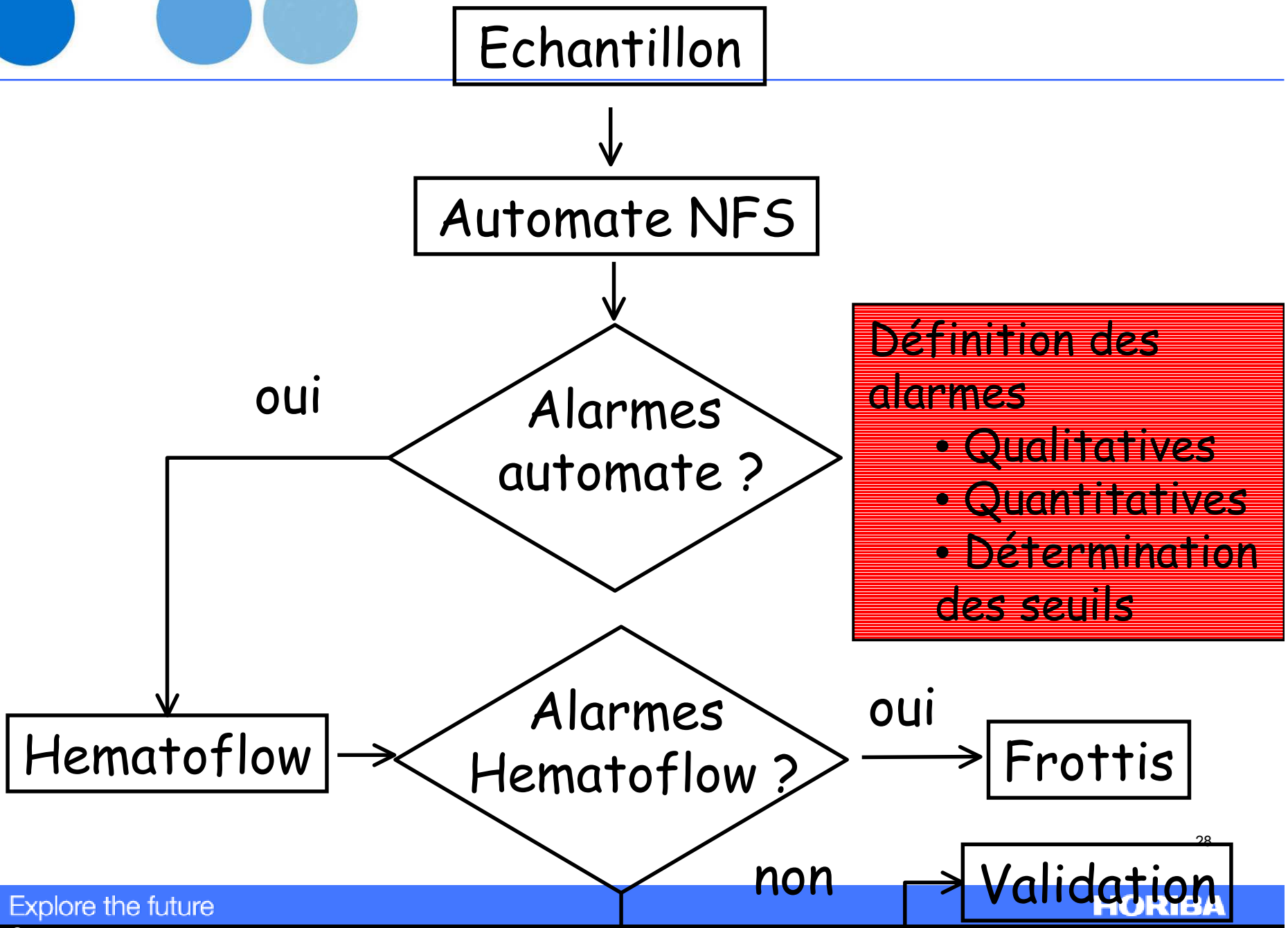

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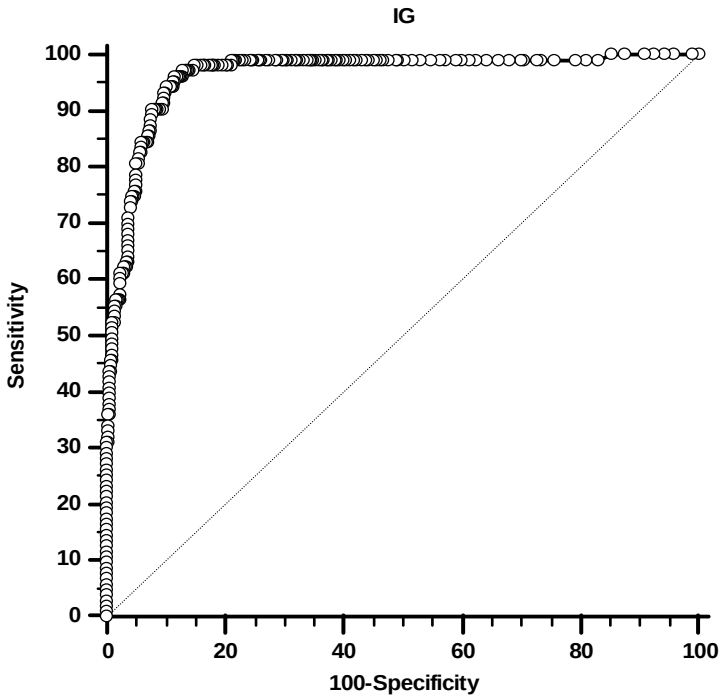
Myélémie ≥2



AUC = 0.963

Seuil IG	Sensitivity	Specificity	+LR	-LR
>1.39 *	96.12	88.17	8.13	0.044

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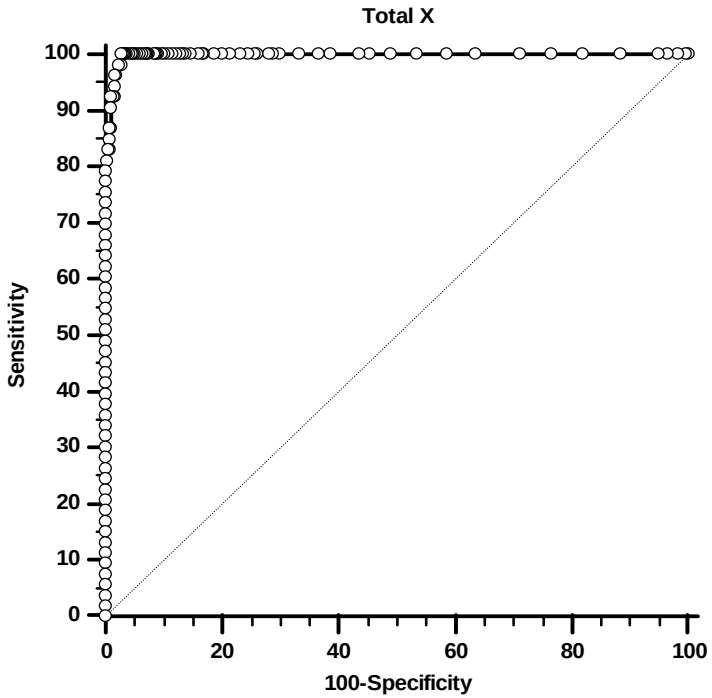
Blastes ≥1



AUC = 0.997

Seuil Total X	Sensitivity	Specificity	+LR	-LR
>1.01 *	100	97.04	33.82	0

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Nomenclature des alarmes (1)

Sous-poulations leucocytaires HF	Hf_Flag GD	Commentaire	HF Flag GP	N° Histo à modif	Sous-populations leucocytaires morpho
Toutes	10	Gating automatique correct	0	-	
PNN	1				**Neutrophiles**
	11	MonoPro classés en PNN	1	3	**Monocytes**
	12	Saturation en SS	1	Stop Count	
PNE	2				**Eosinophiles**
	21	IG ou PNN classés en PNE	2	-	**Myélémie ou PNN**
	22	Saturation en SS	1	Stop Count	
	23	Baisse CRTH2 seule	0	-	
	24	Monos CD36 faible classés en PNE	2	-	**Monocytes**
PNB	3				**Basophiles**
	31	Ly CD16+ classés en PNB	1	6	**Lymphocytes**
	32	Xt (ou Xn) classés en PNB	2	-	**Cell imm**
Lymphos	4				**Lymphocytes**
	41	Xb (Xt ou Xn) classés LyB ou (LyT)	2	-	**Cell imm**
	42	LyB pris pour LyT	1	1	**Lympho**
	43	Pourcentages LyB1,LyB2 ↘ dans LMNH B	0	-	**L atyp ou lympho**
	44	Promonos CD36- classés en LyT	2	-	**Promono**
	45	Monos CD36 faible classés en LyT	2	-	**Monocytes**
	46*	LyBx SSc fort	2	-	**L atyp**
	47*	Plasmo classés en Ly B	2	-	**Plasmo**
	48**	LyTx non reconnus (?)	2	-	**L act**

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Nomenclature des alarmes (2)

Sous-poulations leucocytaires HF	Hf_Flag GD	Commentaire	HF Flag GP	N° Histo à modif	Sous-populations leucocytaires morpho
Monos	5				**Monocytes**
	51	Interférence Mono (IG ou PNE ou PNN CD16-)	1bis	-	**Myélémie ou Eosino ou PNN**
	52	Monoblastes classés en Monos (Grade I)	1	11	**Cell imm**
	53	Monoblastes classés en Monos (Grade II)	2	-	**Cell imm**
Blastes	6				
	61	LyB classés en Xb (ou Xn+Xm)	2	-	**L atyp ou lympho**
	62	PNB classés en Xt (Xn)	2	-	**Basophiles**
	63	Lympho CD16+ mis en Xn, (Xt)	1	6	**Lymphocytes**
	64	Plasmo classés en Xb ou Xn	2	-	**Plasmo**
	65	Monos CD36 faible classés en Xn	2	-	**Monocytes**
IG	7				
	71	PNE classés en IG	1bis	-	**Eosinophiles**
	72	Perte partielle ou totale CD16	1*	3	**PNN ou myélémie**
	73	Blastes LAM3/M5 classés en IG	2	-	**Cell imm**
Débris	8	Blastes Xn classés en débris	2	2	**Blastes**
Multi populations	9	Multi alarmes	2	-	

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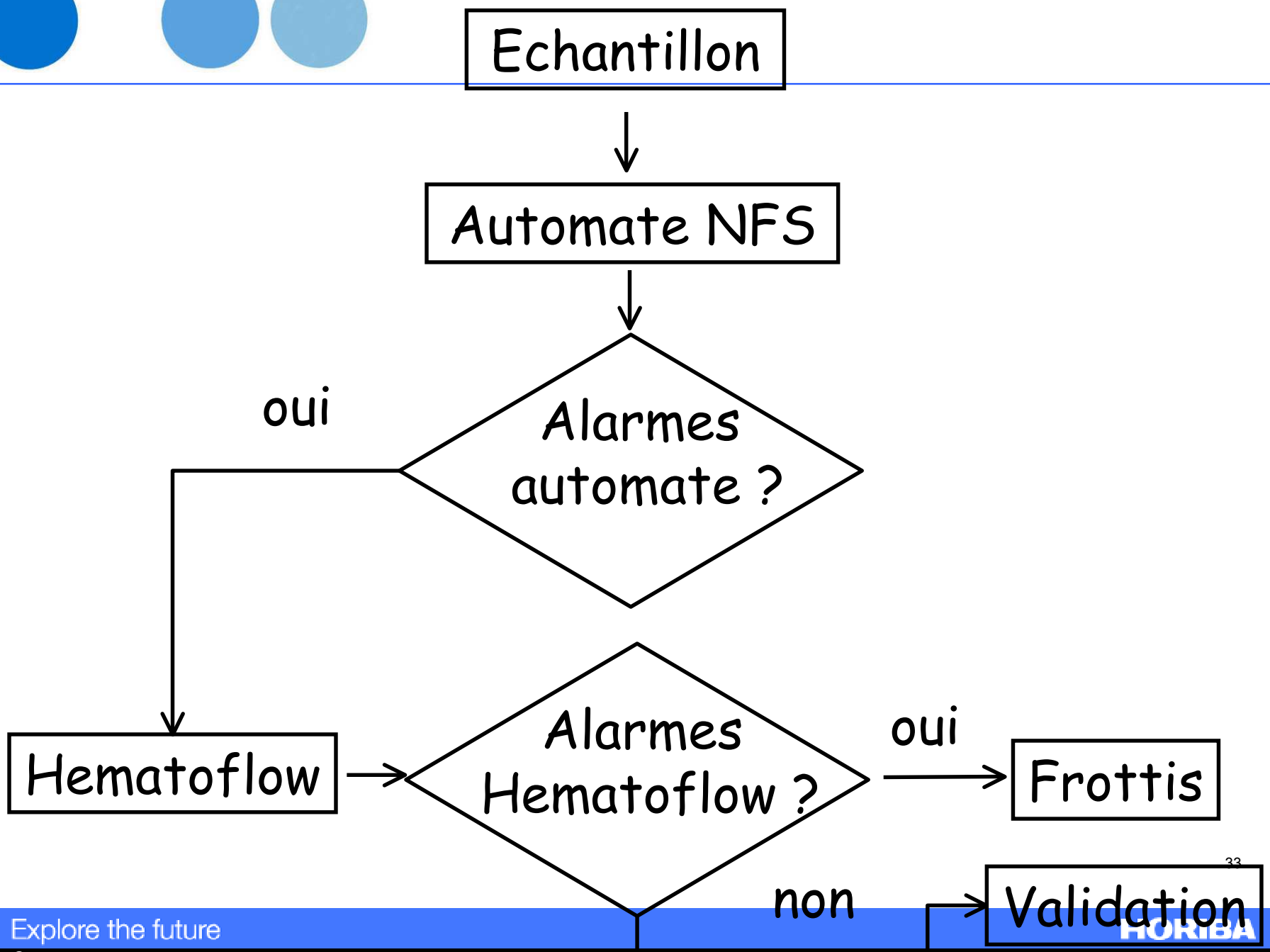

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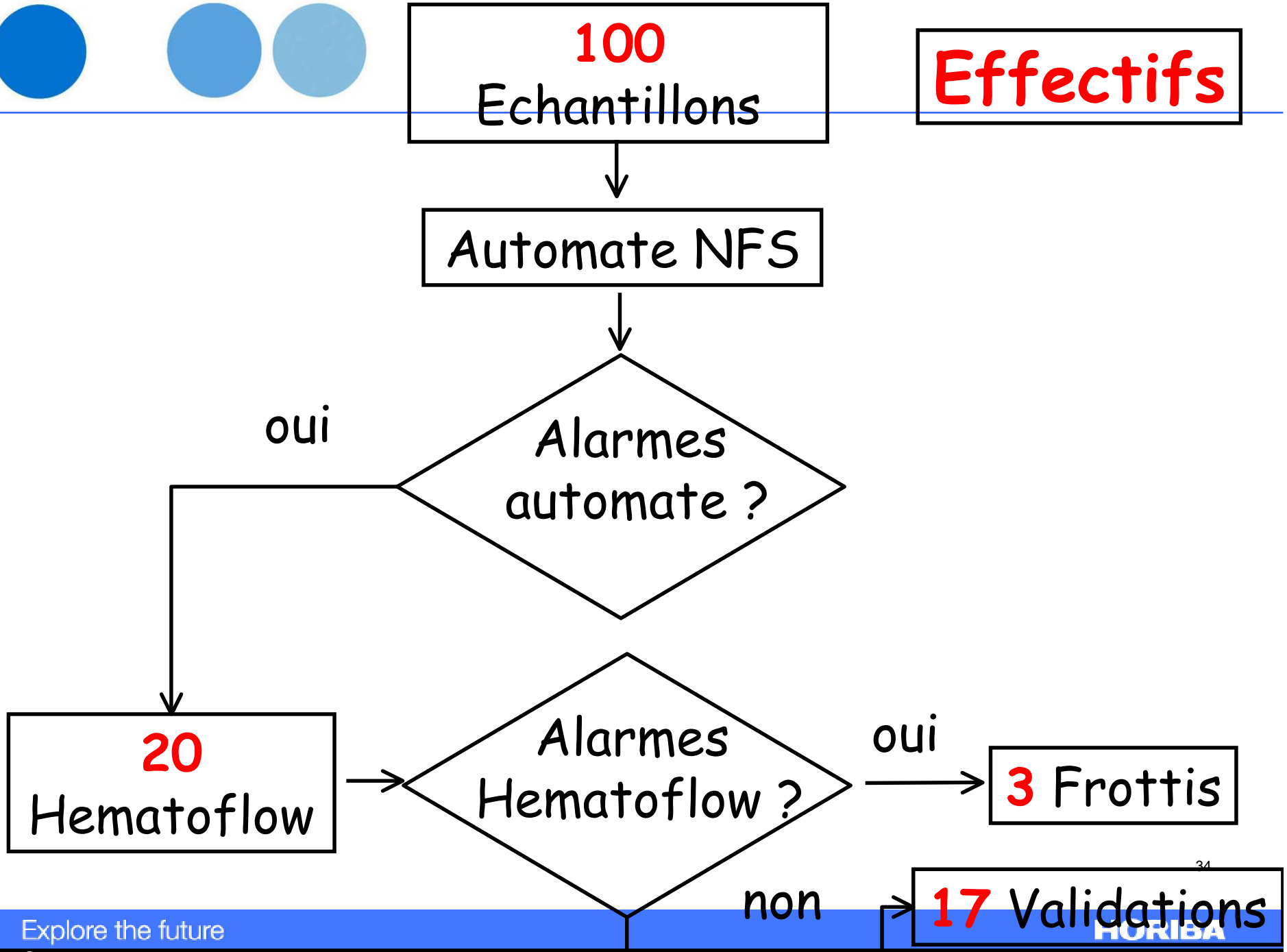
Effectifs



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Hematoflow : nouveaux concepts

- Précision des pourcentages (Table de Rümke)

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Fonte des blastes
(F Lacombe et al. Leukemia 2009)

Leukemia. 2009 ,23(2):350-7

Leukemia (2008), 1–8
© 2008 Macmillan Publishers Limited All rights reserved 0887-6924/08 $32.00
www.nature.com/leu

ORIGINAL ARTICLE

Early clearance of peripheral blasts measured by flow cytometry during the first week of AML induction therapy as a new independent prognostic factor: a GOELAMS study

F Lacombe[1], C Arnoulet[2], M Maynadié[3], E Lippert[1], I Luquet[4], A Pigneux[5], N Vey[6], O Casasnovas[7], F Witz[8] and MC Béné[9]

[1]Hematology Laboratory, CHU Bordeaux, Hôpital Haut Levêque, Pessac, France; [2]Hematology Laboratory, Institut Paoli Calmettes, Marseille, France; [3]Hematology Laboratory, CHU le Bocage, Dijon, France; [4]Hematology Laboratory, CHU Robert Debré, Reims, France; [5]Hematology Clinic, CHU Bordeaux, Haut Levêque, Pessac, France; [6]Hematology Clinic, Institut Paoli Calmettes, Marseille, France; [7]Hematology Clinic, CHU le Bocage, Dijon, France; [8]Hematology Clinic, CHU Brabois, Vandoeuvre-les-Nancy, France and [9]Immunology Laboratory, CHU Brabois, Vandoeuvre-les-Nancy, France

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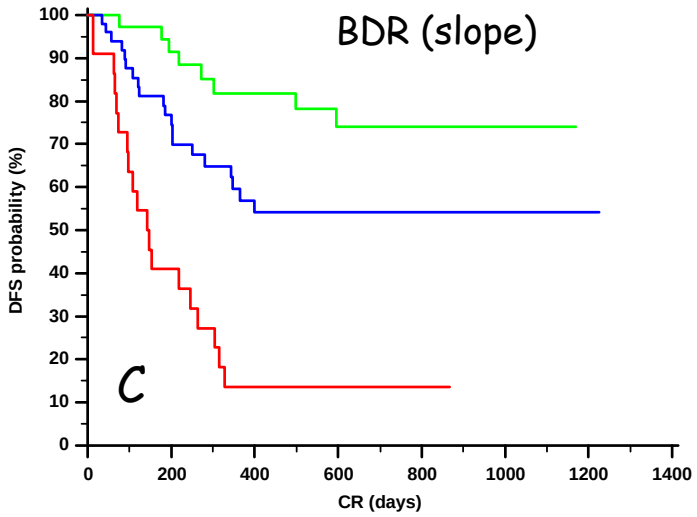
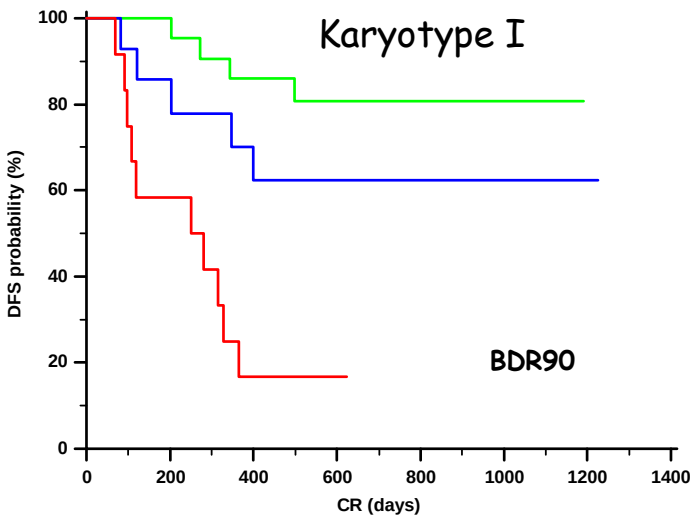
Pour un patient atteint de LAM en traitement d'induction, plus la pente de décroissance des cellules blastiques dans le sang périphérique est forte, plus ce patient a un bon pronostic.





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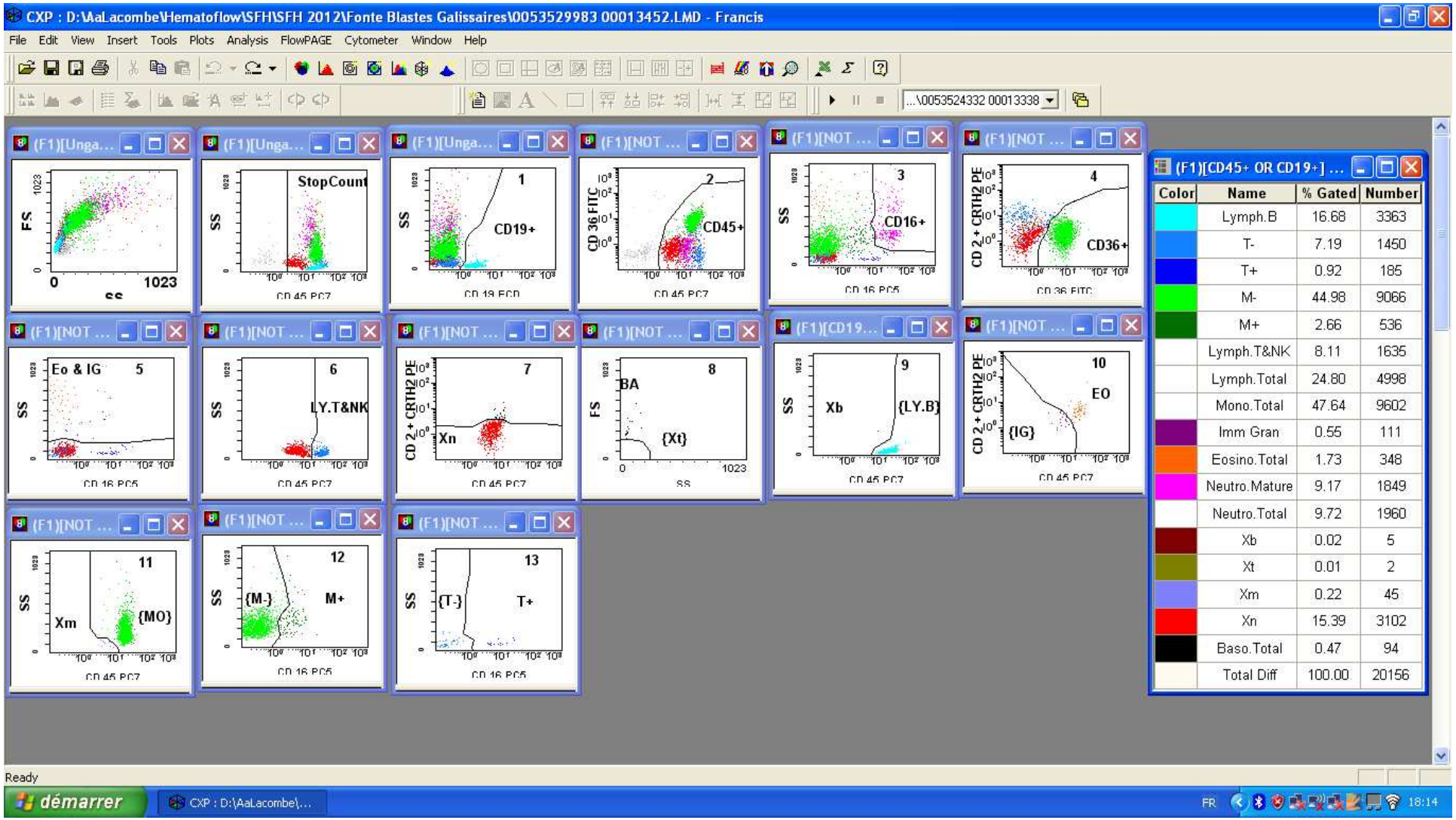
Diagnostic JO



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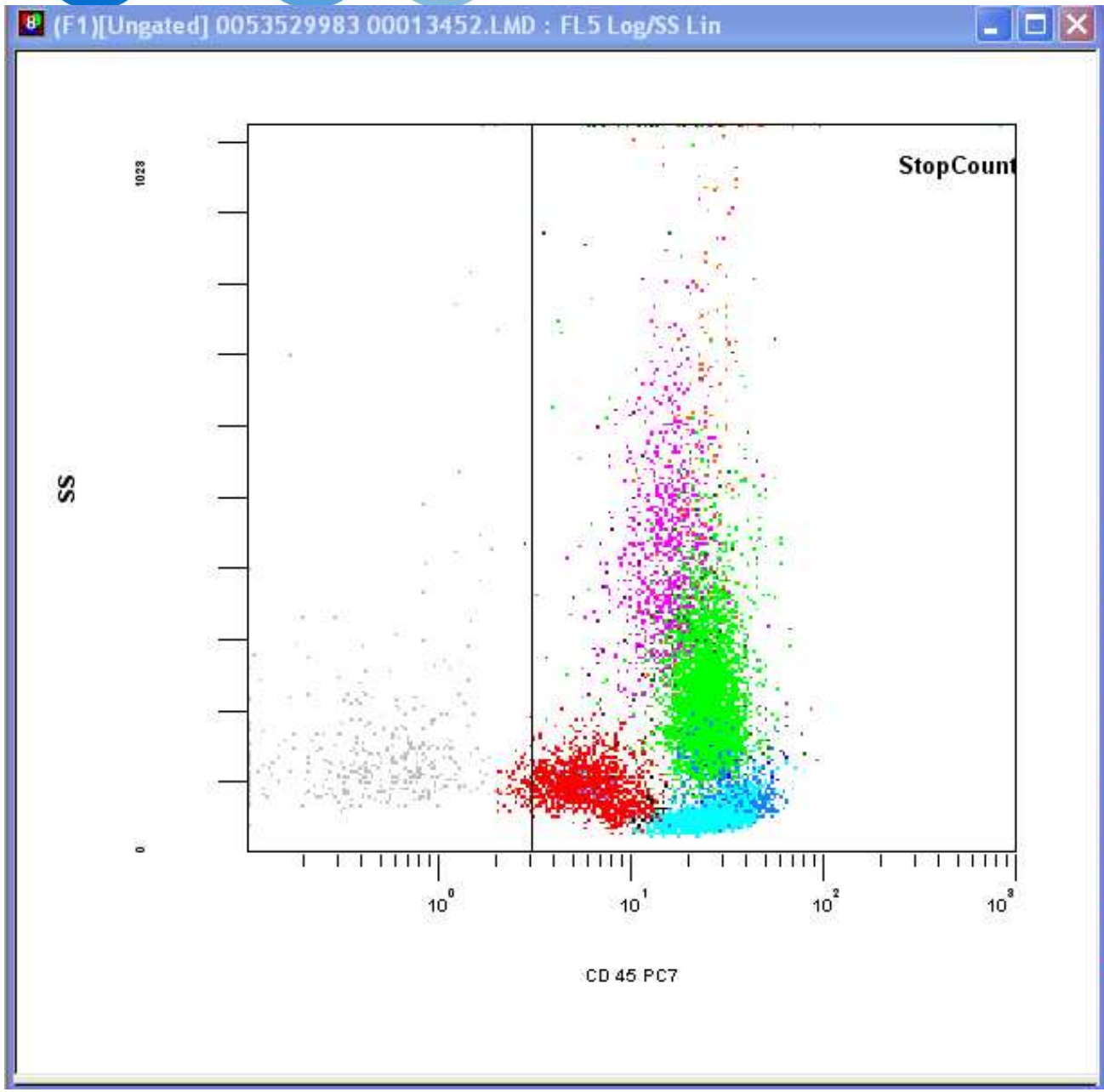
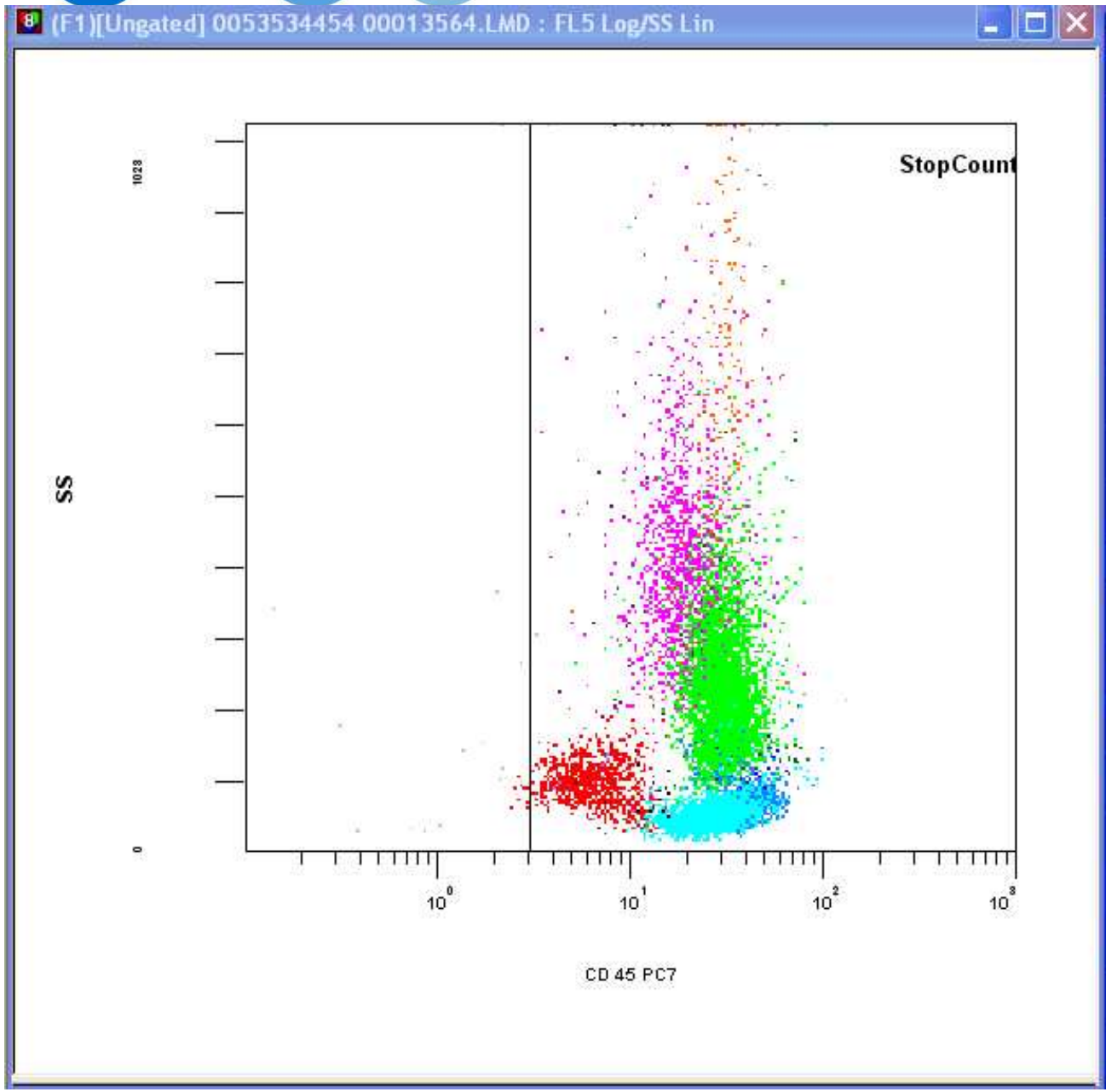
Diagnostic J0



(F1)[CD45+ OR CD19+] 0053...

Color	Name	% Gated	Number
	Lymph.B	16.68	3363
	T-	7.19	1450
	T+	0.92	185
	M-	44.98	9066
	M+	2.66	536
	Lymph.T&NK	8.11	1635
	Lymph.Total	24.80	4998
	Mono.Total	47.64	9602
	Imm Gran	0.55	111
	Eosino.Total	1.73	348
	Neutro.Mature	9.17	1849
	Neutro.Total	9.72	1960
	Xb	0.02	5
	Xt	0.01	2
	Xm	0.22	45
	Xn	15.39	3102
	Baso.Total	0.47	94
	Total Diff	100.00	20156

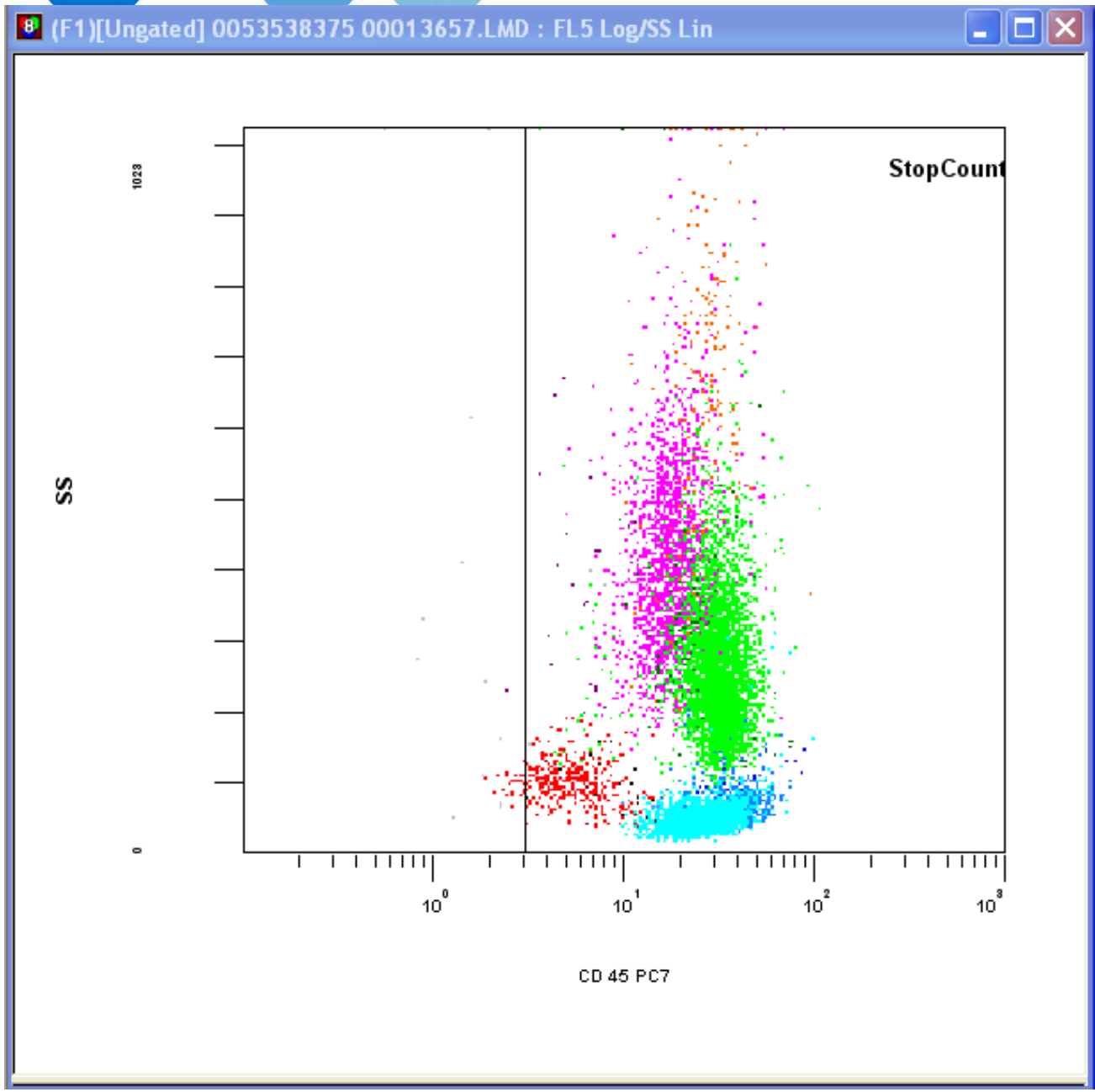
Chimio J1



(F1)[CD45+ OR CD19+] 0053...

Color	Name	% Gated	Number
	Lymph.B	20.65	4133
	T-	7.83	1567
	T+	1.21	243
	M-	43.17	8640
	M+	1.29	258
	Lymph.T&NK	9.04	1810
	Lymph.Total	29.69	5943
	Mono.Total	44.45	8898
	Imm Gran	0.17	35
	Eosino.Total	2.09	419
	Neutro.Mature	13.93	2789
	Neutro.Total	14.11	2824
	Xb	0.04	9
	Xt	0.01	2
	Xm	0.20	41
	Xn	9.14	1829
	Baso.Total	0.25	51
	Total Diff	100.00	20016

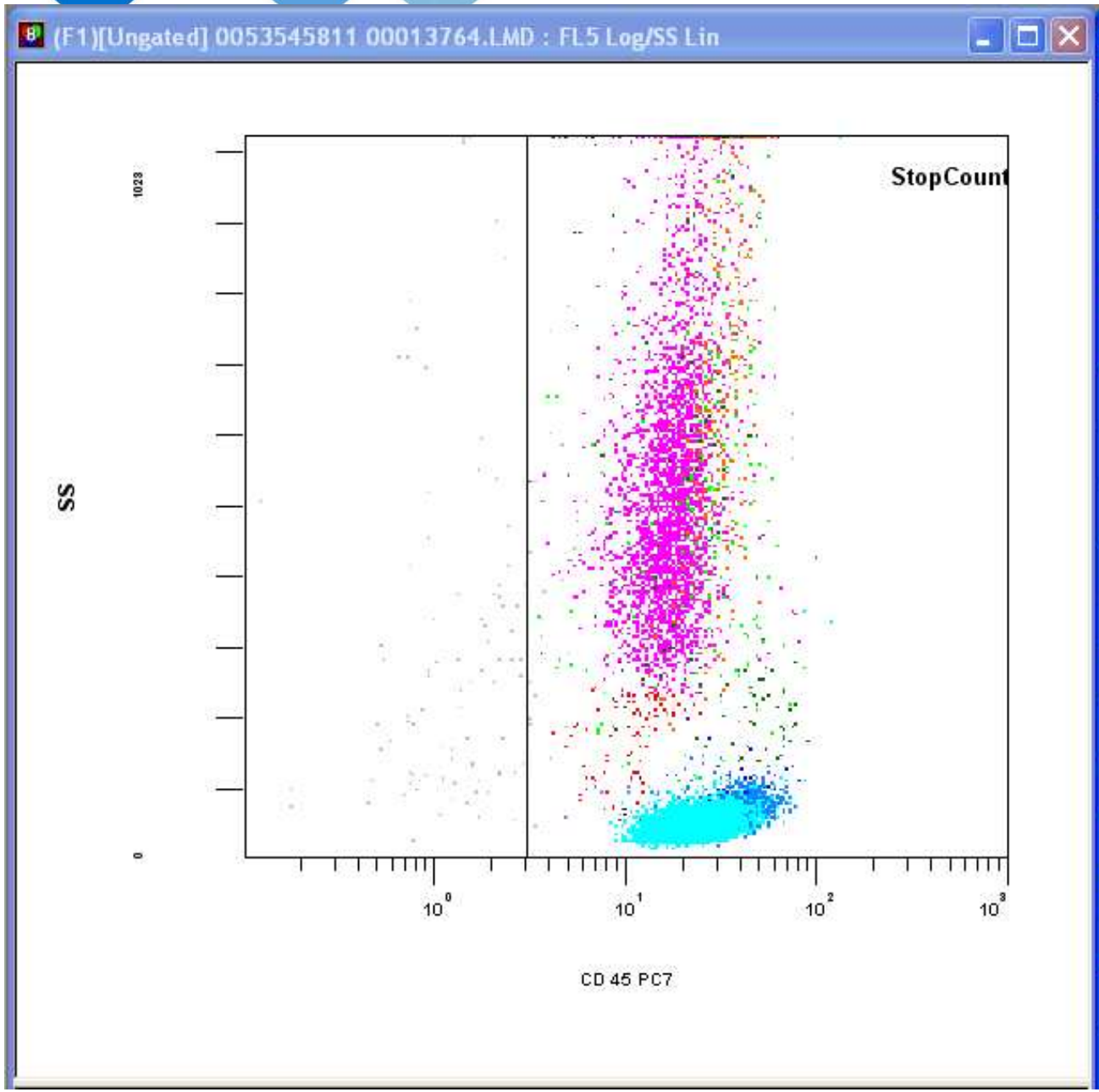
Chimio J2



(F1)[CD45+ OR CD19+] 0053...

Color	Name	% Gated	Number
	Lymph.B	27.71	5556
	T-	6.13	1229
	T+	1.03	206
	M-	38.59	7737
	M+	1.76	353
	Lymph.T&NK	7.16	1435
	Lymph.Total	34.87	6991
	Mono.Total	40.36	8090
	Imm Gran	0.23	47
	Eosino.Total	2.09	419
	Neutro.Mature	18.44	3696
	Neutro.Total	18.67	3743
	Xb	0.03	6
	Xt	0.00	1
	Xm	0.04	8
	Xn	3.70	741
	Baso.Total	0.24	48
	Total Diff	100.00	20047

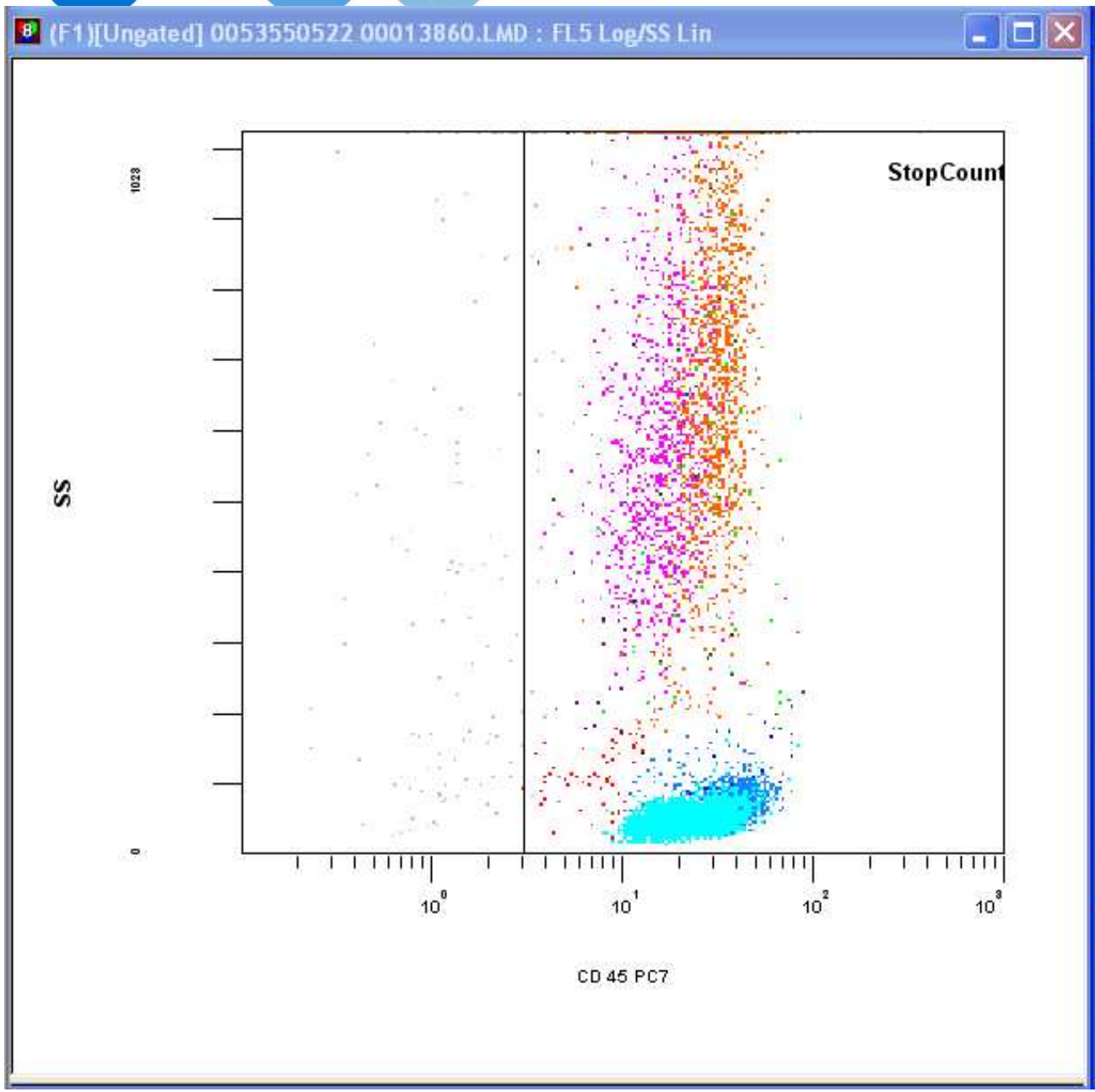
Chimio J3



(F1)[CD45+ OR CD19+] 0053...

Color	Name	% Gated	Number
	Lymph.B	47.31	9445
	T-	11.66	2329
	T+	2.03	406
	M-	2.01	401
	M+	1.41	282
	Lymph.T&NK	13.70	2735
	Lymph.Total	61.00	12180
	Mono.Total	3.42	683
	Imm Gran	0.19	38
	Eosino.Total	3.76	751
	Neutro.Mature	30.63	6115
	Neutro.Total	30.82	6153
	Xb	0.01	2
	Xt	0.00	0
	Xm	0.11	22
	Xn	0.85	169
	Baso.Total	0.03	6
	Total Diff	100.00	19966

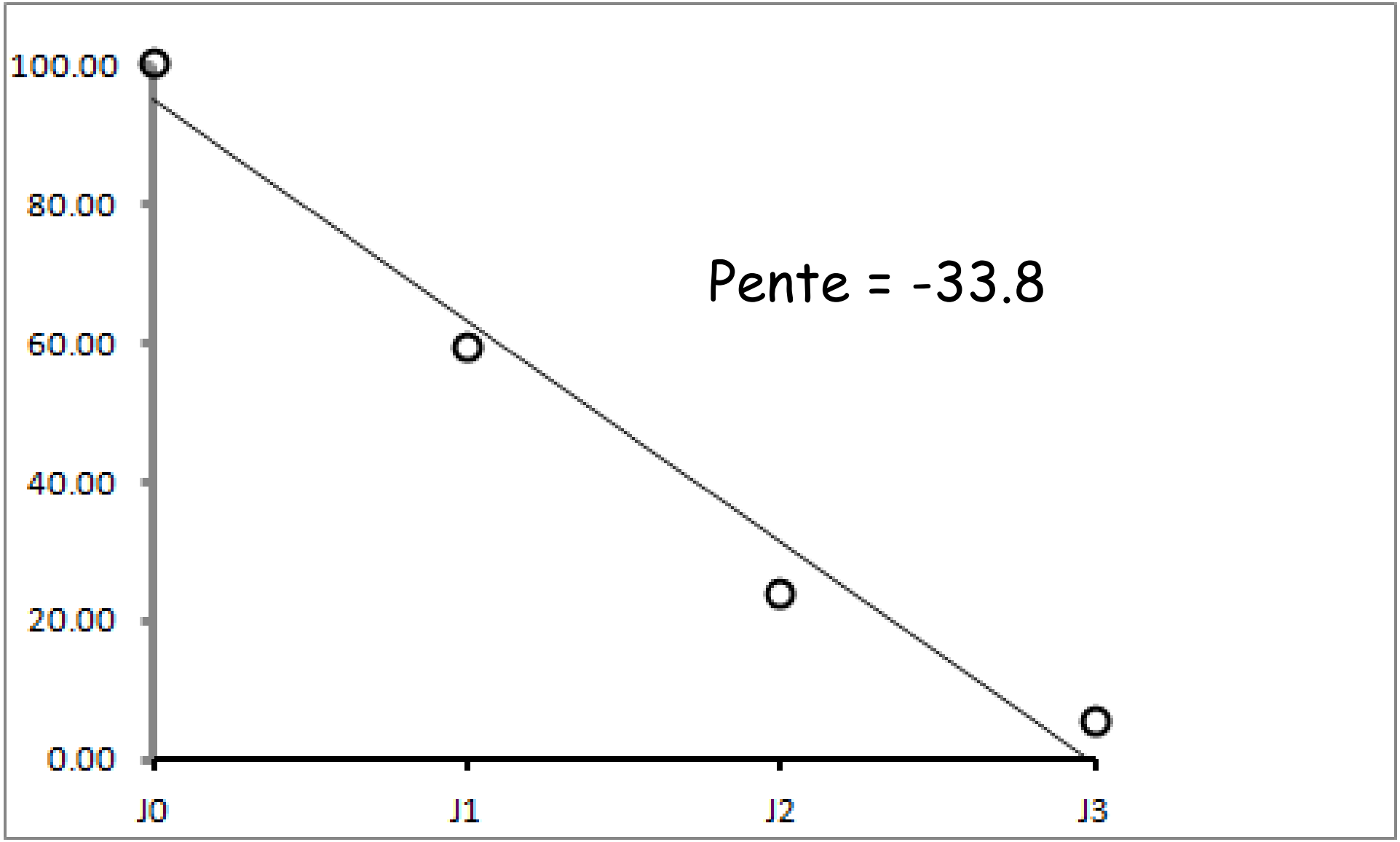
Chimio J4



(F1)[CD45... Space evenly down

Color	Name	% Gated	Number
	Lymph.B	51.67	10307
	T-	19.11	3812
	T+	2.28	454
	M-	0.60	119
	M+	0.40	79
	Lymph.T&NK	21.39	4266
	Lymph.Total	73.06	14573
	Mono.Total	0.99	198
	Imm Gran	0.30	59
	Eosino.Total	14.36	2864
	Neutro.Mature	10.70	2134
	Neutro.Total	10.99	2193
	Xb	0.05	9
	Xt	0.01	1
	Xm	0.03	6
	Xn	0.50	100
	Baso.Total	0.02	3
	Total Diff	100.00	19947

Calcul pente



Hematoflow : nouveaux concepts

- Précision des pourcentages (Table de Rümke)

- Nouvelles sous populations cellulaires

  Monocytes CD16+

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Syndromes inflammatoires

Infections (bacteriennes et virales)

- Sepsis
- HIV…

Maladies autoimmunes

- Polyarthrite rhumatoïde
- Maladie de Kawasaki
- Eczema…

Détection et quantification
Des subpopulations monocytaires

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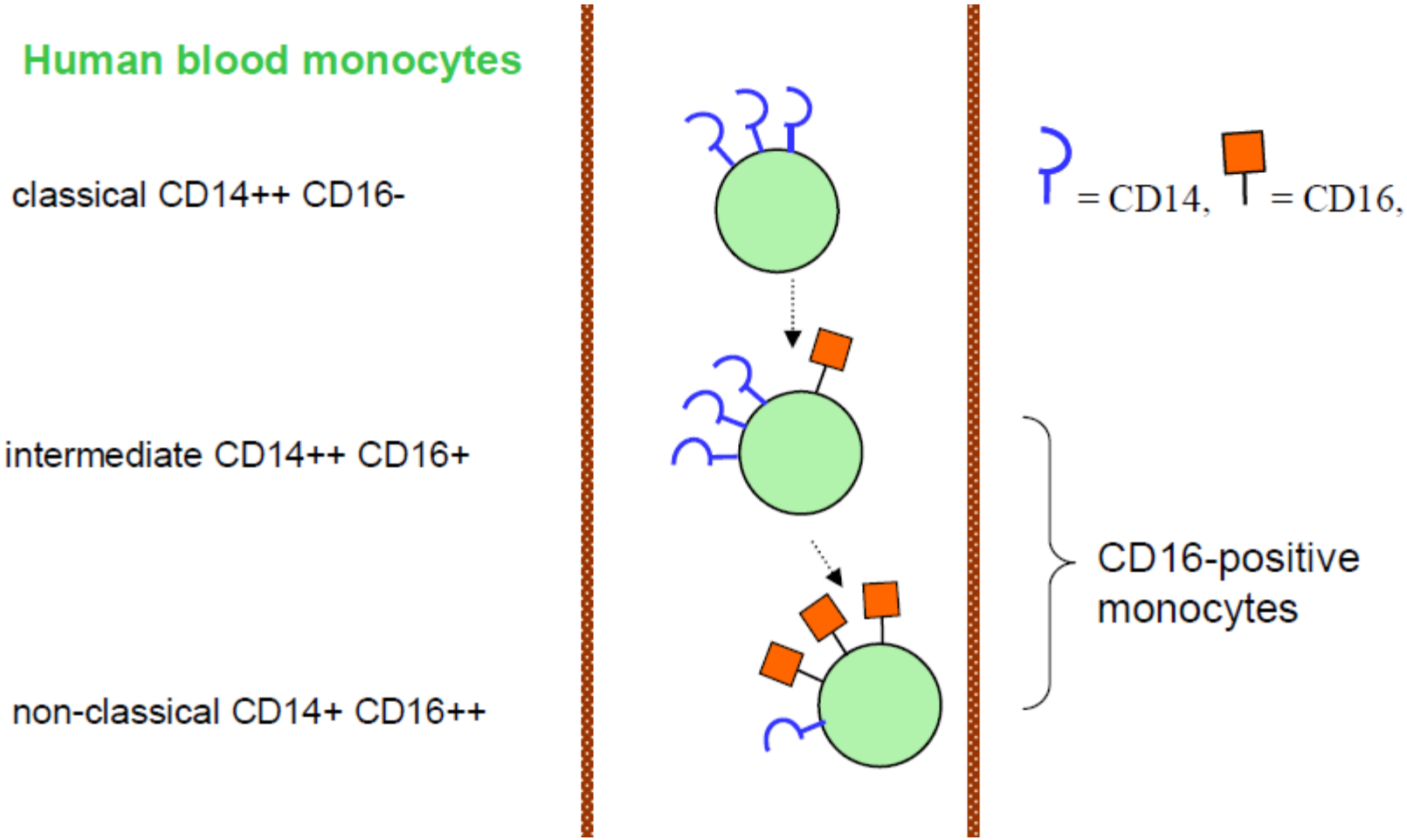
Nomenclature des monocytes et des cellules dendritiques sanguines

Loems Ziegler-Heitbrock et al. Blood, 2010, 116:e74-e80



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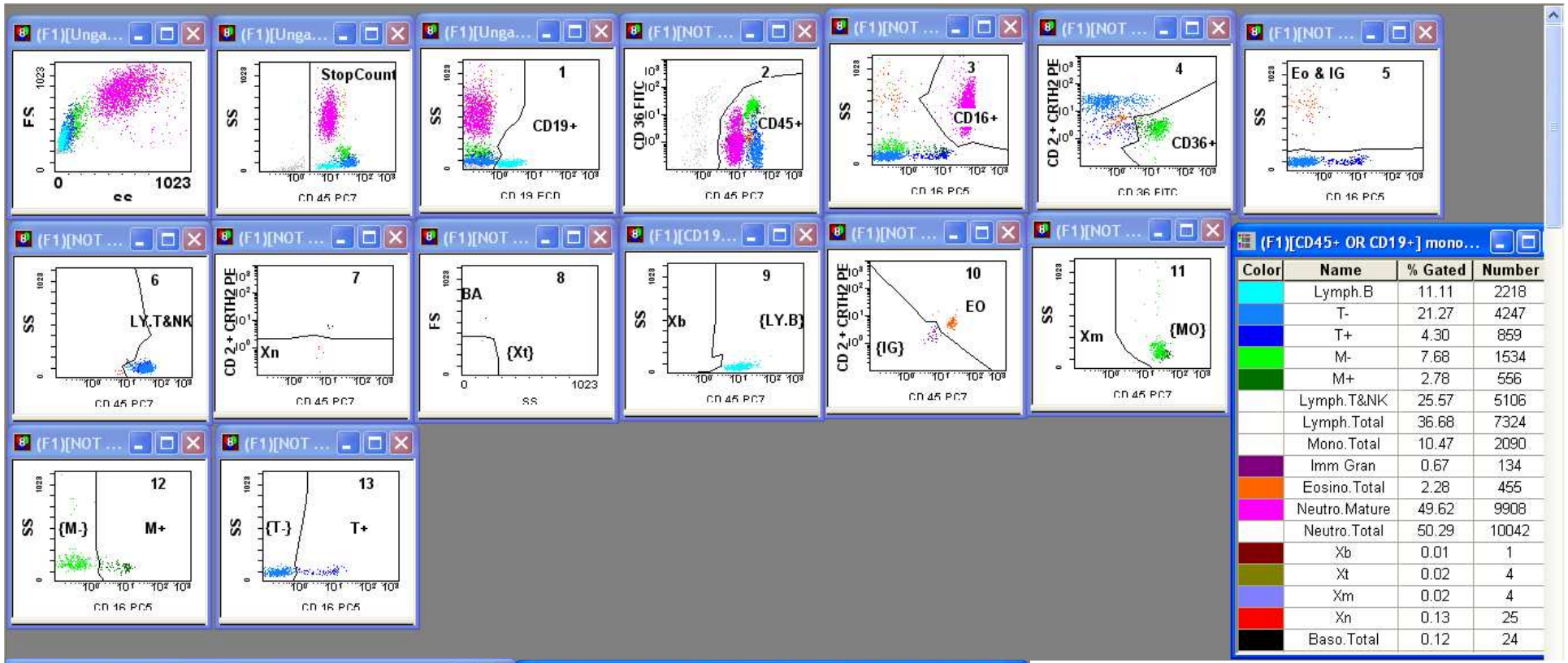
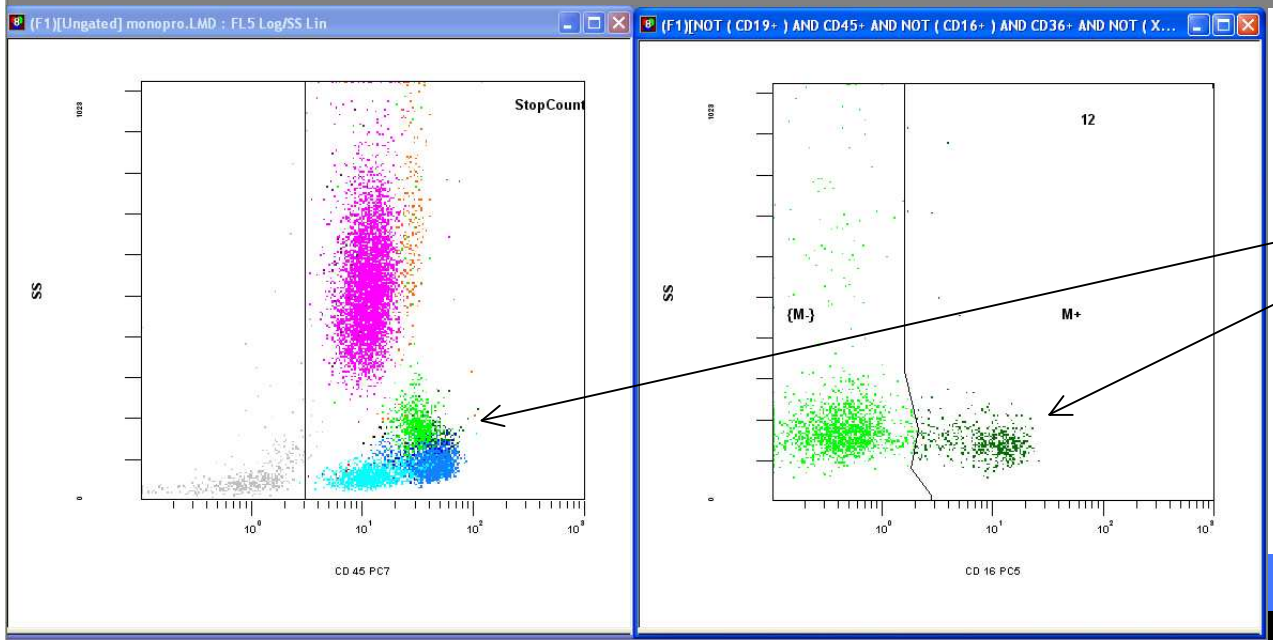


Color	Name	% Gated	Number
	Lymph.B	11.11	2218
	T-	21.27	4247
	T+	4.30	859
	M-	7.68	1534
	M+	2.78	556
	Lymph.T&NK	25.57	5106
	Lymph.Total	36.68	7324
	Mono.Total	10.47	2090
	Imm Gran	0.67	134
	Eosino.Total	2.28	455
	Neutro.Mature	49.62	9908
	Neutro.Total	50.29	10042
	Xb	0.01	1
	Xt	0.02	4
	Xm	0.02	4
	Xn	0.13	25
	Baso.Total	0.12	24



CD16+ monocytes

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Hematoflow : nouveaux concepts

- Précision des pourcentages (Table de Rümke)

- Nouvelles sous populations cellulaires

  Monocytes CD16+

  Granuleux CD16+ variants

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Hematoflow : nouveaux concepts

- Précision des pourcentages (Table de Rümke)

- Nouvelles sous populations cellulaires

  Monocytes CD16+

  Granuleux CD16+ variants

- Formule des sangs leucopéniques (0.35 - 1G/L)

...

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Au CHU de Bordeaux…

Mise en routine depuis le 7 Juillet

- **Site de Pellegrin (600 NFS/j)**
  40 Cytodiff
  6 lames

- **Site de Haut-Lévêque (400 NFS/j)**
  80 Cytodiff (y compris GB < 1G/L)
  15 lames

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Synthèse et conclusions

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Conséquences II

Quel est le rôle exact du frottis dans un laboratoire ?

Méthode de screening ?
Méthode de référence ?
Les deux ?
…

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Réponse I

Quel est le rôle exact du frottis dans un laboratoire ?

- **ni une méthode de référence**
- **ni une méthode de screening**

Mais une technique **spécialisée** d'analyse cellulaire **nécessaire** en particulier pour l'identification des :

- anomalies des érythrocytes
- anomalies rares des leucocytes
- populations cellulaires pathologiques…

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Réponse II

Quel est le rôle exact de la CMF dans un laboratoire ?

- **Sans doute une méthode de référence**
- **Certainement LA méthode de screening**

Elle doit précéder le frottis qui reste irremplaçable pour l'instant comme examen spécialisé de référence en cytologie hématologique

Accréditation assimilable aux automates de NFS

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Réponse III

Quel est le rôle exact de la CMF dans un laboratoire ?

- **Sans doute une méthode de référence**
- **Certainement LA méthode de screening**

Elle peut orienter vers un immunophénotypage spécialisé en oncohématologie.

Elle représente la méthode de référence du comptage plaquettaire (CD61 ou CD41)

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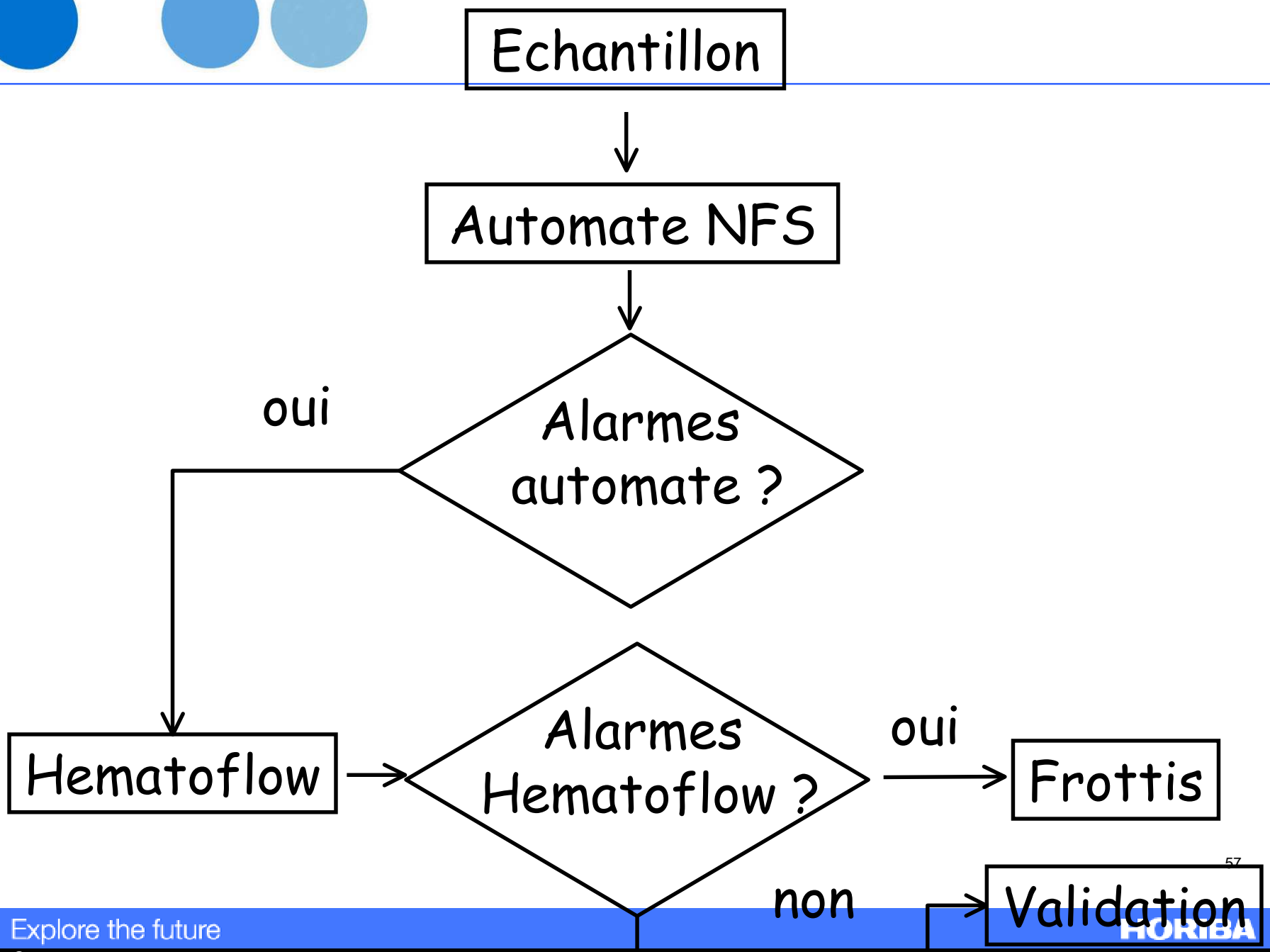

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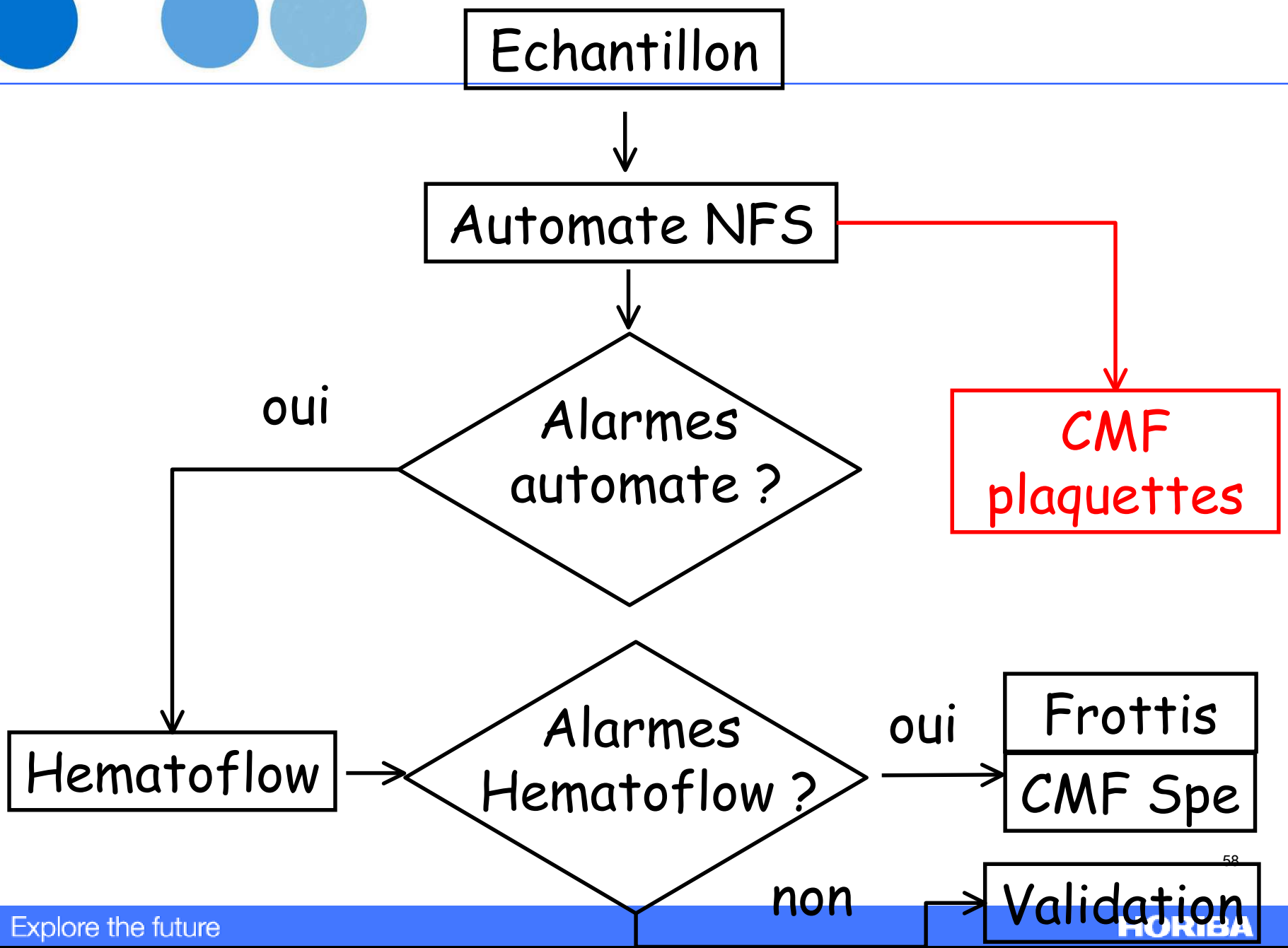

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Remerciements

- L' équipe de cytométrie du laboratoire d'Hématologie Haut-Lévêque (Jean-Philippe Vial, Marie Jeanneteau, Kelly Airiau, Manon Giraudon, Francis Belloc, Alex Briais)
- Les techniciens du PTRR Haut-Lévêque (Josiane, Jean-Pierre, Annie, Nathalie, Sandra)
- Hélène Beaudelle (Ingénieur CHU Bordeaux)
- Hamid Allou
- La Société Beckman Coulter (A Pacheco, JL Vieillefont, E Gautherot, M Yadini Foucart)

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