EQA testing in Belgium: flow cytometry

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CHU Liège – Unilab Lg

	CD34 numeration	Lymphocyte subsets
#surveys	2-3	3
#samples/survey	2	2
type	Stabilized blood (if possible fresh cord blood)	Fresh K_2EDTA blood
Participants	20	49

FC/21357

	Median	SD
% CD34+ cells within total WBC	0.170	0.022
Absolute CD34+ cell count (cells/µL)	10.0	1.3

FC/21559	Median	SD	CV,%	N
WBC 10E9/L	6.80	0.16	2.4	42
Lympho% haematology analyser	35.0	2.0	5.7	41
Lympho% flow cytometer	35.2	2.8	8.0	39
CD3 %	77.4	3.0	3.9	45
CD3 10E9/L	1.837	0.190	10.4	44
CD4 %	53.6	3.7	6.9	45
CD4 10E9/L	1.261	0.127	10.0	44
CD8 %	19.5	1.1	5.7	45
CD8 10E9/L	0.465	0.035	7.5	44
CD19 %	9.0	1.3	14.8	45
CD19 10E9/L	0.208	0.044	21.3	44
NKcells %	13.0	2.5	19.4	45
NKcells 10E9/L	0.309	0.072	23.2	44
Kappa % B lymphocytes	59.8	1.9	3.2	38
Lambda % B lymphocytes	39.9	2.1	5.4	38
Kappa/lambda	1.51	0.13	8.8	38
Sum K+L % B lymphocytes	99.9	0.6	0.6	38
Lymphosum %	99.4	1.0	1.0	45

Clinical context

- Lymphocyte subsets
  - Work-up of lymphocytosis – lymphoproliferative disease screening
  - Screening/monitoring of immune deficiencies
    - Congenital ID (+ T/B cell subclasses)
    - Acquired ID: CD4+ cells in HIV infection
- CD34 cell count
  - Monitoring of stem cell mobilisation/collection procedures (peripheral blood)
  - Cell therapy products QC: apheresis, cord blood

Lymphocyte subsets

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Double vs single platform

Double platform

- **Flow Cytometer**:

  % target cells* relative to total CD45+ leucocytes

- **Hematology analyser**:

  #leucocytes/µL

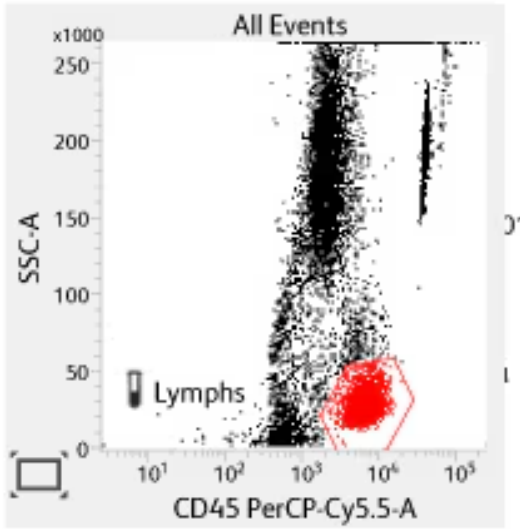
Single platform

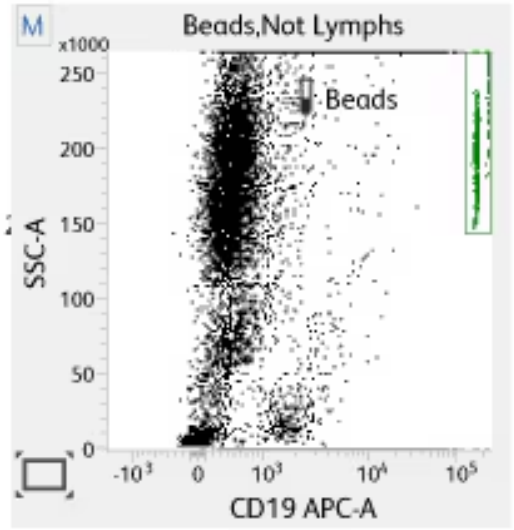
- **Flow Cytometer**: % target cells* relative to beads

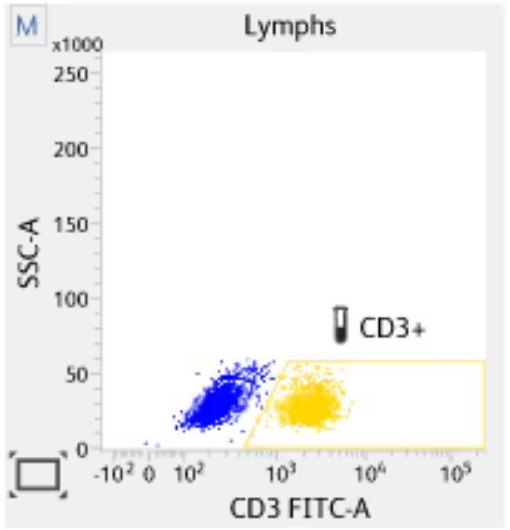
- Beads with defined concentration in #beads/ µL

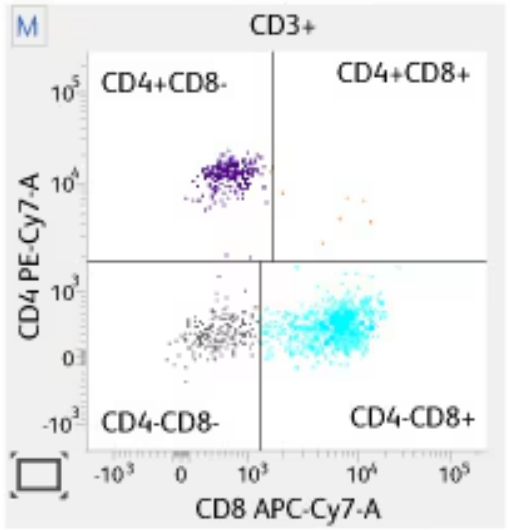
* CD34+ cells or lymphocytes

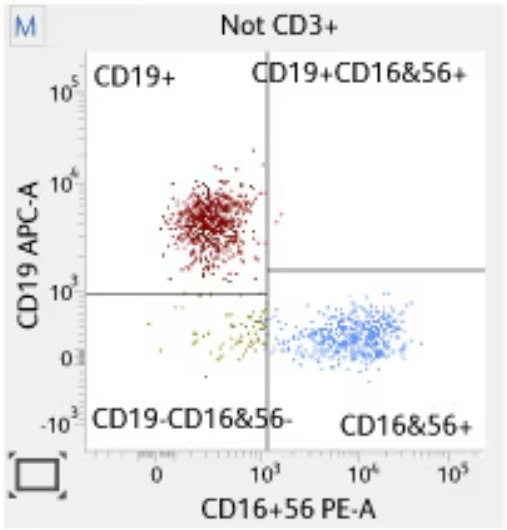
Single platform lymphocyte numeration











BD

Equipment

- 12% single platform, 88% double platform

Haematology analyser	**Number of participants**
Sysmex XN 1000/ XN 2000/ XN 3000/ XN 9000	39
Beckman Coulter UniCel DxH 800 / DxH 900	5
Siemens Advia 2120	1
Abbott Cell-Dyn Ruby	1
Not mentioned	3

Flow cytometer	Number of participants
BD Biosciences FACSLyric	20
Beckman Coulter Navios	10
BD Biosciences FACSCanto II	9
Beckman Coulter DxFLEX	5
Beckman Coulter AQUIOS CL	2
Beckman Coulter Cytomics FC 500	1
BD Biosciences FACSVia	1
Sysmex XF-1600	1

Performance monitoring

- With bead reference material, 100%

Bead material	Number of laboratories
BD Biosciences, cytometer Setup and Tracking beads (CST beads)	29
Beckman-Coulter Flow-Check Fluorospheres	7
Beckman-Coulter Flow-Check Pro Fluorospheres	5
BD Biosciences 7-color setup beads	1
Beckman-Coulter Flow-Set Fluorospheres	1
Not mentioned	6

- Additional cellular material, 80%
  - stabilized cells with target ranges for common cell subsets

Methodology

- T/B/NK assay: 55% lyse no wash

Lysing reagent	Number of laboratories
BD Biosciences FACS Lysing Solution	25
Beckman-Coulter VersaLyse	11
Ammonium chloride (NH_4Cl)	5
Beckman-Coulter Optilyse C	3
BD Biosciences Pharm Lyse	3
Beckman-Coulter Immunoprep reagent system	1

	Number of participants				
	$CD3^+$	**$CD4^+$**	**$CD8^+$**	**$CD19^+$**	**NK**
6 colours	23	23	23	23	23
7 colours	10	10	10	10	10
8 colours	12	12	12	12	12
10 colours	3	3	3	3	3

- κ/λ assay: 100% labs used ≥ 2 washing steps

Results (CV%)

	% lymphs	# 10^9/L
CD3	<5	5-10
CD4	~5	~10
CD8	~5	~10
CD19	10-15	10-15
NK	10-15	10-15
Kappa %B lymph	5-10	NA
Lambda %B lymph	5-10	NA

Technical validation:

- lymphosum (sum of CD3+% plus CD19+% plus CD3-CD16+ and/or CD56+%) should equal the total lymphocytes gate ± 5%
- sum of kappa and lambda (expressed as a % of CD19+ B-cells) should be between 90 and 110

Methodological issues affecting performance

Parameter	Number of evaluated results		% results >3 SD	
	Double platform	Single platform	Double platform	Single platform
CD3 10^9/L	268	28	6%	25%
CD4 10^9/L	268	28	6%	18%
CD8 10^9/L	268	28	5%	18%
CD19 10^9/L	268	28	7%	14%
NK cells 10^9/L	268	28	7%	14%

Parameter	Number of evaluated results		% results >3 SD	
	Lyse and wash	Lyse no wash	Lyse and wash	Lyse no wash
CD3 %	142	158	6%	4%
CD3 10^9/L	138	158	8%	8%
CD4 %	142	158	10%	3%
CD4 10^9/L	138	158	9%	6%
CD8 %	142	158	8%	2%
CD8 10^9/L	138	158	8%	5%
CD19 %	142	158	8%	4%
CD19 10^9/L	138	158	9%	6%
NK cells %	142	158	6%	4%
NK cells 10^9/L	138	158	7%	8%
Lymphosum	142	158	5%	6%

CD34+ cell numeration

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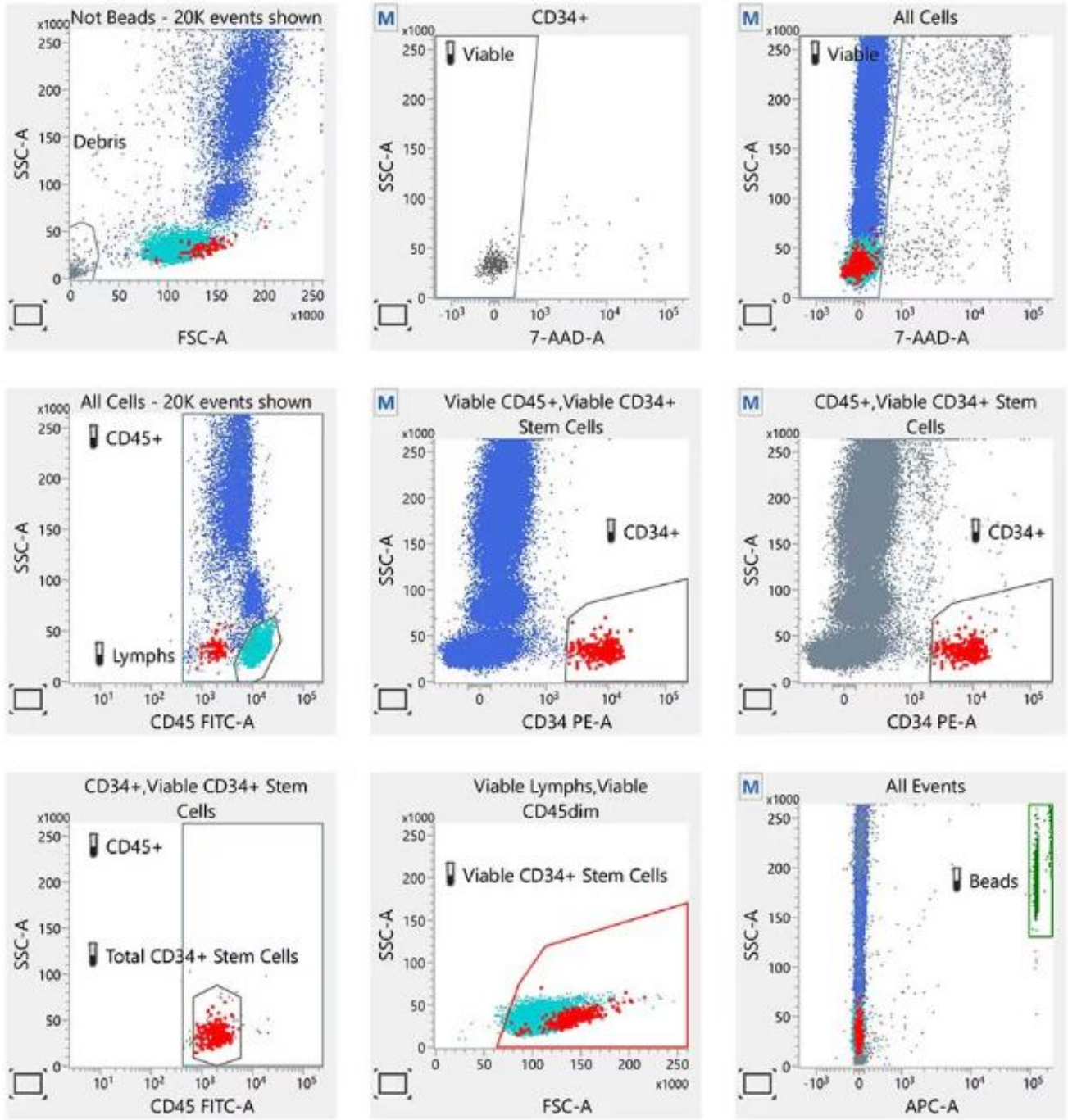
ISHAGE-derived single platform protocol



BD

Methodology

- 75% single platform

Flow cytometer	Number of laboratories
BD Biosciences FACSLyric	9
Beckman-Coulter Navios	4
BD Biosciences FACSCanto II	4
Beckman Coulter AQUIOS CL	2
Miltenyi Biotec MACSQuant analyzer	1

- 14/20: ISHAGE protocol
- 4 BD stem cell enumeration kit
- 1 BD procount
- 1 stem kit Coulter

Results (%CV), 2024

Sample	Median % CD34+ cells within total WBC	CV %	N	Median CD34+ cells/µL	CV %	N
FC/20466	0.160	12.7	20	9.9	13.5	20
FC/20467	0.640	8.1	20	39.7	11.5	20
FC/20615	0.130	9.4	20	8.3	9.8	20
FC/20616	0.489	5.1	20	30.1	4.4	20

10 cells range: CV ~ 11,65% (median UK NEQAS 12%)
35 cells range: CV ~ 7,95% (median UK NEQAS 8%)

Total error in DP versus SP CD34+ cell enumeration (UK NEQAS)

%aTE = [Absolute Value (Reported Result–Median)/Median] × 100

		Median CD34+ cells/µL	(Exercises, n) entries, n	Median %aTE (interquartiles)
Dual platform 2004–2014	A	0–10.0	(19) 1,712	12.47 % (5.91–22.50)
	B	10.1–25.0	(37) 3,258	9.81 % (4.54–18.78)
	C	25.1–50.0	(29) 2,057	8.31 % (3.70–15.03)
	D	>50.1	(35) 2,429	8.15 % (3.74–15.29)
Single platform 2004–2014	A	0–10.0	(19) 2,820	11.57 % (5.26–21.97)
	B	10.1–25.0	(37) 4,857	8.94 % (4.12–16.91)
	C	25.1–50.0	(29) 5,193	7.50 % (3.46–13.78)
	D	>50.1	(35) 6,196	6.77 % (2.96–12.26)

In the 10-25 CD34+ cells/µL range:
delta aTE |DP-SP| = 0,87 % i.e. <0,1-0,25 cell/µL

Brando, CCLM 2025, 63:1190

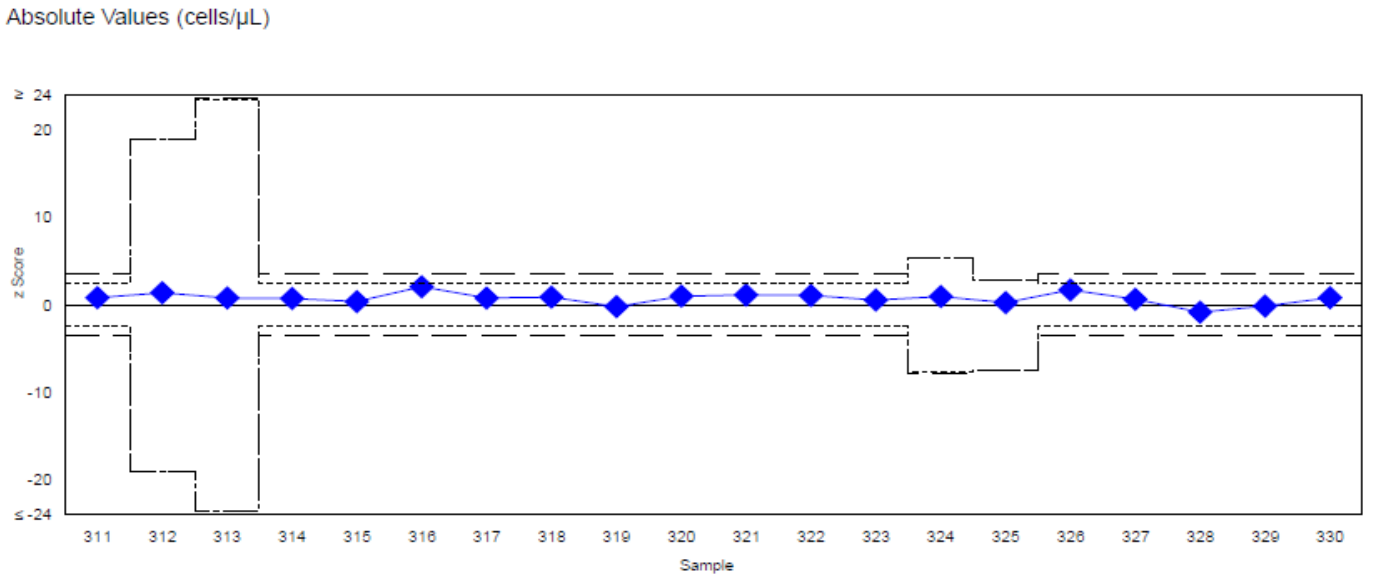
Where does EQA fit in a 15189 QA program?

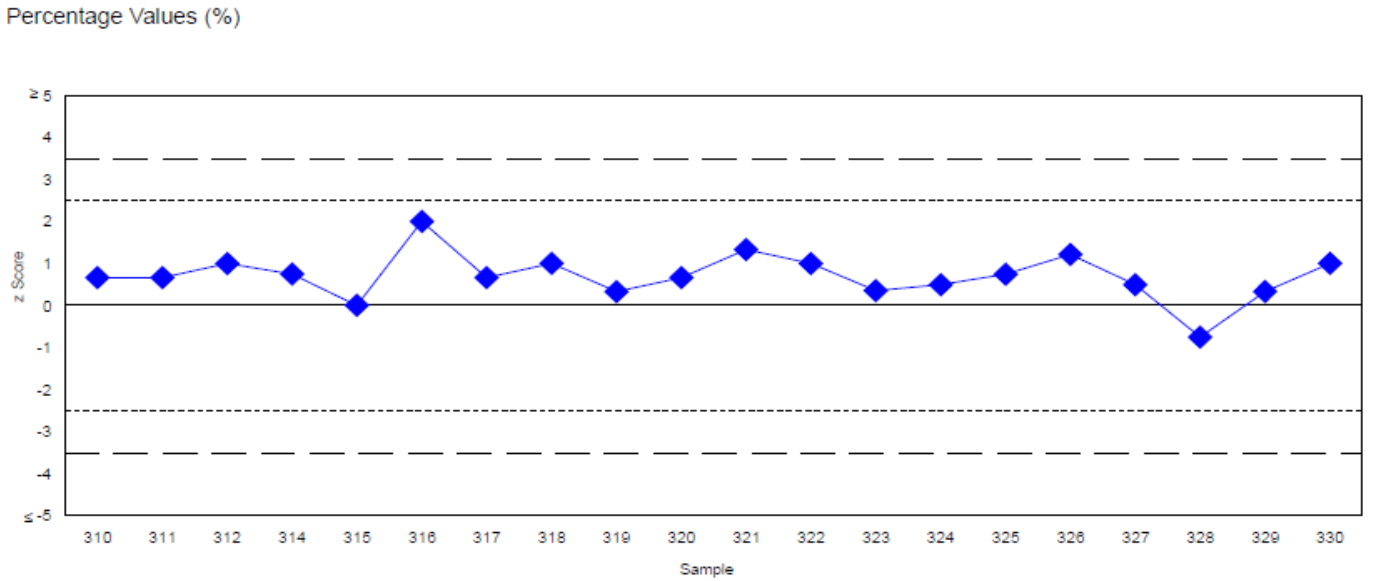
1. Performance monitoring
2. Method validation (UM)

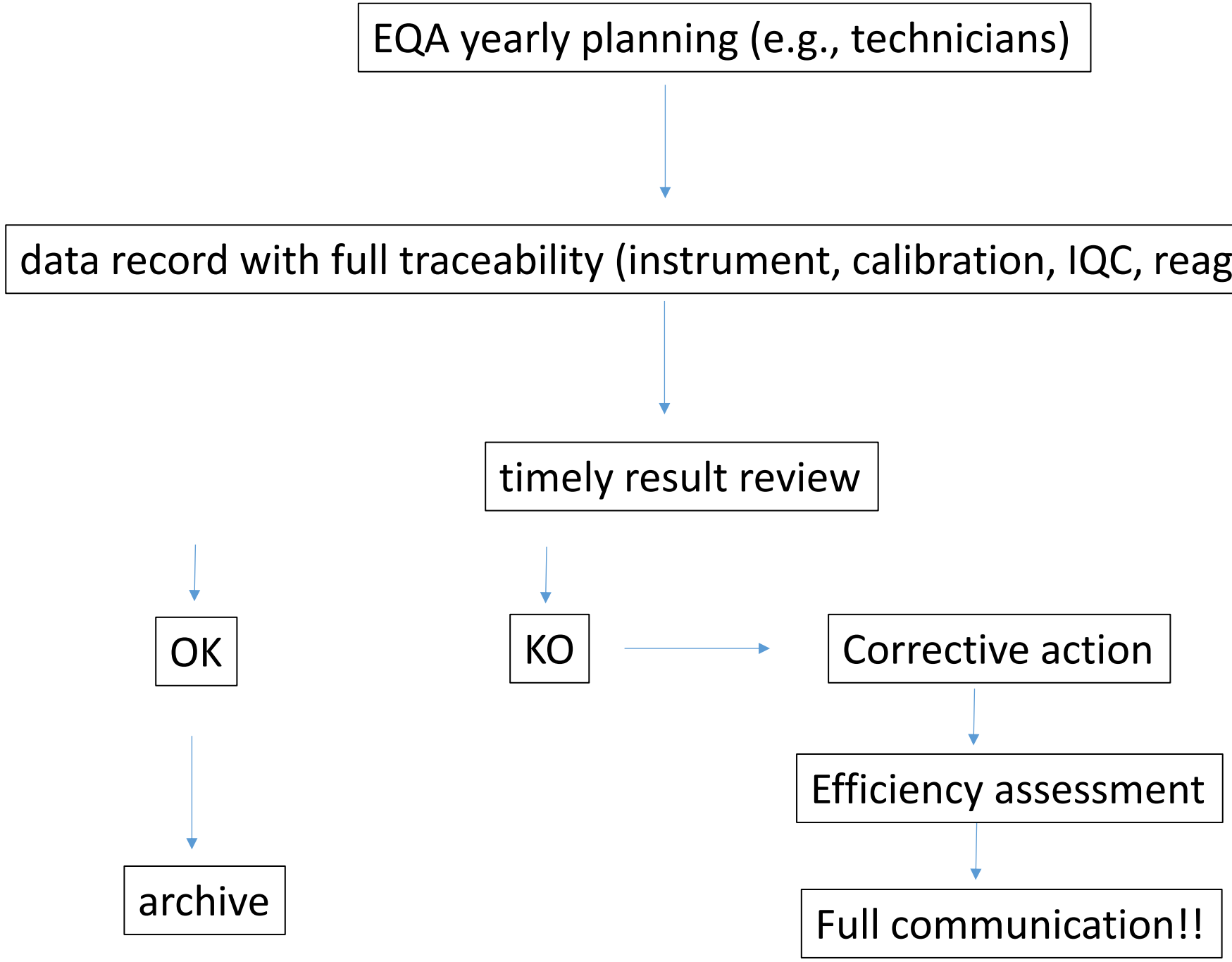
Performance monitoring

EQA: Requirements of ISO 15189 §7.3.7.3

a) Performance monitoring by comparison with other laboratories
   - EQA programs for (all) tests and interpretation of results






EQA yearly planning (e.g., technicians)
data record with full traceability (instrument, calibration, IQC, reagent lot, operator)
timely result review
OK
KO
Corrective action
Efficiency assessment
archive
Full communication!!

Non-Conformité

Id 105339 Date 31/07/2025 Enregistré par GOTHOT André

Lié à [105338] [QCE.PRO - 3 - Rapport QCE]

Date réelle ou date de détection du problème 31/07/2025

Mot(s)-Clé(s) CD34 - 2025 - 2 TEST

Secteur concerné BCL - HIH - H - Typage hématopoïétique et Thérapie cellulaire

☐ Diffuser la NC au laboratoire

Enregistrement | Documents | Analyse | Actions correctives | Evaluation de l'efficacté | Historique | Traçabilité

Observation

KO

FC21357: z score > 3
overestimation by wrong CD45 gating

FC21358: OK

Fournisseur concerné Sciensano

Sous-Traitant concerné

Est-ce que ce problème est déja survenu ? Oui

La NC a un impact sur

ISO 15189	ISO 17025	Item
7.3.7		Garantie de la validité des résultats d'examen(s)

Origine de la NC

Laboratoire / Secteur commun

QCE

Actions immédiates prises

check IQC and patient results

check competency monitoring of TN and CS
review CD34 procedure with TN and CS (sample prep, analysis)

communication to all techs

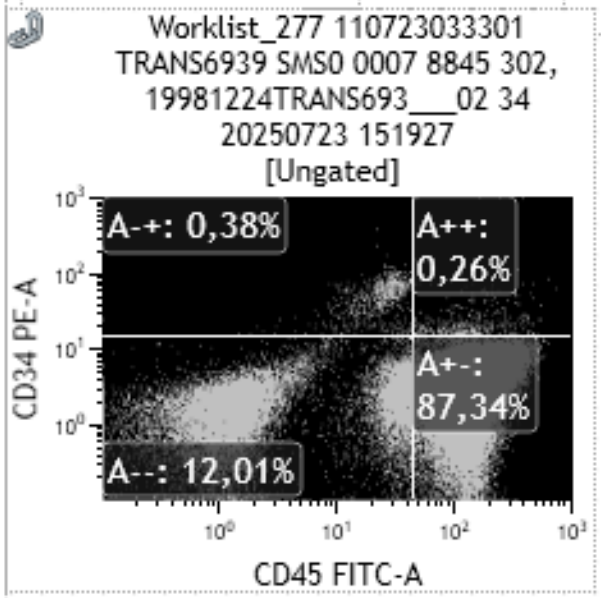
La Non-Conformité nécessite une analyse des causes profondes ?

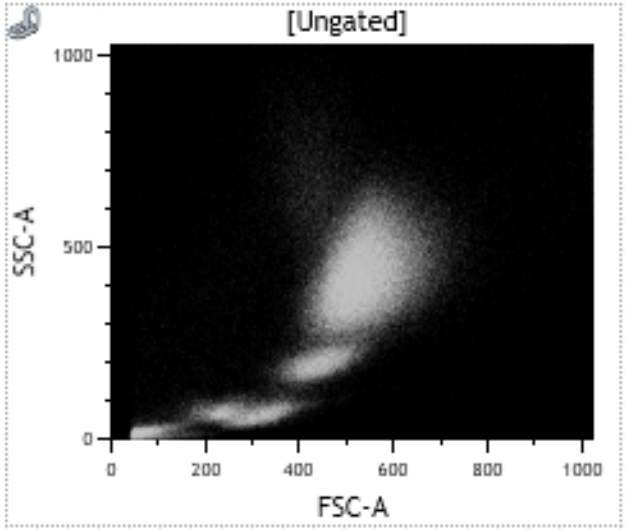
◉ Oui ○ Non

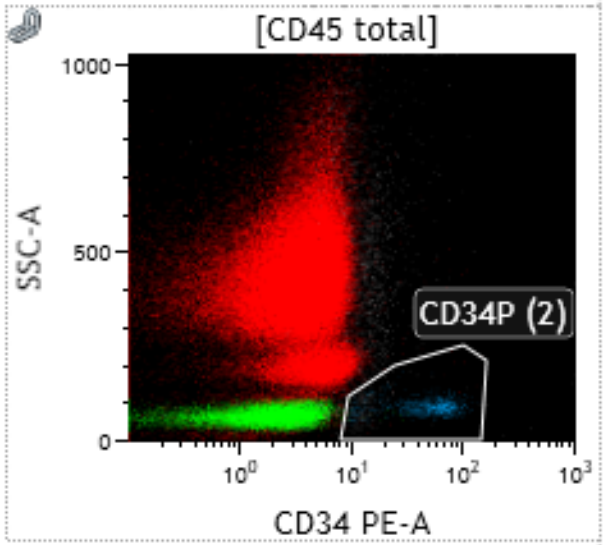
Numération CD34+ viables (FCM.NUM34.ANA)

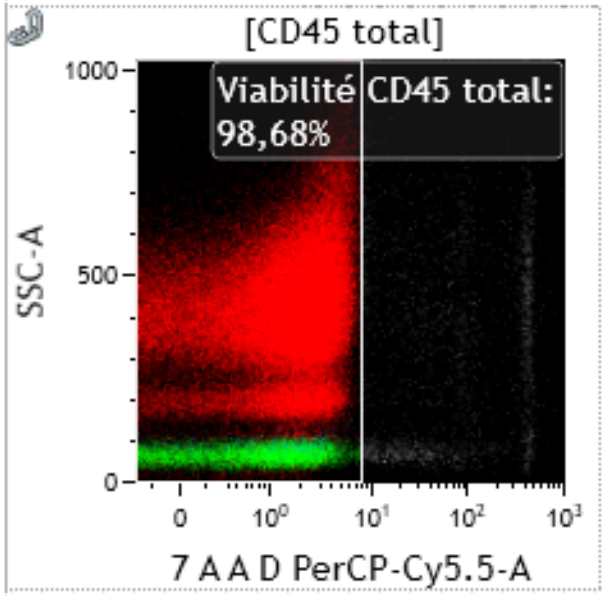
vendredi 1 août 2025 09:15

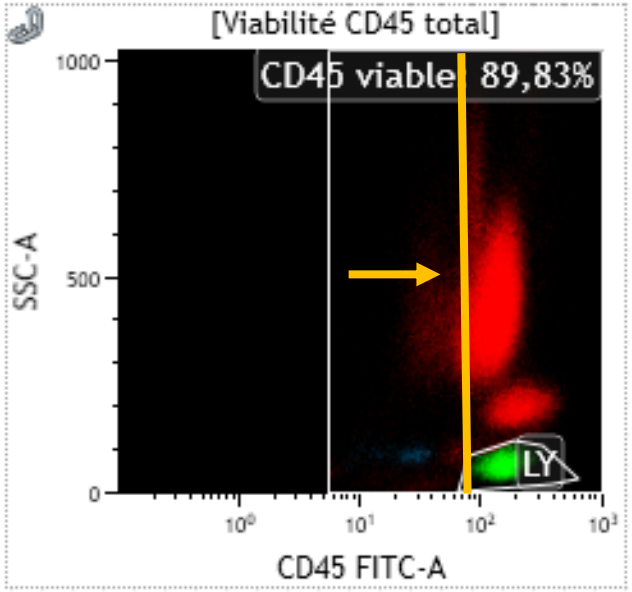
KALUZA V2.1
PROTOCOLE V1

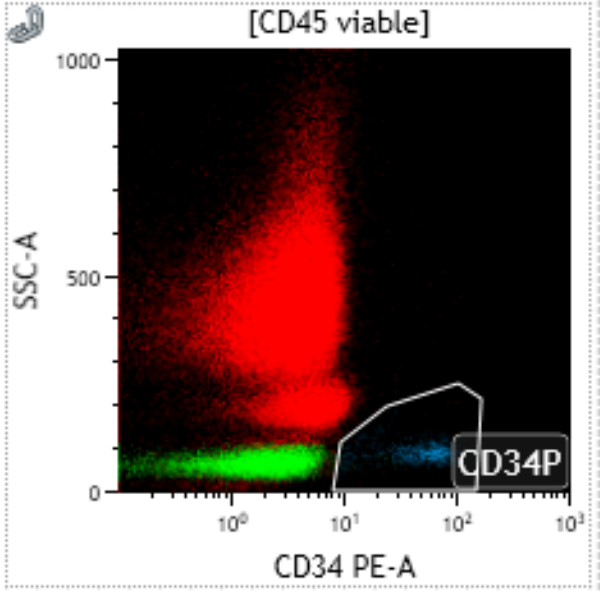


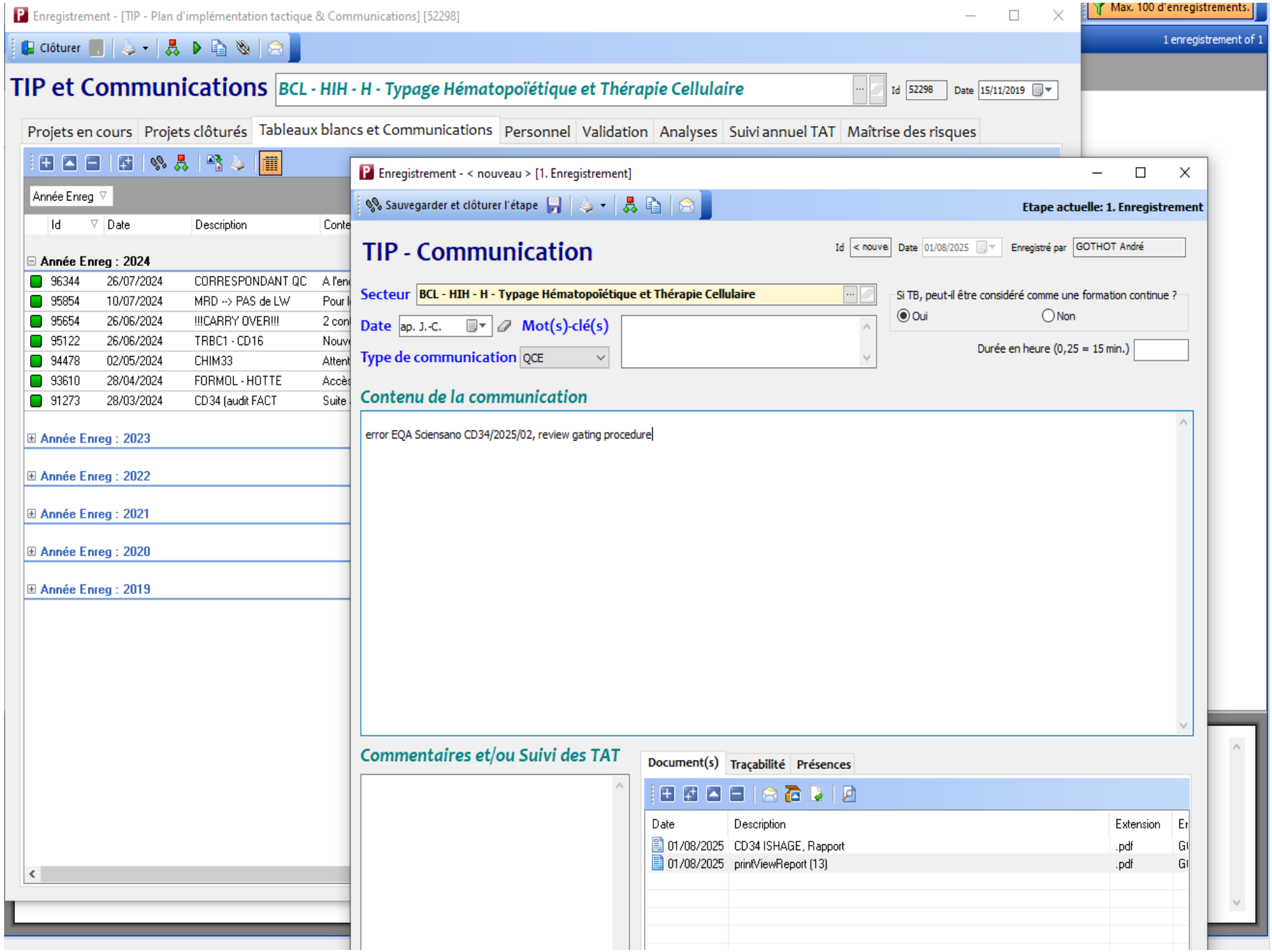











Enregistrement - [TIP - Plan d'implémentation tactique & Communications] [52298]
Clôturer
TIP et Communications
BCL - HIH - H - Typage Hématopoïétique et Thérapie Cellulaire
Id 52298 Date 15/11/2019
Projets en cours
Projets clôturés
Tableaux blancs et Communications
Personnel
Validation
Analyses
Suivi annuel TAT
Maîtrise des risques
Année Enreg
Id
Date
Description
Année Enreg : 2024
96344 26/07/2024 CORRESPONDANT QC
95854 10/07/2024 MRD --> PAS de LW
95654 26/06/2024 !!!CARRY OVER!!!
95122 26/06/2024 TRBC1 - CD16
94478 02/05/2024 CHIM33
93610 28/04/2024 FORMOL - HOTTE
91273 28/03/2024 CD34 (audit FACT
Année Enreg : 2023
Année Enreg : 2022
Année Enreg : 2021
Année Enreg : 2020
Année Enreg : 2019
Max. 100 d'enregistrements.
1 enregistrement of 1
Enregistrement - < nouveau > [1. Enregistrement]
Sauvegarder et clôturer l'étape
Etape actuelle: 1. Enregistrement
TIP - Communication
Id < nouve Date 01/08/2025 Enregistré par GOTHOT André
Secteur BCL - HIH - H - Typage Hématopoïétique et Thérapie Cellulaire
Si TB, peut-il être considéré comme une formation continue ?
Oui
Non
Date ap. J.-C.
Mot(s)-clé(s)
Type de communication QCE
Durée en heure (0,25 = 15 min.)
Contenu de la communication
error EQA Sciensano CD34/2025/02, review gating procedure
Commentaires et/ou Suivi des TAT
Document(s)
Traçabilité
Présences
Date
Description
Extension
01/08/2025 CD34 ISHAGE, Rapport .pdf
01/08/2025 printViewReport (13) .pdf

EQA and method validation

EQA and method validation ISO 15189 7.3.4

- 7.3.4 Measurement uncertainty evaluation (MU)

MU for measured quantitative values shall be evaluated and maintained for its intended use, if relevant. MU shall be compared to performance specifications and documented.

$$\mathrm{U(c)} = \sqrt{u^2\,(IQC) + u^2(K_{EQC})}$$

- u(IQC) = between-run imprecision, stdev IQC
- u(K_{EQC}) = bias, max bias /√3

COFRAC, GTA 14

BIAS EQC

	% CD34		
	result	median	bias
2023/1	0,556	0,58	-0,024
2023/2	0,55	0,534	0,016
2023/3	0,569	0,593	-0,024
2024/1	0,574	0,595	-0,021
2024/2	0,54	0,64	-0,1
2024/3	0,487	0,489	-0,002
2025/1	0,442	0,477	-0,035
2025/2	0,488	0,488	0
MAX			-0,035
$u(K_{EQC})$			0,020
$u^2(K_{EQC})$			0,0004

IMPRECISION IQC

Run	CD45 c.	CD34 c.	%CD34
1	153.670	869	0,5655
2	139.824	770	0,5507
3	154.144	861	0,5586
4	155.935	891	0,5714
5	156.621	894	0,5708
6	156.052	899	0,5761
7	153.162	916	0,5981
8	154.455	934	0,6047
9	154.633	888	0,5743
mean			0,5745
u(IQC)			0,0173
u^2(IQC)			0,00030

$$\mathrm{U(c)} = \sqrt{u^2\,(IQC) + u^2(K_{EQC})}$$

$$\mathrm{U(c)} = \sqrt{0{,}0004 + 0{,}00030}$$

U	0,0266
$U_{95\%}$ (k=2)	0,0532

Absolute error	0,5745 ± 0,0532
Relative error	0,5745 ± 9,23%

Additional EQA programs - flow cytometry

- UK NEQAS – INSTAND (DE)
  - CD34+ stem cell enumeration
  - Leukemia/lymphoma immunophenotyping
  - Paroxysmal nocturnal hemoglobinuria
  - Measurable residual disease (MRD): ALL, AML, CLL, MM (UK)
  - CSF immunophenotyping (UK)
- Euroflow
  - Lymphocyte screening tube (LST) (w*)
  - MRD ALL (w*/d)
  - MRD MM (d)
  - Primary immunodeficiency orientation tube (PIDOT) (w*/d)
    - *Wet lab includes standardisation of fluorescence intensities
    - Digital: analysis of raw data


THANKS FOR YOUR ATTENTION