Basic workshop MB&C26:
Automation in and by flow cytometry: user experiences

AQUIOS CL

Jonathan Vanbesien
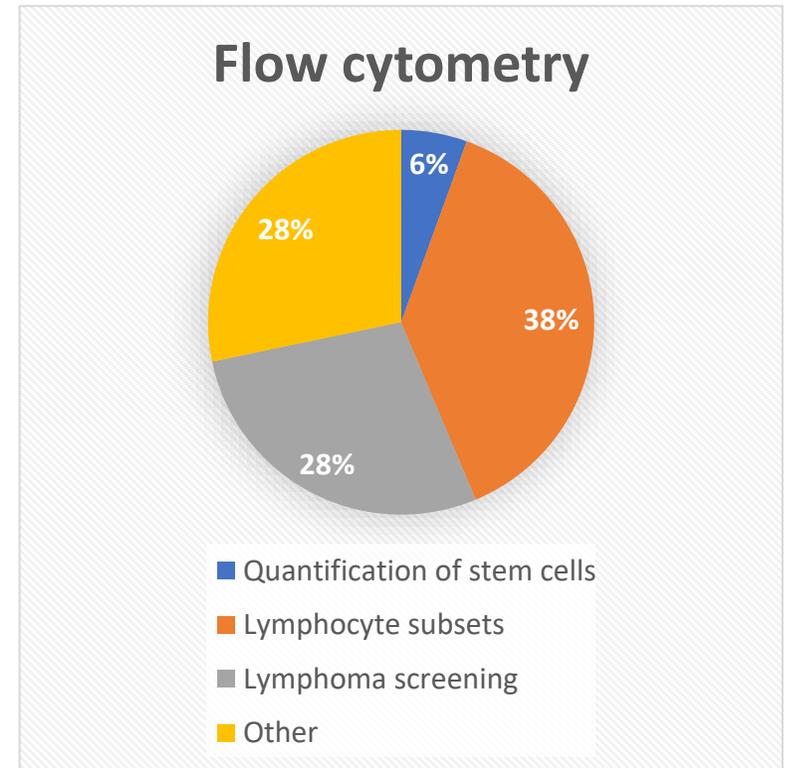AZ Delta

AZ Delta

– Flow cytometers
  - Aquios CL
  - Dx-Flex
  - CytoFlex SRT

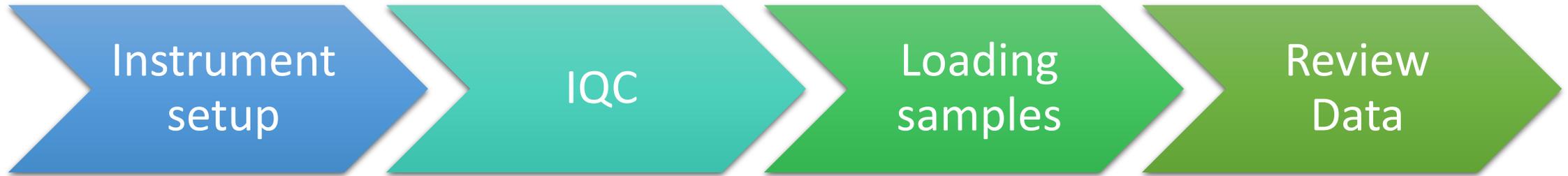– Sample Preparation Workstations
  - NizonPLUS
  - Aquios CL

Flow cytometry

- 6%
- 38%
- 28%
- 28%

- Quantification of stem cells
- Lymphocyte subsets
- Lymphoma screening
- Other

AQUIOS CL

– LOAD & GO flow cytometer

 ▪ Automated sample preparation
 ▪ Automated analysis
 ▪ (Bi-directional) LIS link

AQUIOS CL

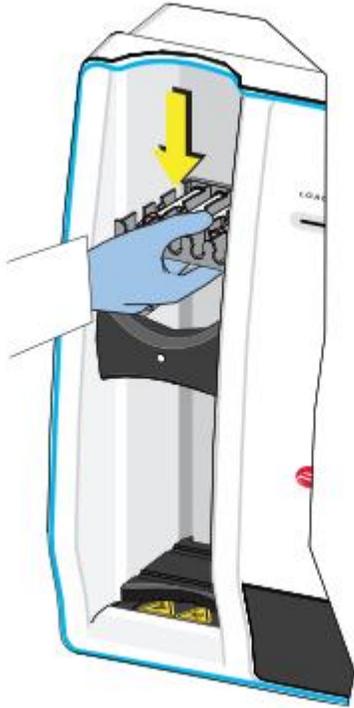Instrument setup → IQC → Loading samples → Review Data
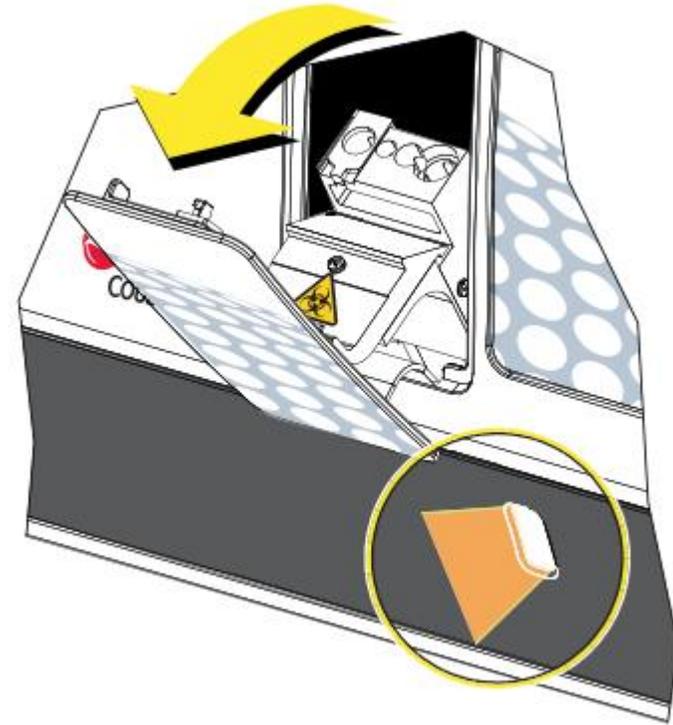
Loading samples

Cassette loader:
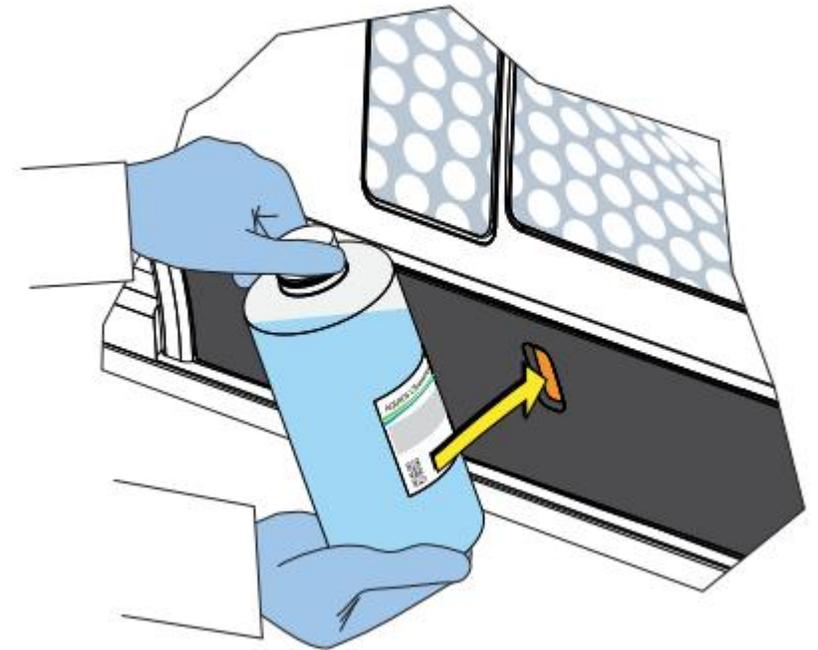- Different cassettes
- Closed tubes

Single tube loader:
- Open/closed tubes
- Dilutions
- Urgent samples

Reagents

– Barcoded
  - Markers, lysing solutions… => automatic scanning
  - Cleaning agent, sheath => manual scanning

– Expiration date
 - Closed vial
  - Open vial
  - On board

AQUIOS CL

– Applications:

- TETRA (lymphocyte subsets)

- STEM (quantification of CD34+ stem cells)

Lymphocyte subsets (TETRA)

– Monitoring of HIV infection

– Investigation of primary and secondary immune deficiency

– Immune monitoring following immunosuppressive therapy

Lymphocytes

T-cells   B-cells   NK-cells

Helper   Cytotoxic
T-cells   T-cells
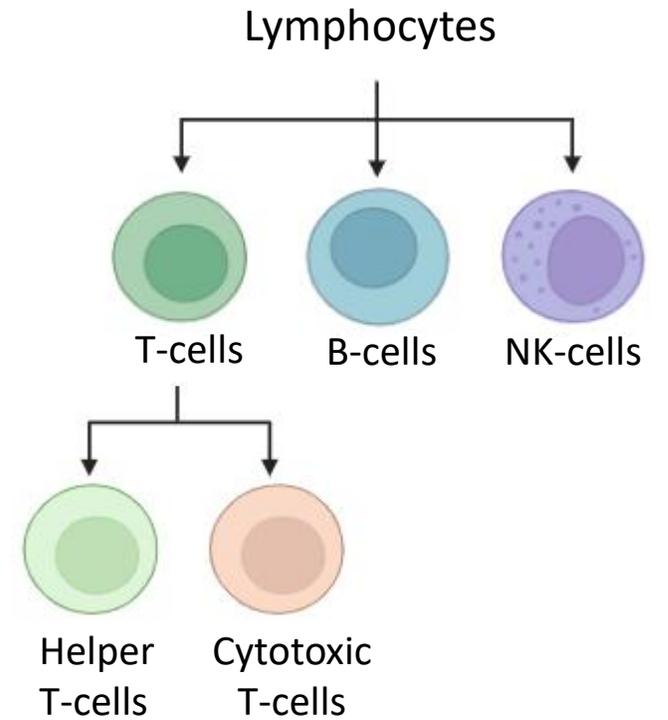
TETRA Application

– IQC:
  - IMMUNOTROL cells
  - IMMUNOTROL low cells (CD4+ low)

– Tests:
  - TETRA 1 (CD45/CD3/CD4/CD8) => T-cells
  - TETRA 2+ (CD45/CD3/CD19/CD16+CD56) => TBNK
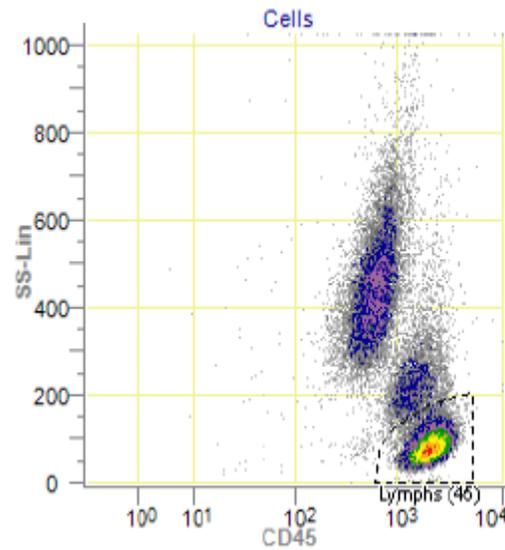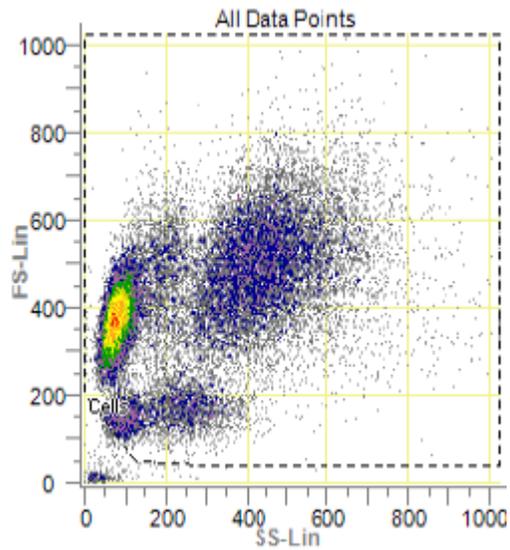 - TETRA COMBO (1 & 2+)

– Principle:
  - Lyse no wash
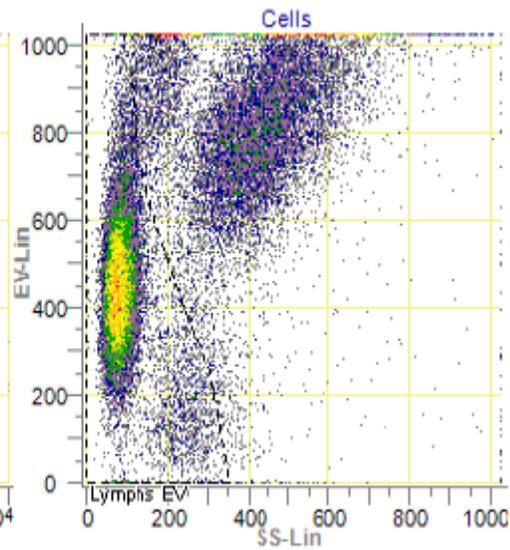 - Single platform
    - Volumetric absolute count

TETRA 1 & 2+
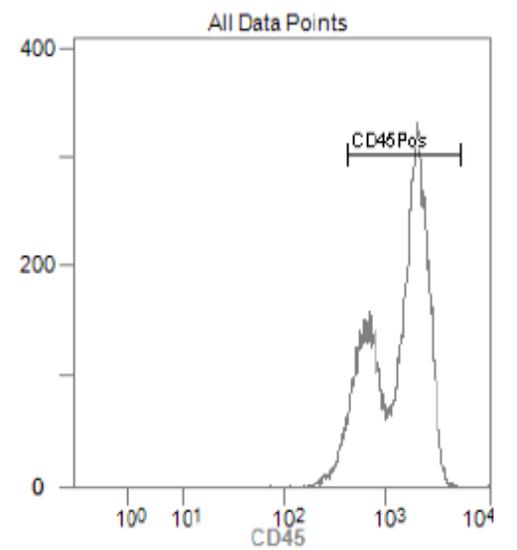
– Gating algorithm:
  - "Broad" lymphocyte gate: A OR (B AND C)
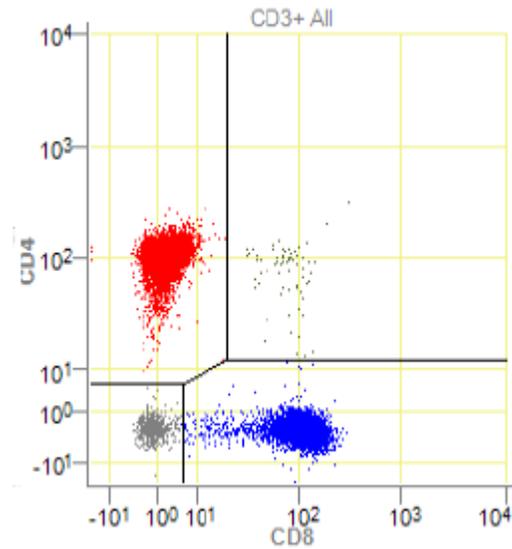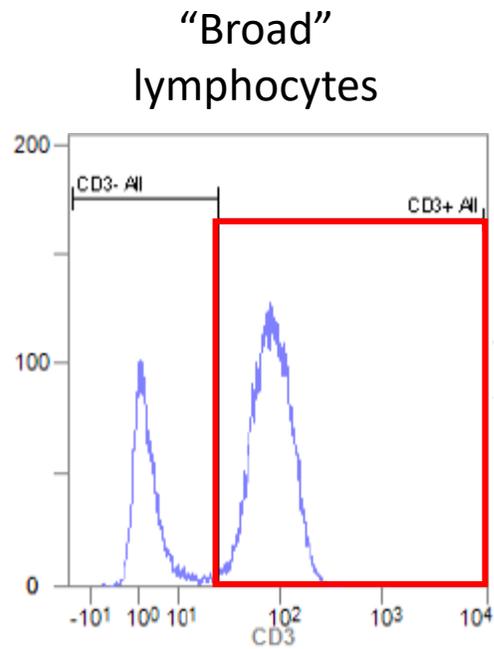 - "limited" lymphocyte gate: A AND B

A   B   C
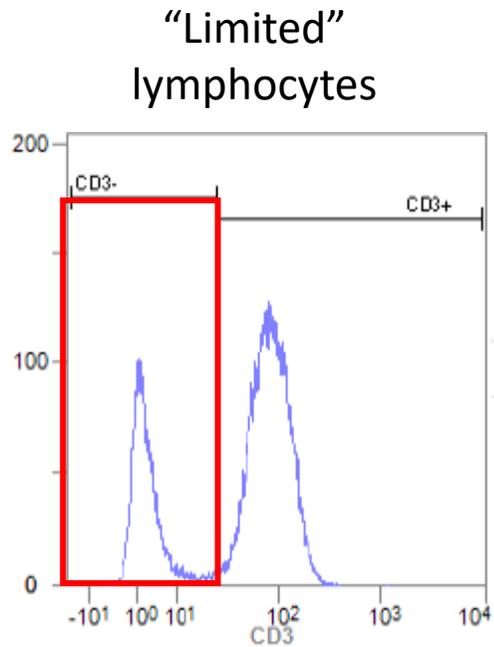
TETRA 1

– Gating algorithm:

- T-Lymphocytes: CD3+ All

"Broad" lymphocytes

TETRA 1

– Gating algorithm:

■ B-Lymphocytes & NK cells: CD3- OR CD3- Capture

"Limited" lymphocytes

"Broad" lymphocytes

TETRA 2+

– Gating algorithm:

- T-Lymphocytes: CD3+ All

"Broad"
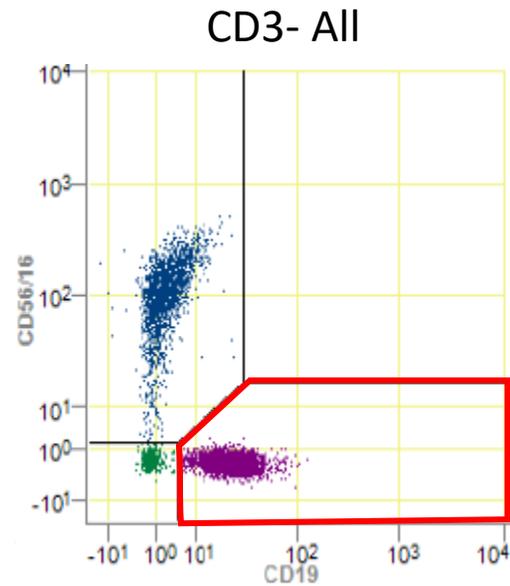lymphocytes

TETRA 2+

– Gating algorithm:
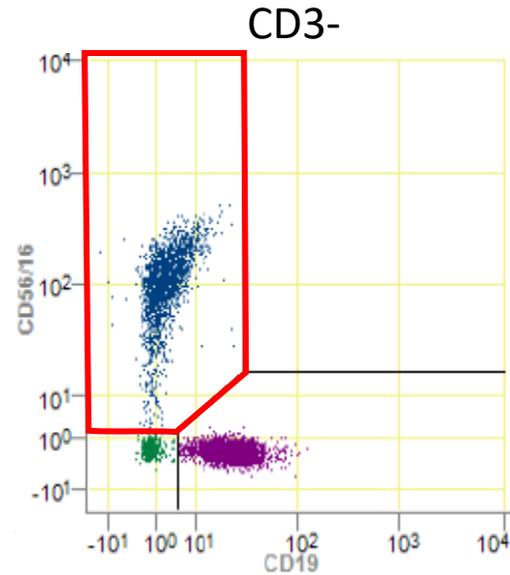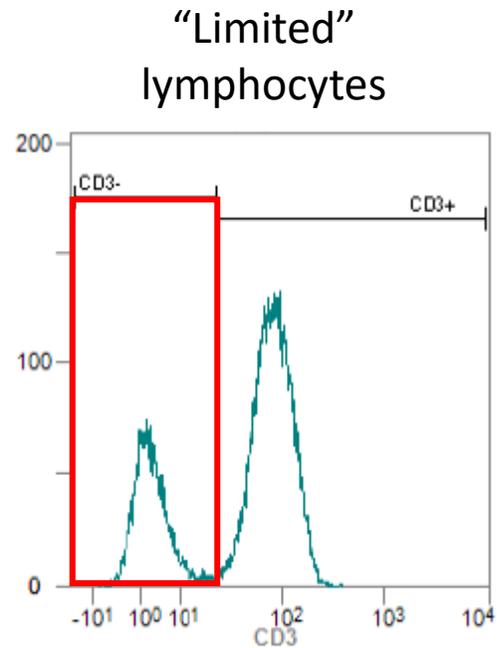- B-Lymphocytes: CD19+CD56/16-
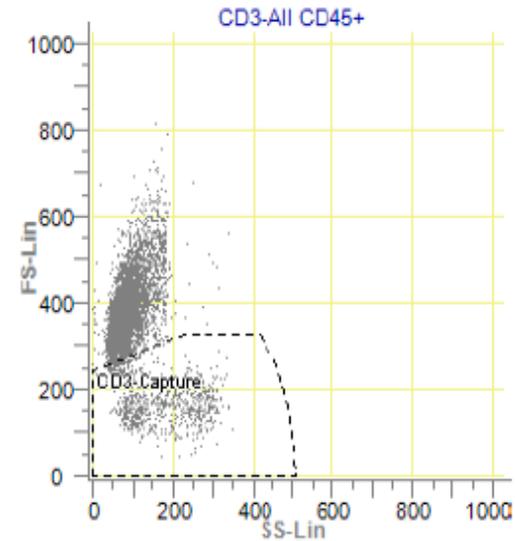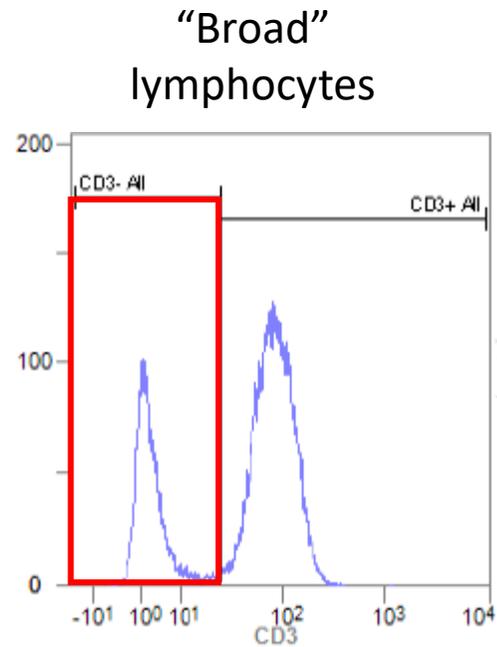
"Broad"
lymphocytes

CD3- All
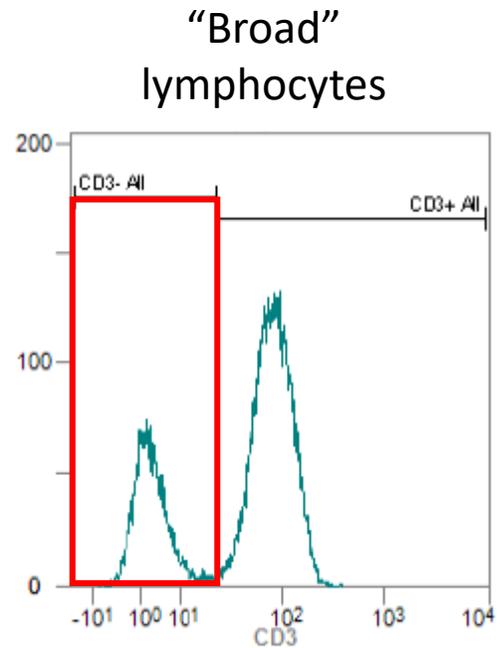
TETRA 2+

– Gating algorithm:
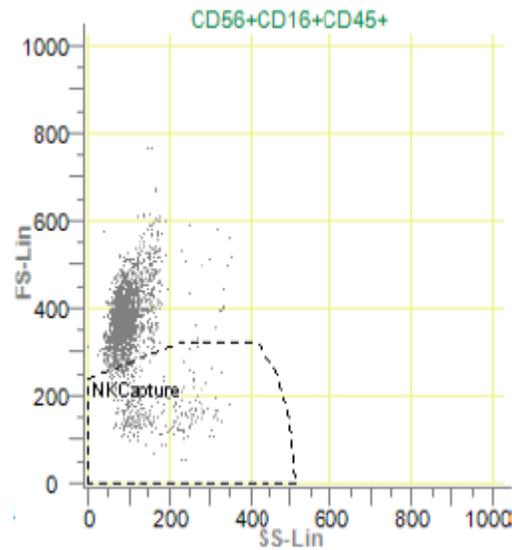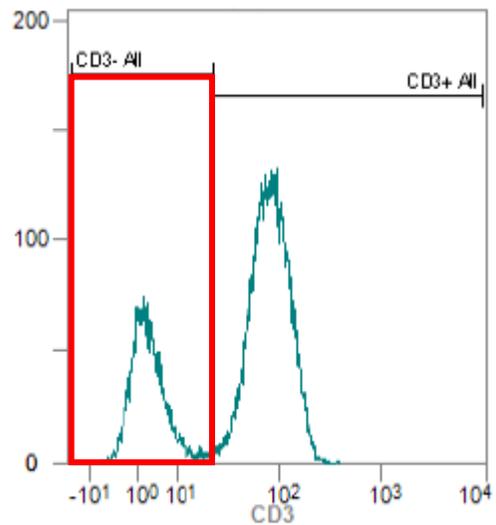- NK-cells: CD19-CD56/16+ OR NK Capture

TETRA 2+

– Gating algorithm:

  ▪ NK cells: CD19-CD56/16+ OR NK Capture

"Broad" lymphocytes

Result workflow

Review

- Flags
- Notifications

Flags and notifications

– Flags
  - Missing QC
  - Clog or Bubble
  - Insufficient Lysing
  - Insufficient Lymphs
  - Inter Panel Count Error
  - Onboard Expiration
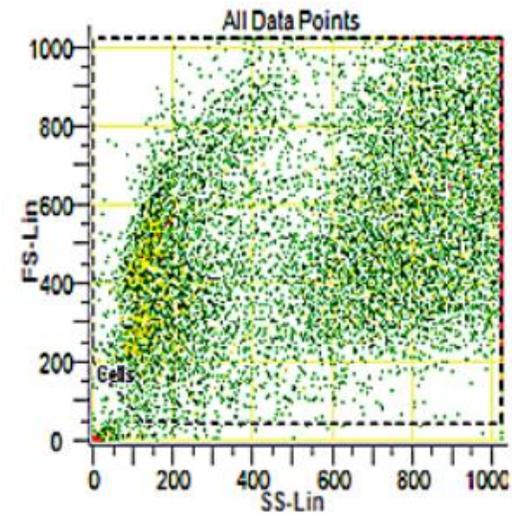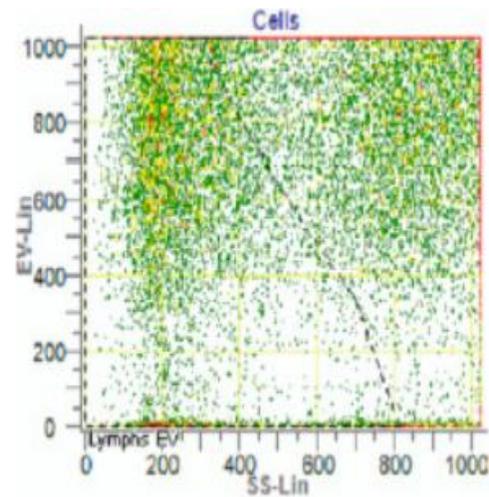 - Expired Reagent

– Notifications
  - Under Lysed
  - Low CD8 positives
  - Low CD3 positives
  - Low CD3 negatives
  - **Potential sample or gating issue**

Reviewing sample gating
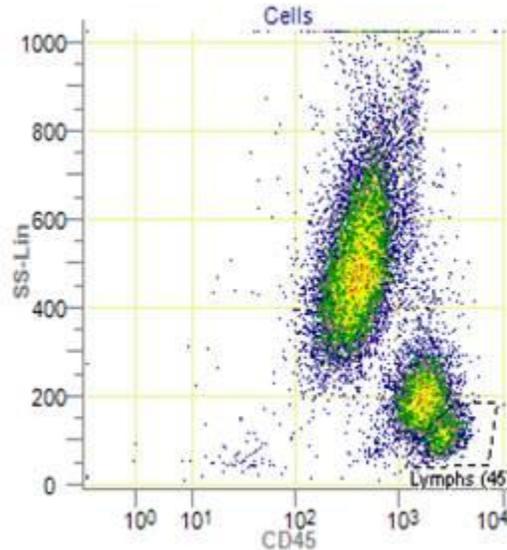
– "Potential sample or gating issue"

- 40% of samples AZ Delta
- Sample anomaly
  - Light scatter and EV patterns
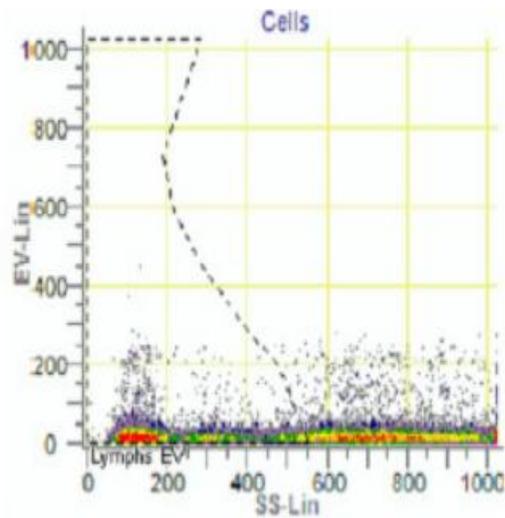
Reviewing sample gating

– "Potential sample or gating issue"

- 40% of samples
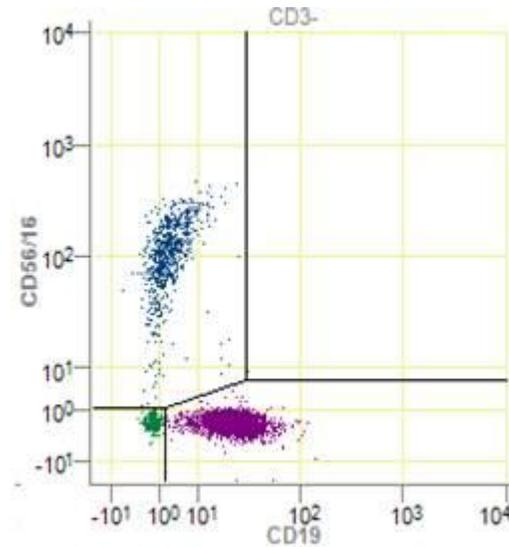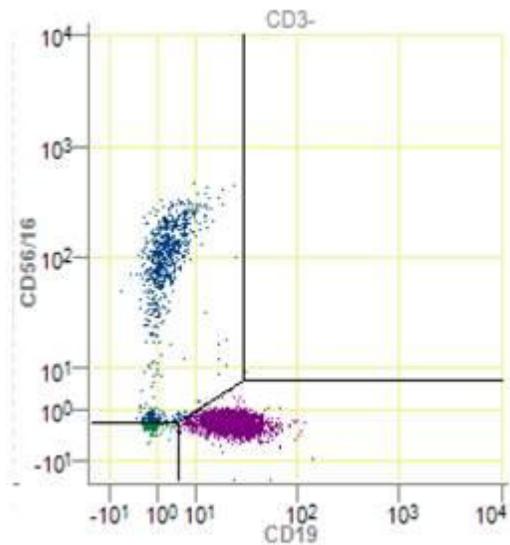- Sample anomaly
  - Light scatter and EV patterns
- Gate misplacement
  - 2/3 have to be adjusted
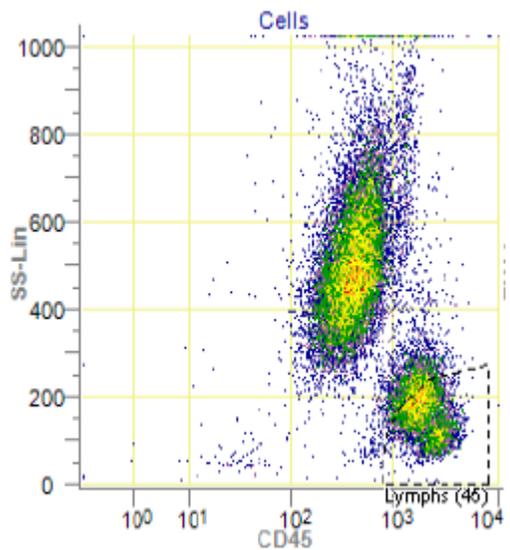
Reviewing sample gating

– "Potential sample or gating issue"

- 40% of samples
- Sample anomaly
  - Light scatter and EV patterns
- Gate misplacement
  - 2/3 have to be adjusted

Result workflow

Review
- Flags
- Notifications

Result transmission
- LIS Link
- Consistency checks

azdelta
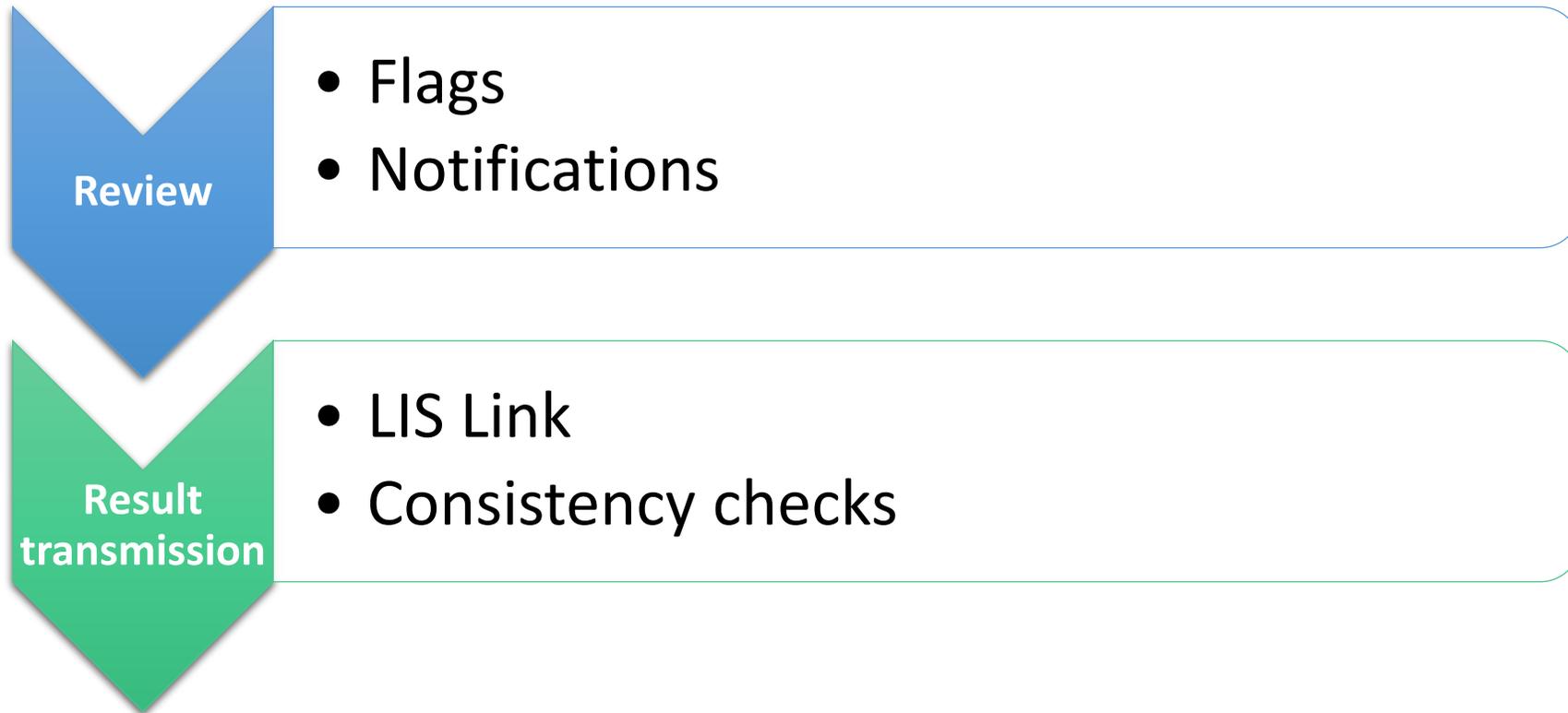
Consistency checks

- Leucocyte count
  - vs haematology analyser
  - <20%

- % Lymphocytes
  - vs haematology analyser
  - <20%

- Lympho sum (TBNK)
  - 95% - 105%

- Intrapanel check
  - Comparison of CD3+ in Tetra 1 & 2+
  - <3,5%

Result workflow

Review
- Flags
- Notifications

Result transmission
- LIS Link
- Consistency checks
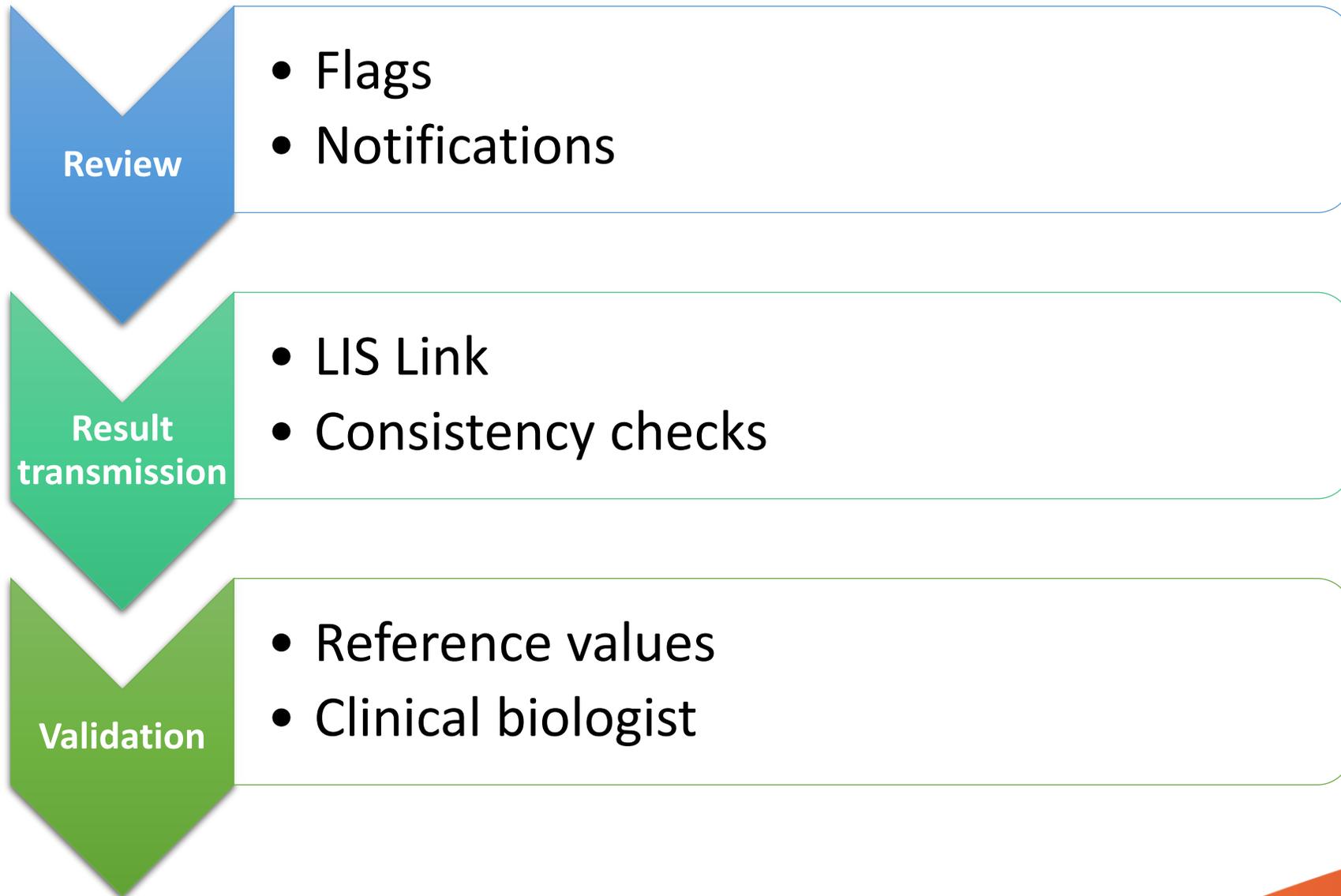
Validation
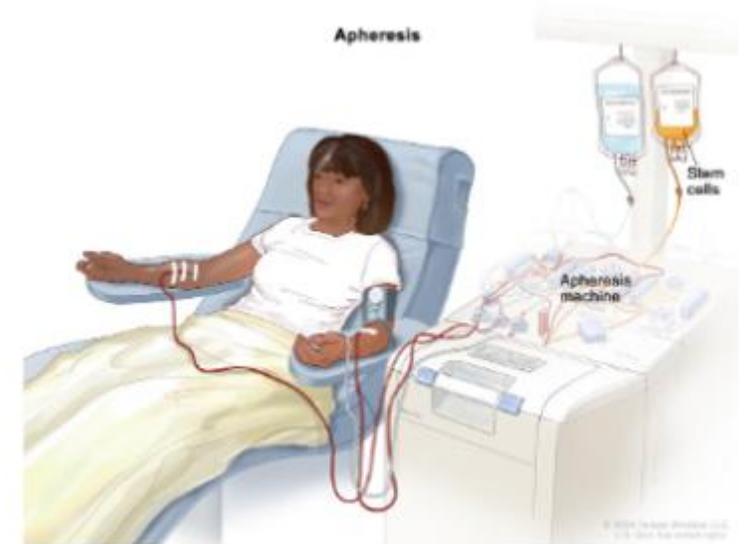- Reference values
- Clinical biologist

Advantages and limitations
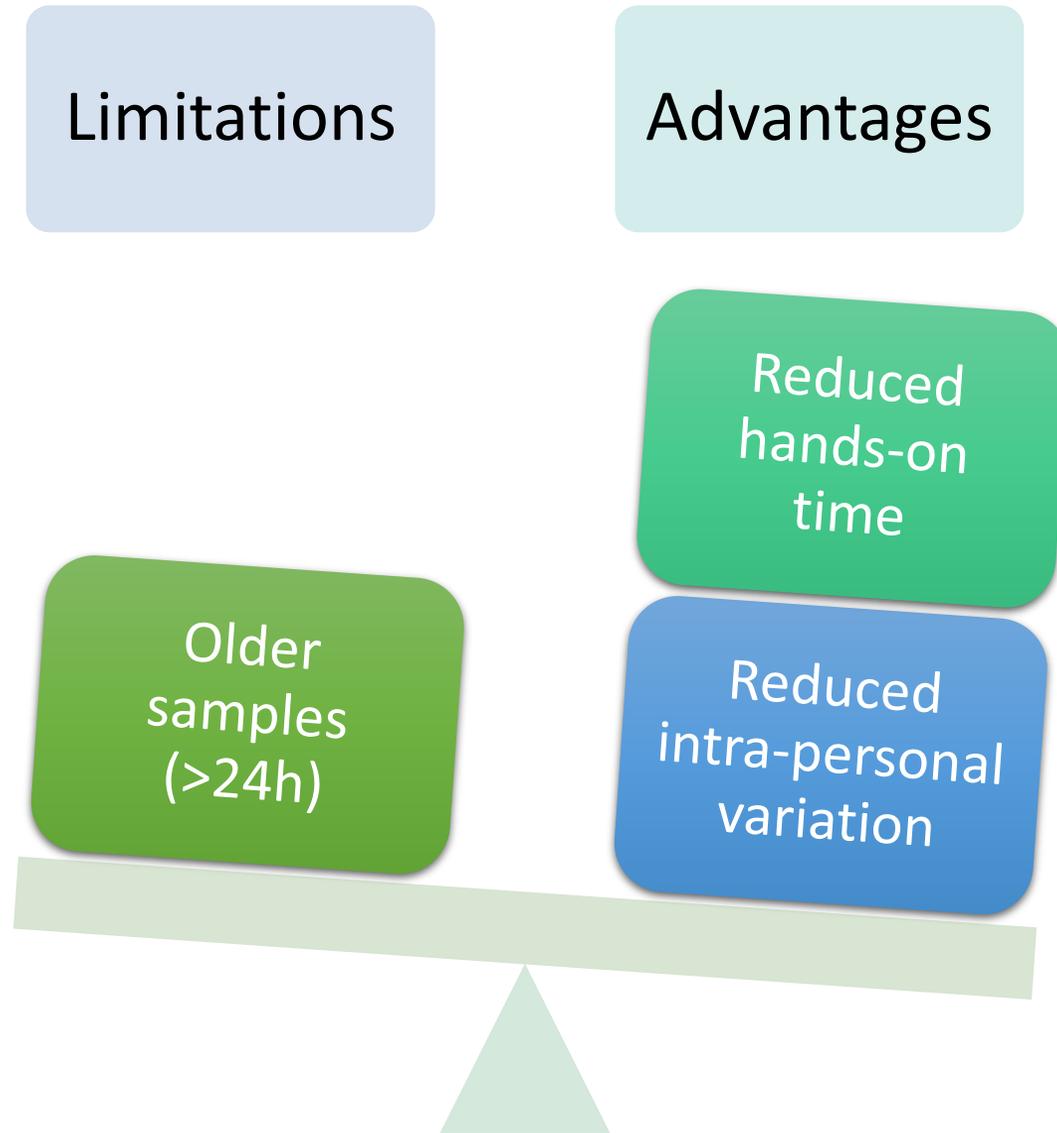
STEM Application

– Quantification of CD34+ stem cells

- Mobilized blood:
    - >10 CD34+ stem cells/µL => start apheresis

- Apheresis product:
    - Yield stem cell collection in CD34+ stem cells/kg
      => Need for another collection day

STEM Application

– IQC:
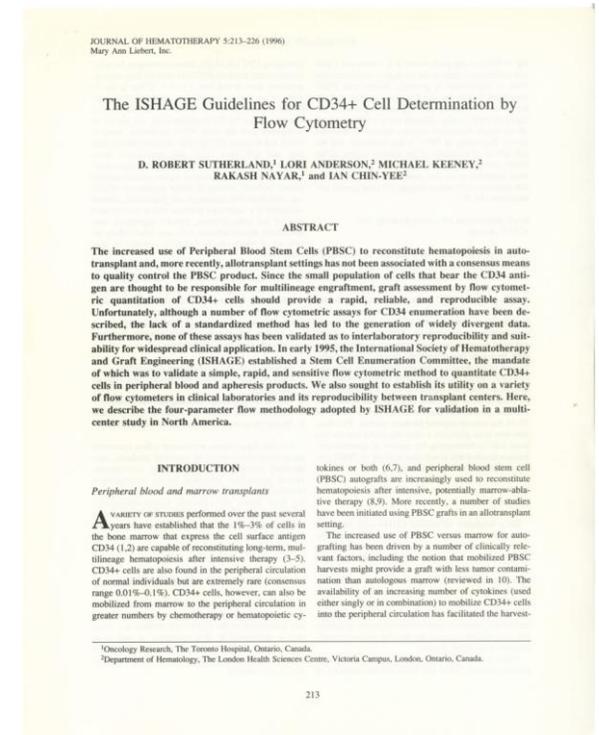 - STEM Control level 1 & 2
  - Flow check alignment

– Tests:
  - STEM Single
  - STEM duplicate
  - STEM panel (+ isoclonic control)

STEM

– Based on the (modified) ISHAGE guidelines

- Antibodies and reagents
  - CD34-PE/CD45-FITC
  - 7-AAD for Cell Viability

- Method
  - Lyse no Wash
  - Single platform
    - Absolute count by beads

- Gating strategy
  - Sequential gating

JOURNAL OF HEMATOTHERAPY 5:213–226 (1996)
Mary Ann Liebert, Inc.

The ISHAGE Guidelines for CD34+ Cell Determination by Flow Cytometry

D. ROBERT SUTHERLAND, LORI ANDERSON, MICHAEL KEENEY, RAKASH NAYAR, and IAN CHIN-YEE

Sutherland et al, Journal of Hematotherapy 1996
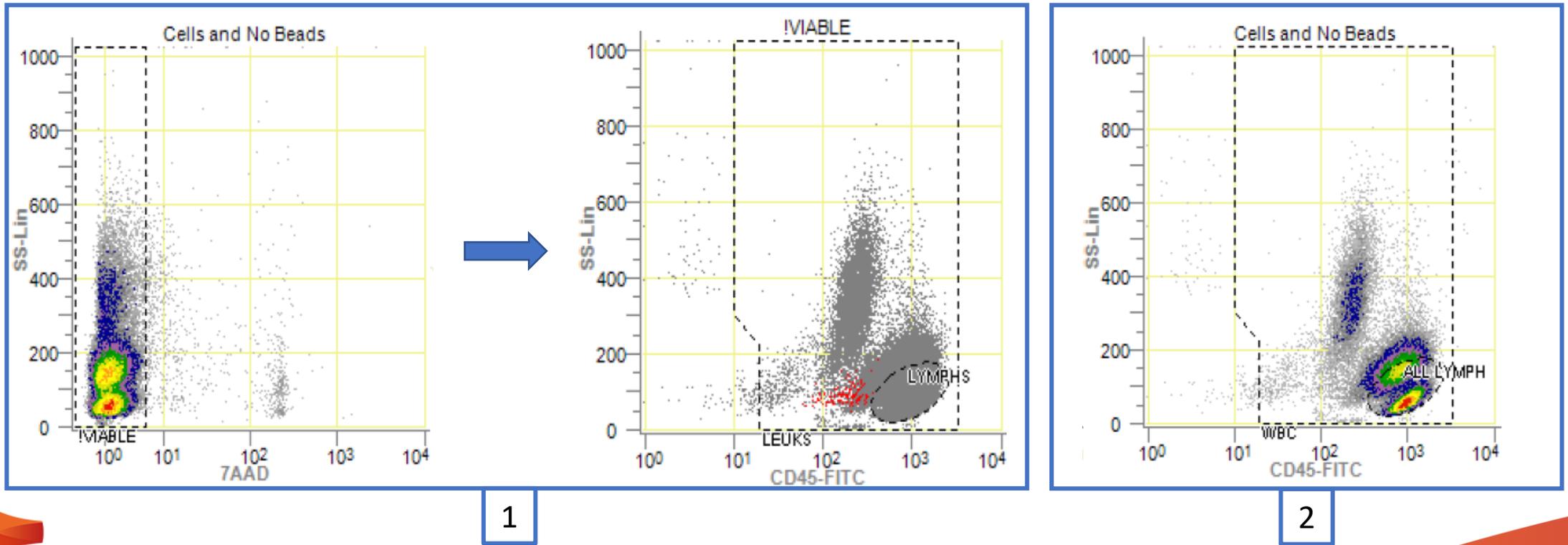Keeney et al, Cytometry 1998

STEM – Gating Algorithm

– Bead selection
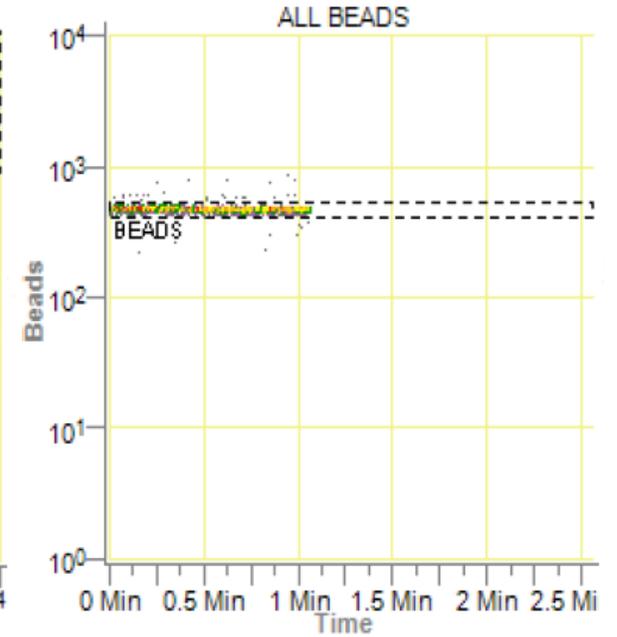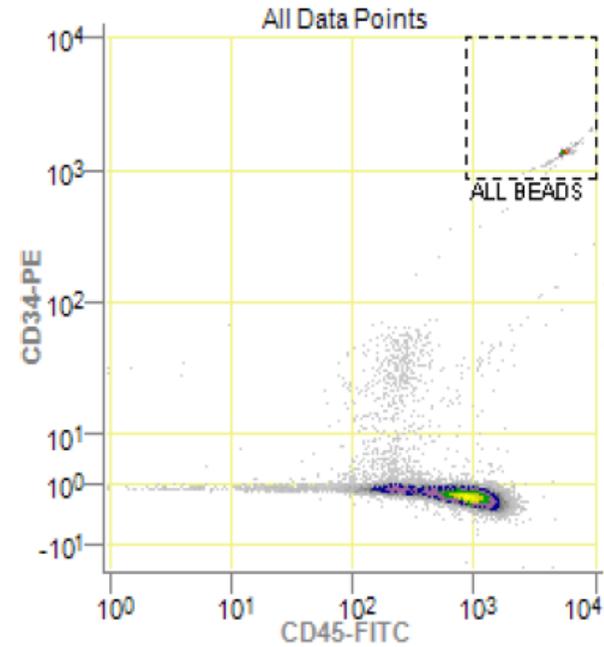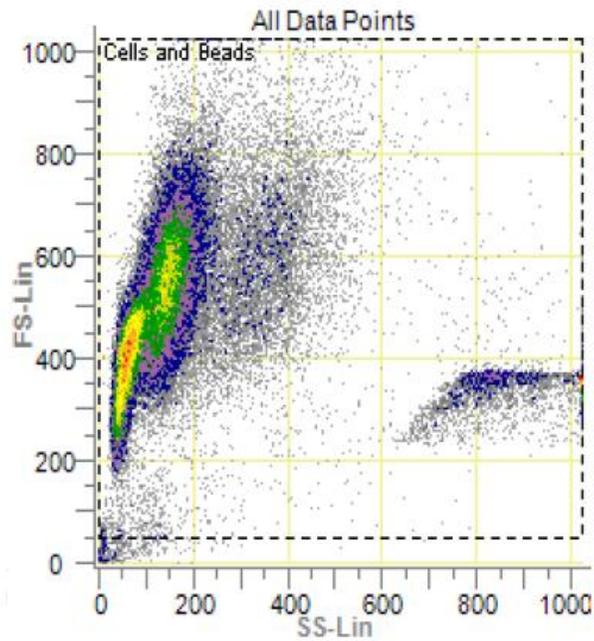
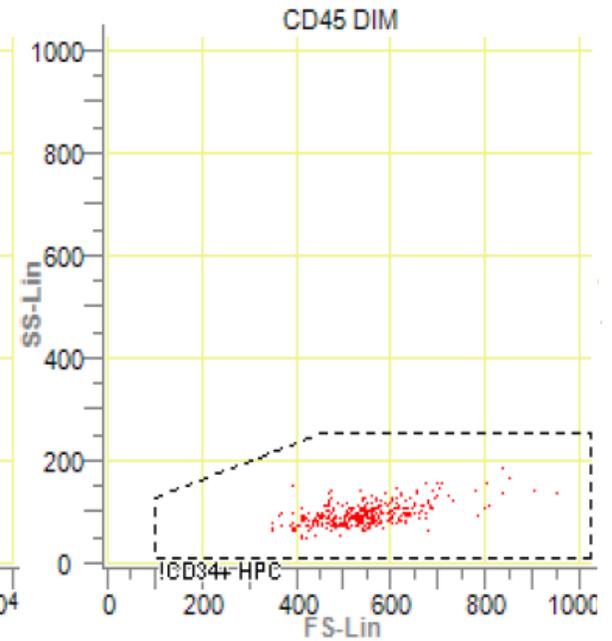# STEM – Gating Algorithm

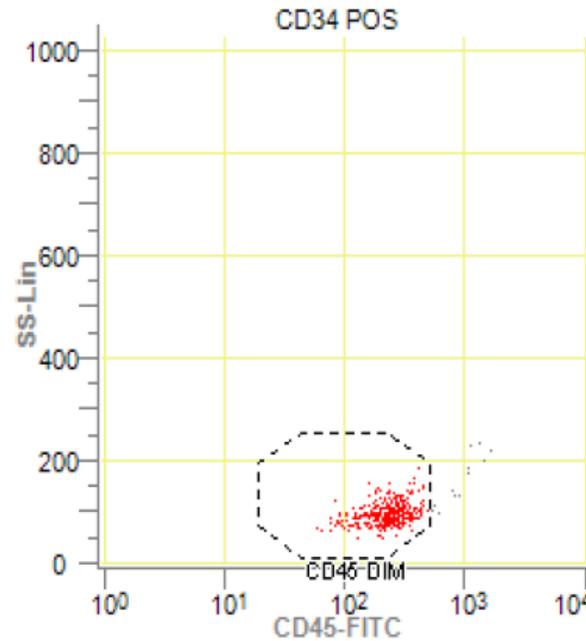– Two stem cell gating pathways:
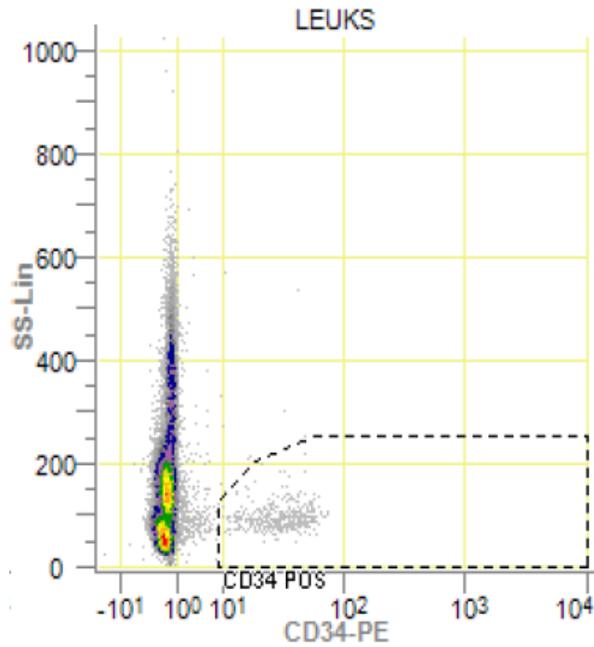1. Viable WBC
2. Total WBC

STEM – Gating Algorithm
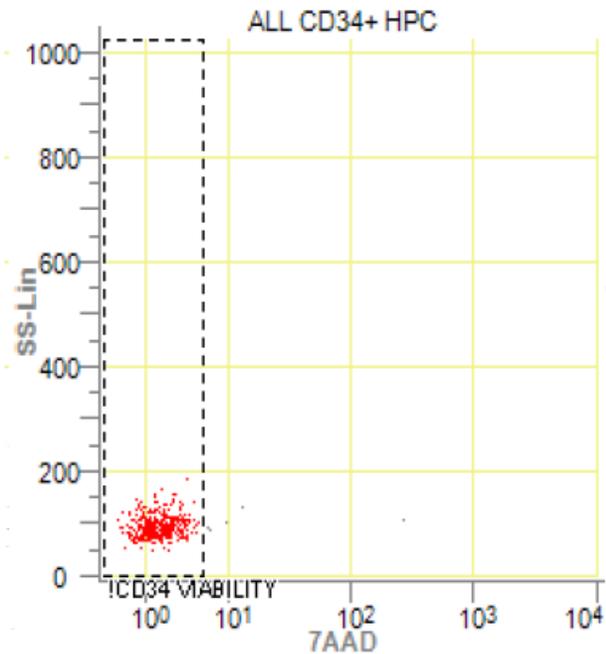
– Viable CD34+ stem cell selection

- Dinstinguish from lymphocytes
- Meet all the fluorescence and light scatter criteria of ISHAGE Guidelines

STEM – Gating Algorithm

– Total CD34+ stemcell selection

- Identical to viable CD34+ stem cell selection
- Viability of stem cells

Result workflow

Review

- Flags
- Notifications

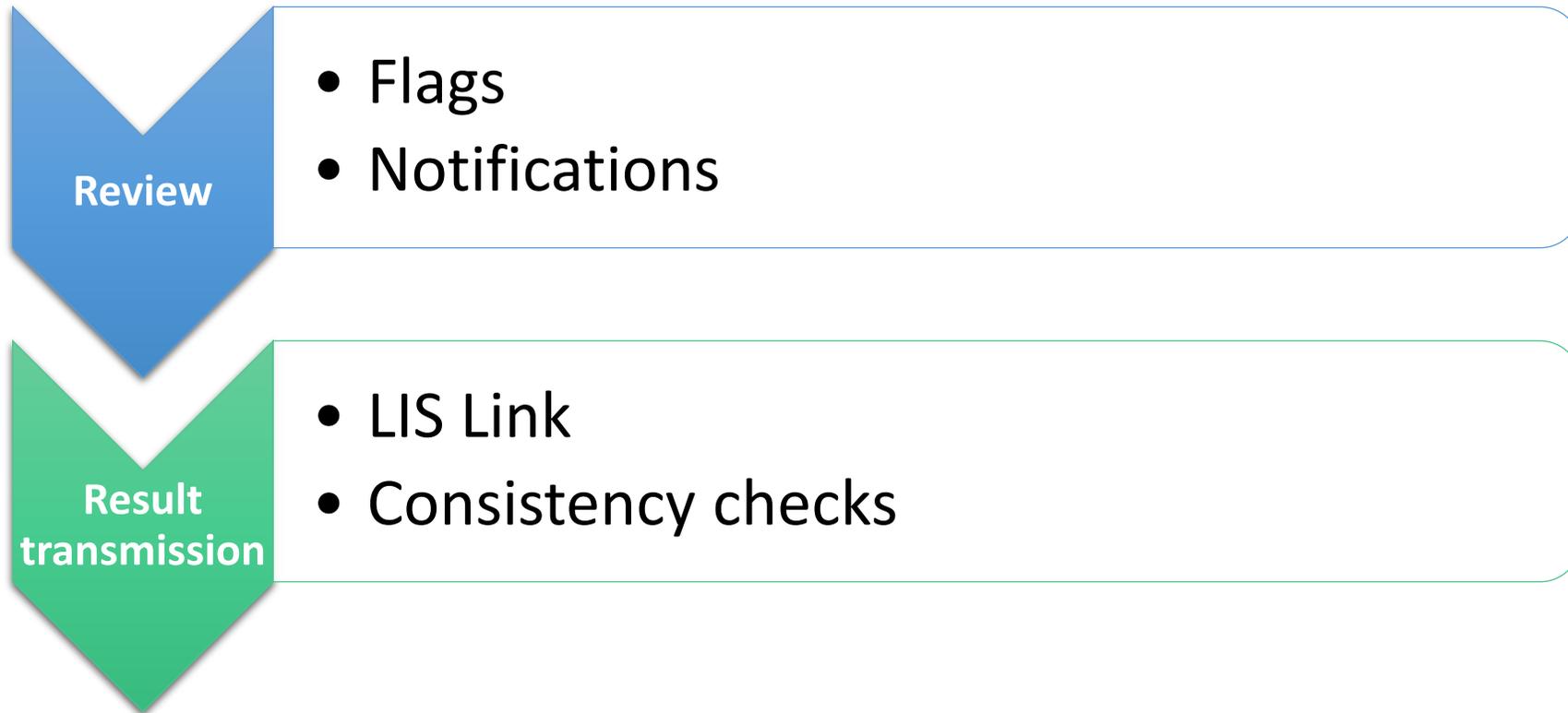# Flags and notifications

– Flags
  - Missing QC
  - High Count Rate
  - Over (Lyse) Incubation
  - Clog or Bubble
  - AQUIOS STEM-Count Mix Time Expired
  - Onboard Expiration
  - Expired Reagent

– Notifications
  - Less than 100 CD34+ events acquired

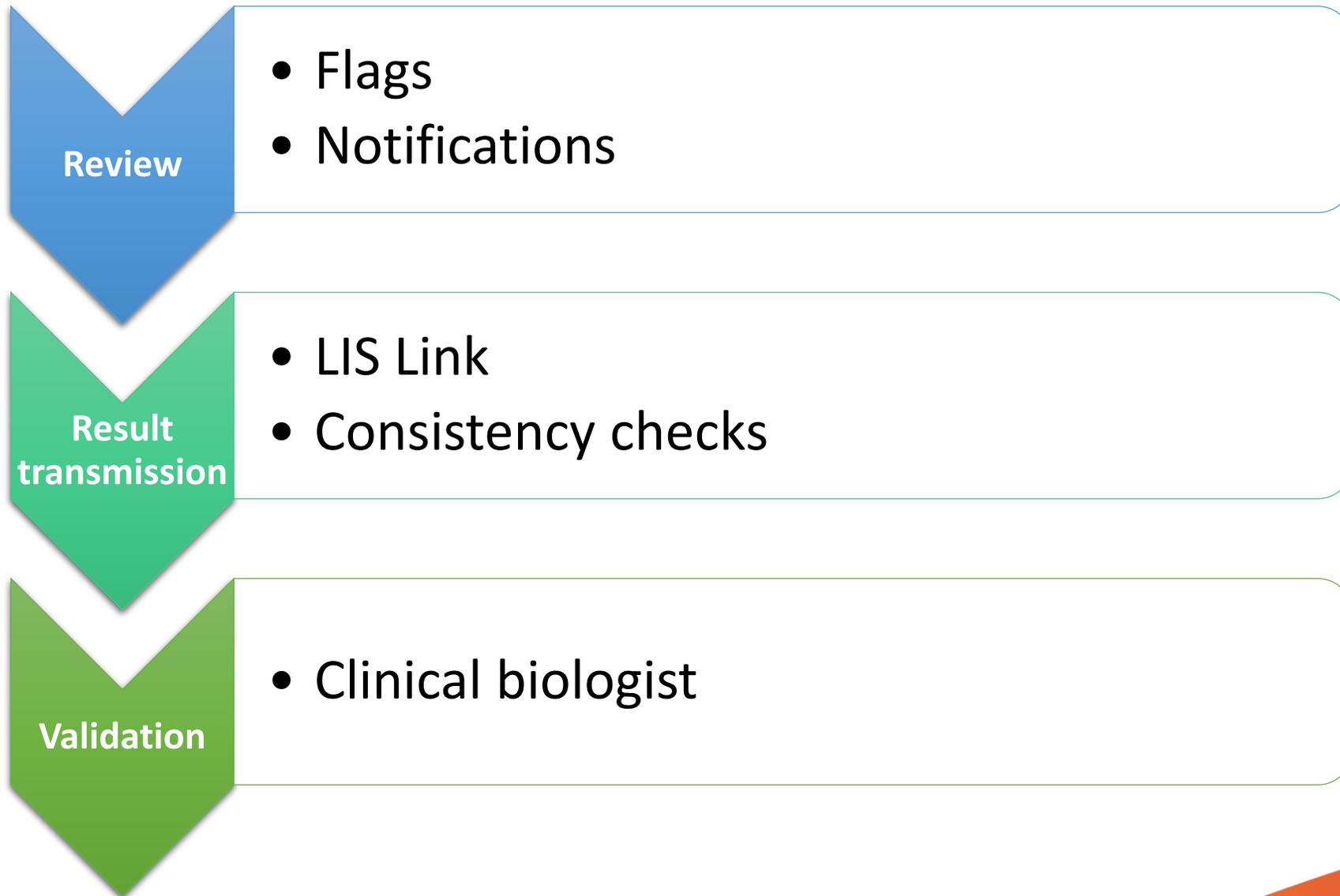# Result workflow

Review
- Flags
- Notifications

Result transmission
- LIS Link
- Consistency checks

azdelta

Consistency checks

– Leucocyte count
  - vs haematology analyser
  - <20%

– CD34+ stem cell duplicate measurement
  - <20%

Result workflow

Review
- Flags
- Notifications

Result transmission
- LIS Link
- Consistency checks

Validation
- Clinical biologist

azdelta

Advantages

– Reduced hands-on-time

– Reduced intra-personal variation

– Simple workflow
  ▪ Allows analysis by lab tech with limited flow cytometry experience
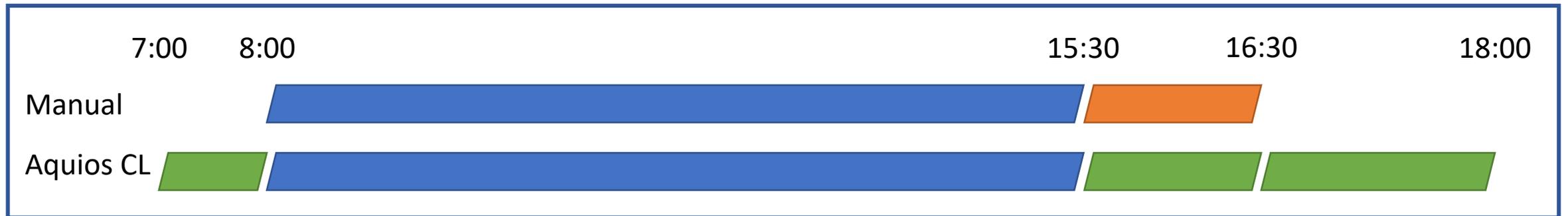
Questions?

jonathan.vanbesien@azdelta.be