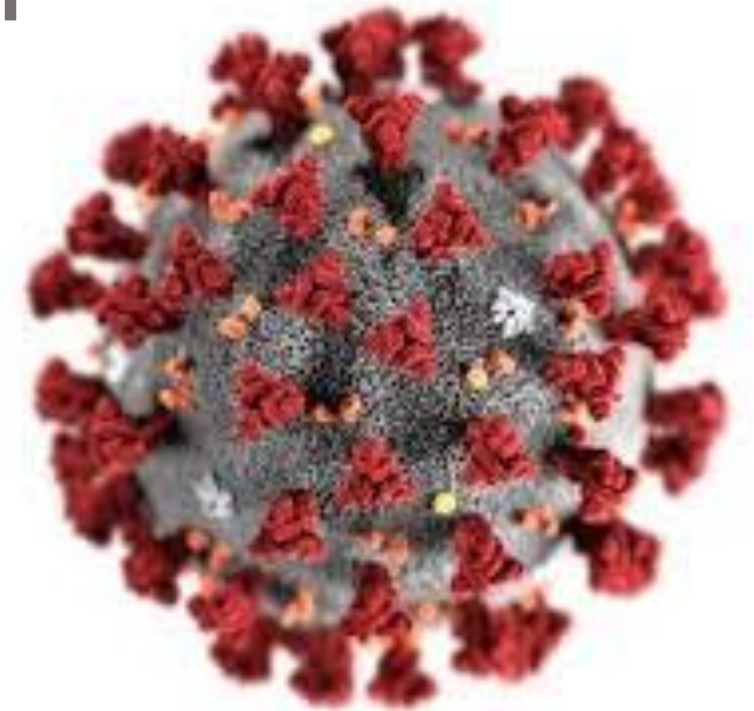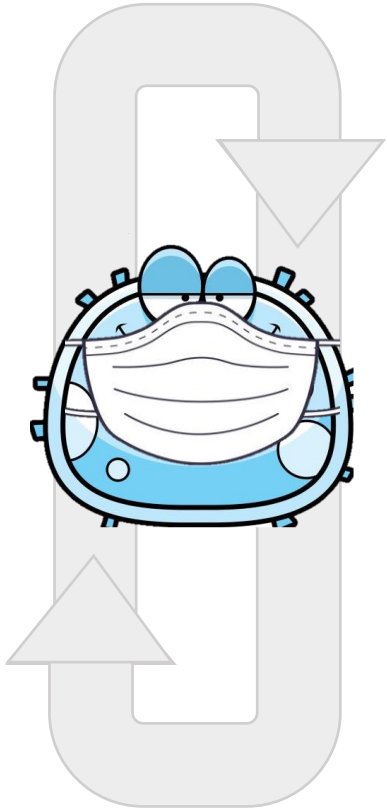# Safely prepare COVID-19 and infectious samples for vaccine research.

# How does Laminar Wash technology help our Vaccine customers?

"All technical procedures should be performed in a way that minimizes the generation of aerosols and droplets."

World Health Organization Laboratory biosafety guidance related to the novel coronavirus (2019-nCoV)

"Just Stop Making Aerosols"
Jack Dunne, Consultant at Miltenyi, Formerly with BD

How does Laminar Wash technology help our Vaccine customers?
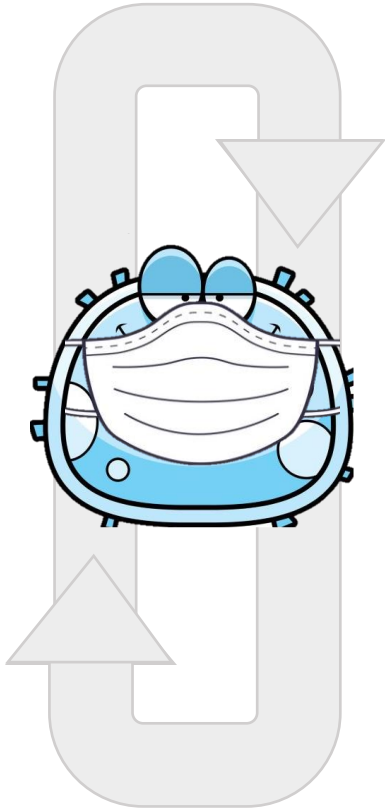
*Centrifugation is a High Risk Task to create aerosols*

TABLE 2. Risk prioritization of selected routine laboratory tasks				
Task or activity	**Exposure risk**			
	Potential hazard	**Likelihood**	**Consequence**	**Risk rating**
Subculturing blood culture bottle	Needle stick — percutaneous inoculation	Likely	Infection; medical treatment	High
	Aerosols — inhalation	Moderate	Infection; medical treatment	Medium
	Splash — direct contact with mucous membranes	Moderate	Infection; medical treatment	High
Centrifugation	Aerosols — inhalation	Likely	Infection; medical treatment	High
Performing Gram stain	Aerosols from flaming slides	Moderate	Colonization; infection	Moderate
Preparing AFB smear only	Aerosols from sputum or slide preparation	Likely	Illness; medical treatment; disease	High
Performing catalase testing	Aerosols — mucous membrane exposure	Unlikely	Colonization; infection	Low
AFB culture work-up	Aerosols — inhalation	Likely	Illness; medical treatment; disease	High
Abbreviation: AFB = acid-fast bacillus.				

From the CDC Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories - Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel

How does Laminar Wash technology help our customers?
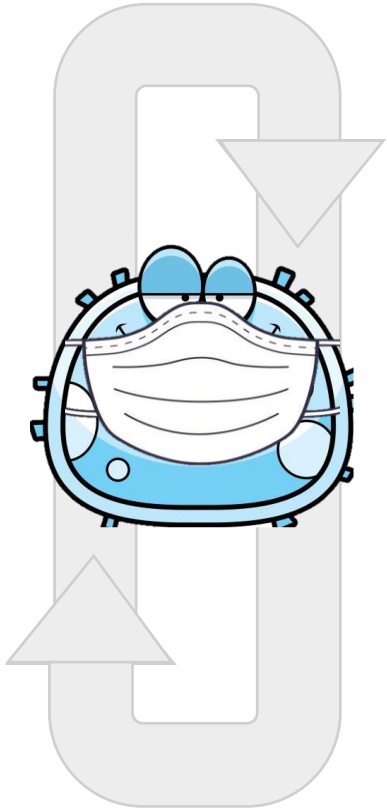
Retain

Biosafety: No centrifugal aerosols and enable safety hood installation.
Control: Eliminate plate flicking and cross contamination.
Sample quality: Cell viability, cell numbers and cell separation.

Reduce

Cell stress: Sample debris, cell clumping and cytometer clogging.
Data variation: And operator inconsistency.
Workflow steps: And time to results.

Repeat

And optimise: For multiple sample types, protocols and applications
And integrate: With laboratory automation.

"Just Stop Making Aerosols"
Jack Dunne, Consultant at Miltenyi, Formerly with BD

How does Curiox technology help our customers?

"The Laminar Washer is **safer than flicking (minimal exposure to infectious materials**) and requires less space than centrifuges. Our samples are fragile and prone to cell death but the LW promotes cell viability and stability and has **proven consistency** at low cell counts producing reliable and reproducible results and also **prevents cross-contamination** of samples. **It saves time, easy use and fewer lab techs are needed** and has proven operator consistency Inter & Intra-Plate. Requires minimal maintenance and is also cost effective."

**Dr Blanca Ponce-Ngo,
Montefiore Medical Center**

Proteona uses Laminar Wash™ by Curiox
For all of its clinical samples.

The Proteona workflow with Curiox Laminar Wash System

1 Binding Proteona Antibodies

2 Curiox Laminar Wash

3 Single Cell Barcoding of RNA and Protein

4 Sequencing and Analysis

5 Clinical report generation
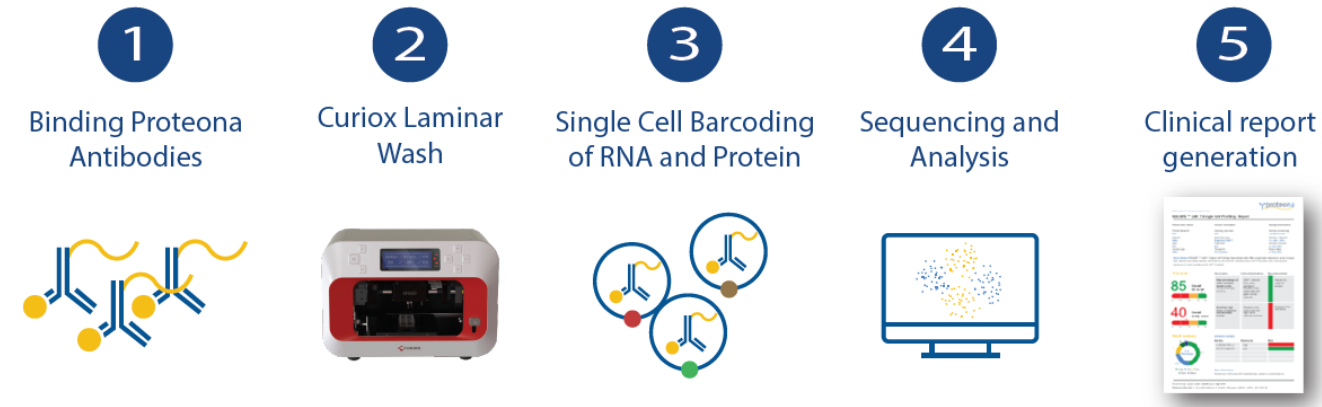
"At Proteona, we have turned to the Curiox Laminar Wash System to standardize our sample prep for single cell proteo-genomic analysis.

The mini-washer allows us to achieve consistent results with less hands-on time and provides superior cell retention compared to the centrifugation of samples".

Reduced cell loss

Laminar Wash System improved cell retention

% Cell retention vs Starting cell number per sample (1,000,000 / 200,000 / 40,000)

Legend: ■ Centrifuge ■ Laminar Wash
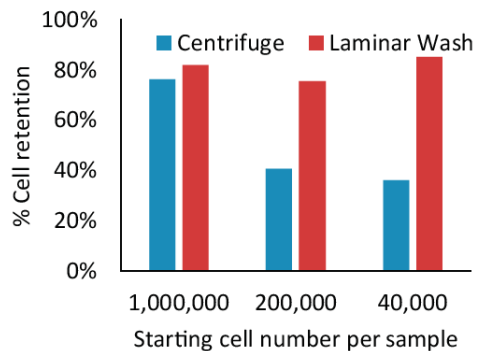
With low cell numbers often obtained in clinical samples, eliminating cell loss is key to successful experiments.

Proper cell washing leads to repeatable, high quality clinical data. Combined with high quality analysis tools, such as provided by MapCell™, data from small clinical samples can be unlocked.

High quality data

Laminar Wash System-prepared sample produces high quality single cell proteogenomics data

Legend:
- B Cells
- DCs
- Monocytes
- NK Cells
- CD4+ T Cells
- Plasma Cells
- CD8+ T Cells

proteona Precision Medicine

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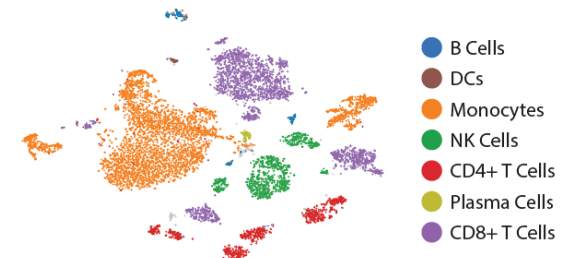
How does Curiox technology help our Vaccine customers?

"With the Curiox Laminar Wash™ we **retain more cells** with much **less data variation** between samples than our centrifuge process. The Laminar Wash retained populations that disappear in centrifugation. Significantly we also **reduce our hands-on time** and also reduce our antibody use by over 50% and still preserve signal and reproducibility. Using the Laminar Wash is a good way to get efficiency".
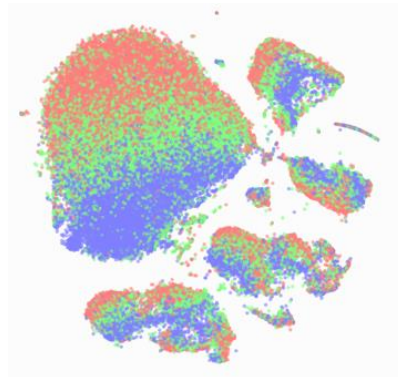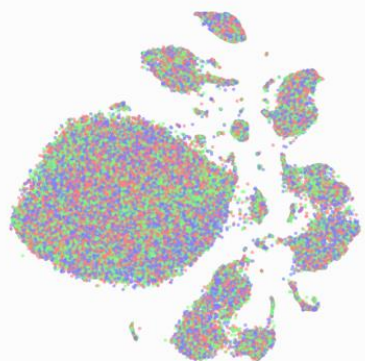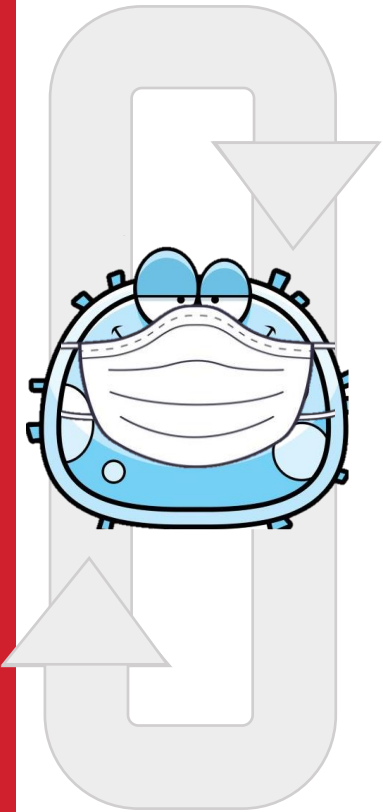
Dr Jorgen Adolfsson, Linkoping University

How does Curiox technology help our customers?

"The Laminar Wash is now part of our workflow in analytical development and all of our subsequent flow cytometry assays will be developed using the Laminar Wash and then transferred to QC. We now load a plate, push a button and walkway. **The Laminar Wash's performance allows us to get very tight control over populations with very tight CV's** largely due to the fact of the clear separations between populations. The maintained ability to detect rare populations is important as we detect the next round of cell therapy products".
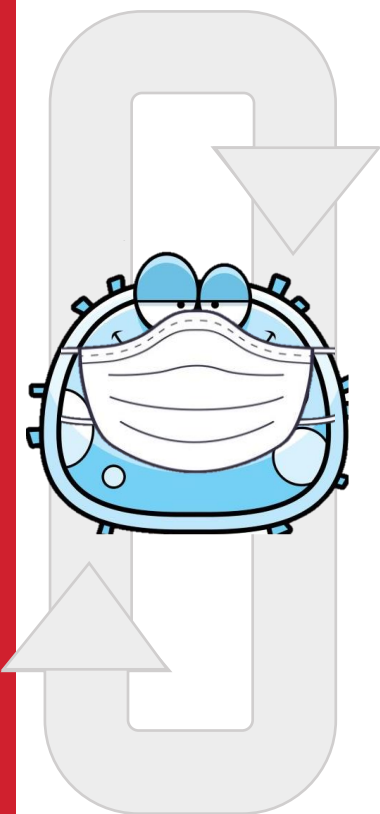
Dr Arnaud Colantonio.
Associate Director Analytical development. **AdiCet Bio, Now with Kite Biopharm**

How does Curiox technology help our customers?

 "We can now do a high throughput inhibitor screen to identify new mediators of TCR signaling. A problem we had before (with centrifugation in the workflow) was trying to gate on the correct populations which would correspond to the different thymocyte developmental stages especially since some of our critical antigens have very low cell surface expression. **Now with the Laminar Washer™ (relative to centrifugation), we are now getting higher MFI values allowing us to gate our populations better and also allowing for automation.**

We also use the washer in testing how long IL-7 induced STAT5 phosphorylation will persist after IL-7 has been removed. **We are now removing the IL-7 much more efficiently yielding the real biology** relative to what we see.

Centrifugation sample prep is now an experimental artifact and **now we are getting three times as many washes in one tenth of the time**. (Centrifugation-3 washes in 20 minutes, now the Laminar Washer™ 9 washes in just two and half minutes)."

Dr. Joanna Brzostek
Dr Chen Weihua Elijah.
Prof Nicholas Gascoigne's Lab

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What is actually happening in Laminar Wash?

Laminar Wash™ Technology for Flow Cytometry

WATCH ON YOUTUBE

HT1000 Laminar Wash Promotes Biosafety, Improves Reproducibility

- No aerosolization or flicking
  - Safer and reduces chance of cross contamination

- Improved workflow with less hands-on time
  - Multiple steps with centrifuge reduced to One

- Improved reproducibility and consistency

- Better debris removal

- No pelleting of cells
  - Reduces clumping clogging of cytometer

- Faster and more complete washing
  - Higher stain index for better resolution of populations

The Auto1000 –
Safely prepare samples for flow cytometry

- Off the shelf ready to use
  - Eliminate development time/cost

- GUI designed for Flowcytometry Protocols
  - Easy to use and program

- Less manual sample handling
  - Safer, more time left for analysis

- For Surface stains and ICS

- Fully Automated

- Electronic Reports for Sample Tracking

Curiox Biosystems and STEMCELL Technologies Announce New Method to Prepare Immune Cell Populations in COVID-19 Samples

A new configuration of the Laminar Wash™ HT1000 System packaged with STEMCELL Technologies' EasySep™ RBC Depletion Reagent.

- Containment of blood sample processing within a biosafety cabinet from start to finish

- Washing without centrifugation, eliminating aerosolization of potentially infectious samples

- Superior data analysis because cells are not stressed by lysis or centrifugation and show improved staining index
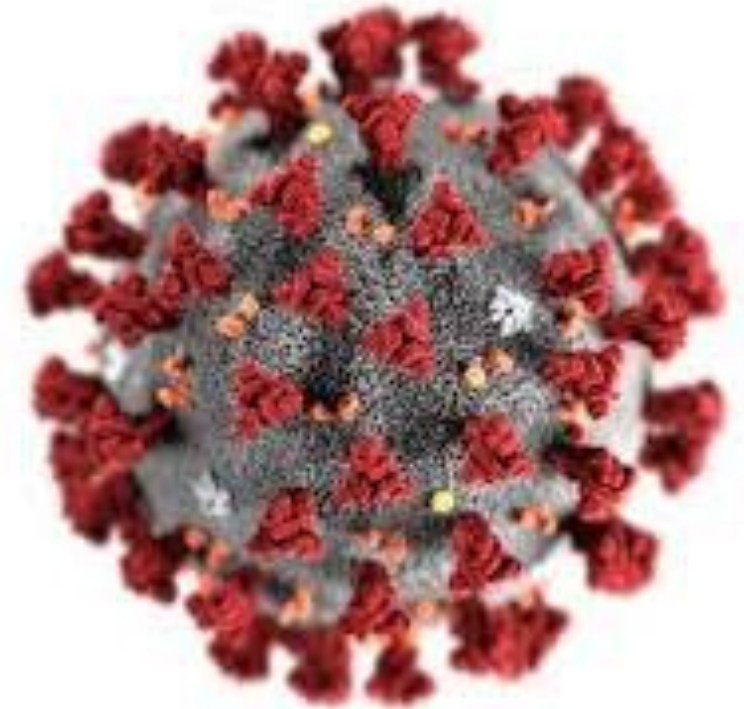
Materials and Reagents Needed

	Material
1	EasyPlate magnet (STEMCELL Technology)
2	96-well U-bottom plate (non-tissue culture treated)
3	Plate vortexer
4	Tube vortexer
5	Laminar Wash LW96 plate
6	LW96 Big Volume Lid

	Reagents
1	EasySep RBC Depletion Reagnet (STEMCELL Technology)*
2	PBS containing 2% FBS and 12mM EDTA
3	Human whole blood
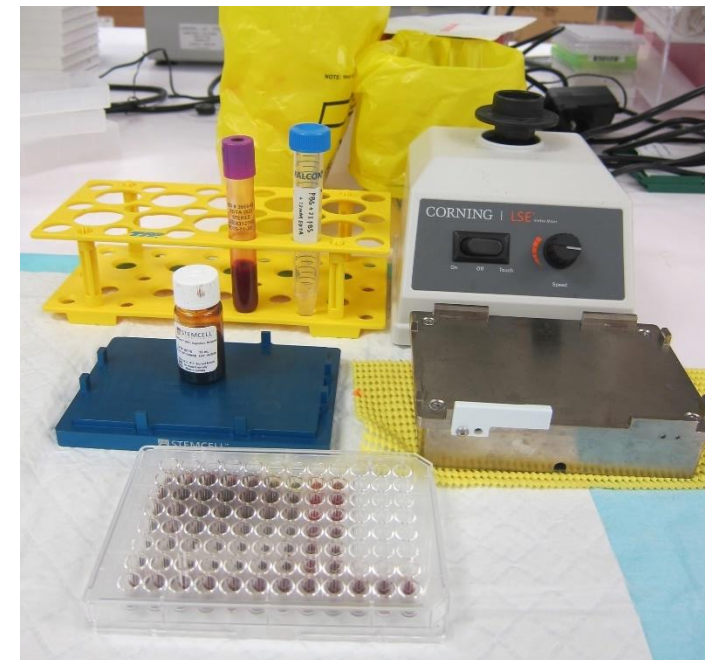
*antibody-based magnetic beads – only for human whole blood

Protocol for Handling Samples within a Biosafety Cabinet
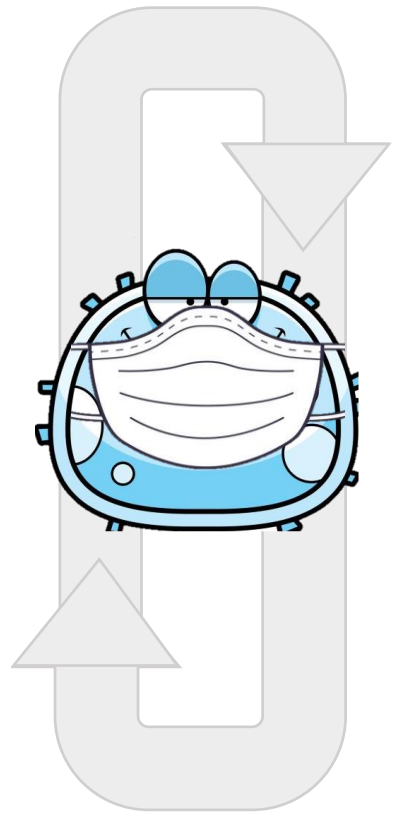
Procedure
Prepare sample – add 100uL FACS buffer containing EDTA into each well of 96-well U-bottom plate. Add 100uL whole blood. Vortex 3 seconds to mix.
Vortex RBC Depletion Beads thoroughly for 30s. Transfer 5uL of beads into each well. Vortex 3 seconds and immediately transfer to EasyPlate magnet without plate lid. Incubate 3 min.
Using pipette set to 150uL, carefully remove supernatant and transfer to fresh well in plate.
Vortex RBC Depletion Beads. Transfer 5uL of beads into each well. Vortex 3s and immediately transfer to Easyplate magnet. Incubate 3 min.
Prepare LW96 with Big Volume Lid
Using pipette set to 150uL, carefully remove supernatant and transfer to new well in LW96. Incubate at least 40min for settling. Wash 9x with HT1000.

Magnetic depletion of erythrocytes with LW gave clear resolution of cell populations
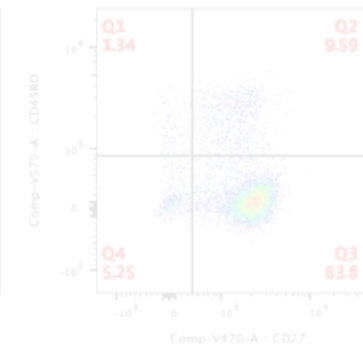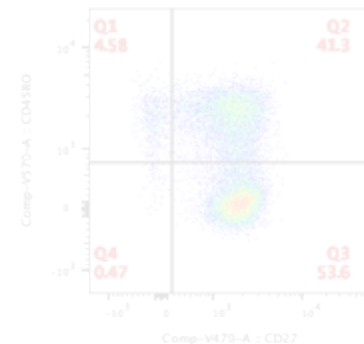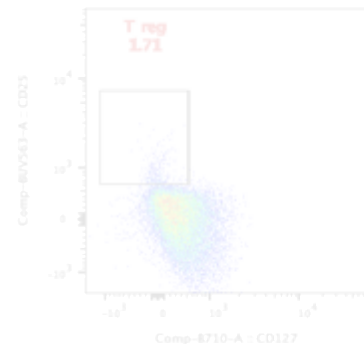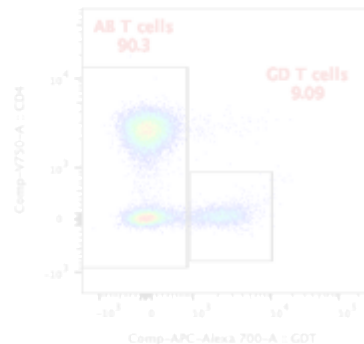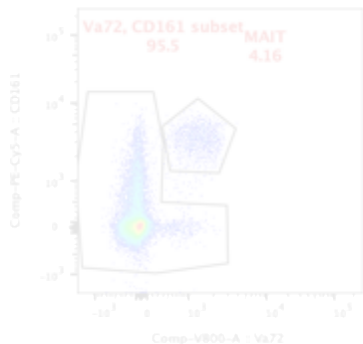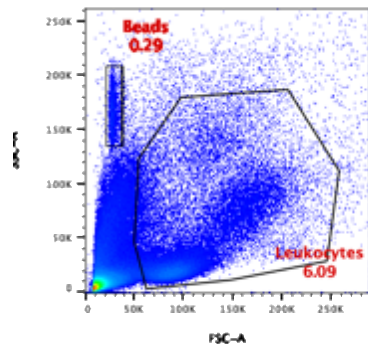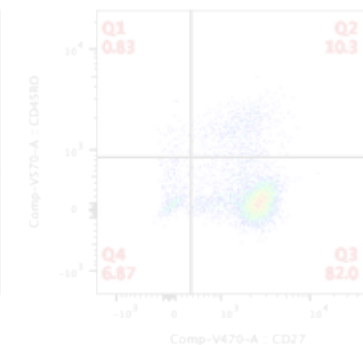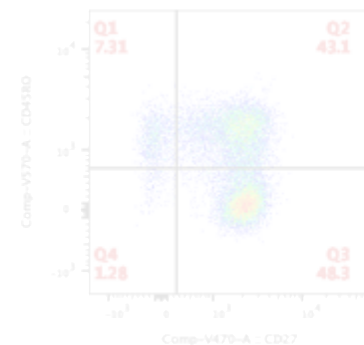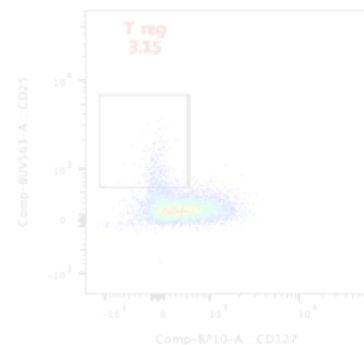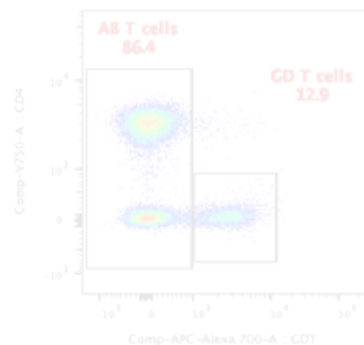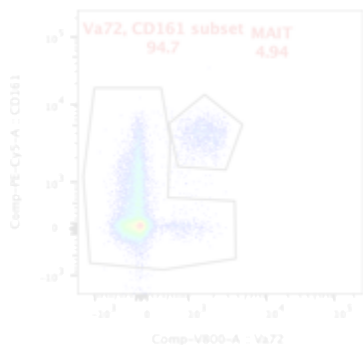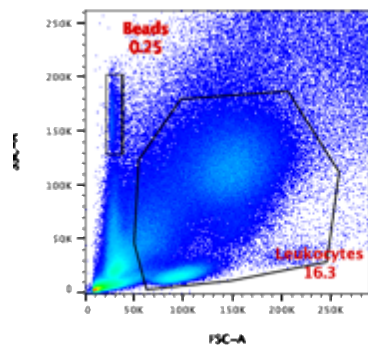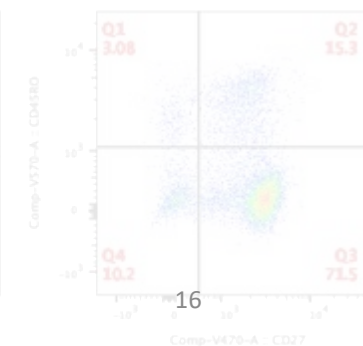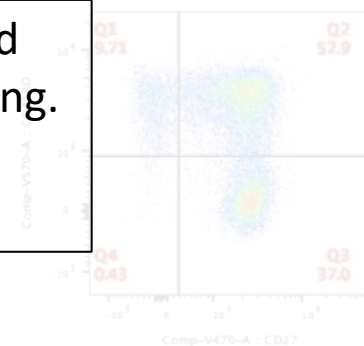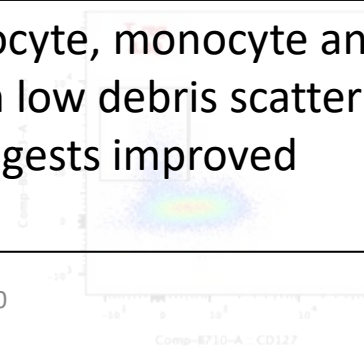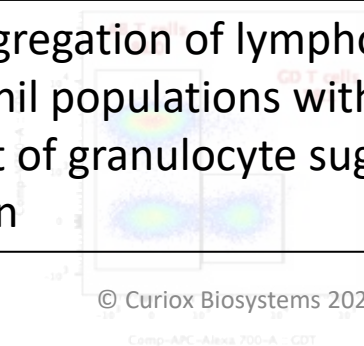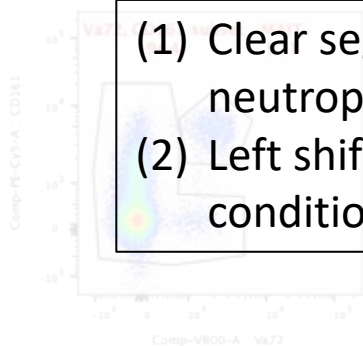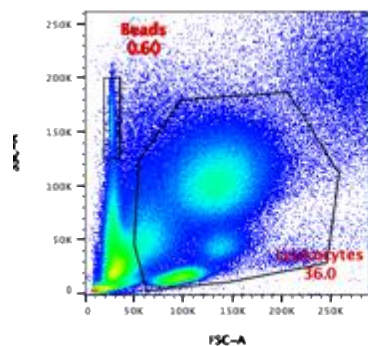
(1) Clear segregation of lymphocyte, monocyte and neutrophil populations with low debris scattering.
(2) Left shift of granulocyte suggests improved condition

Magnetic depletion of erythrocytes with LW gave clear resolution of cell populations


Parent gate | All events | Single, live CD3$^+$ | Va7.2$^-$ | $\alpha\beta$ T cells | CD4$^+$ T cells | CD8$^+$ T cells
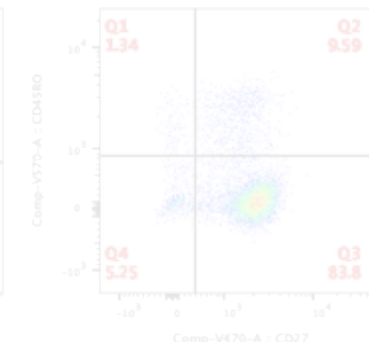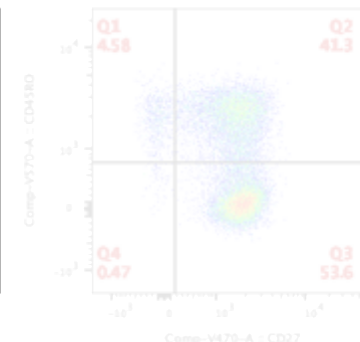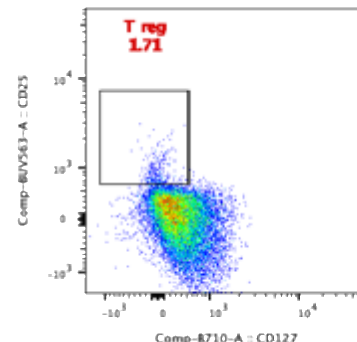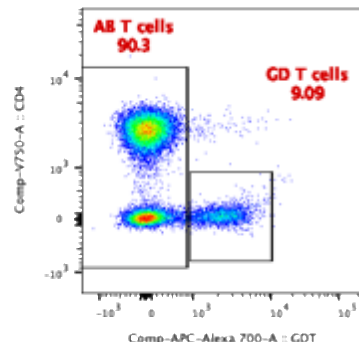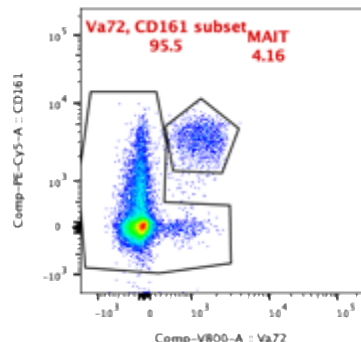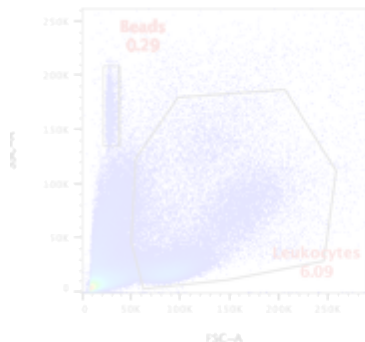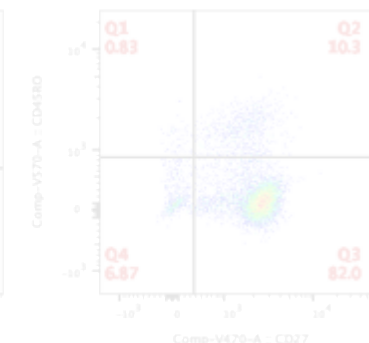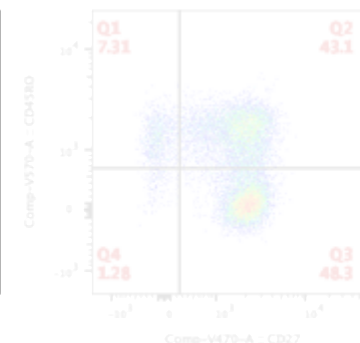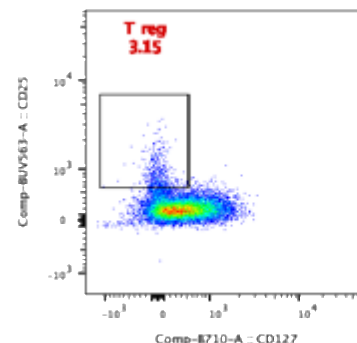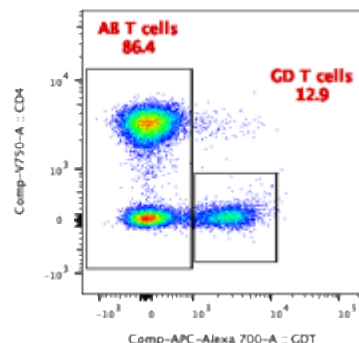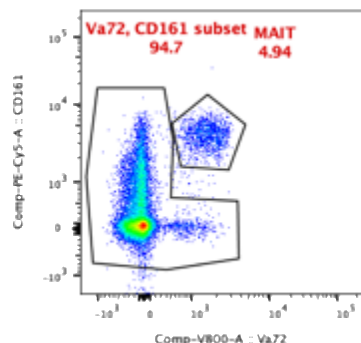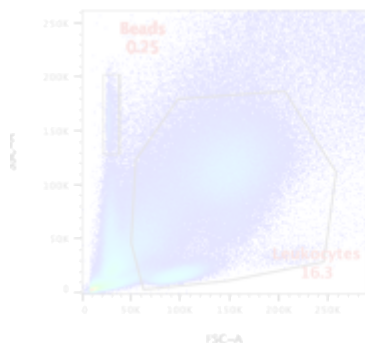
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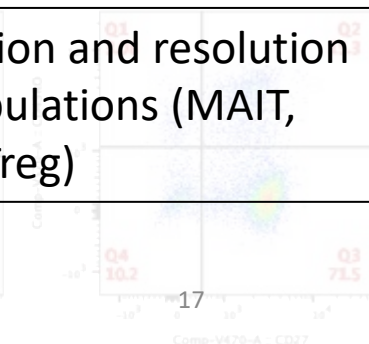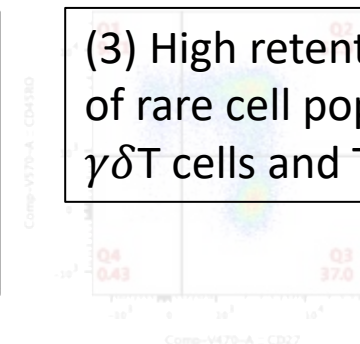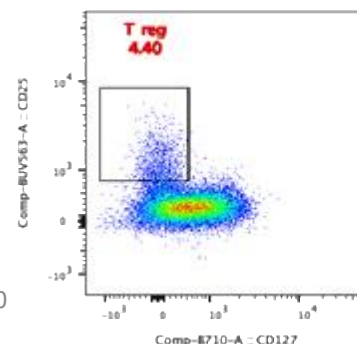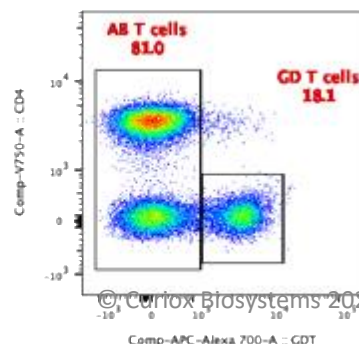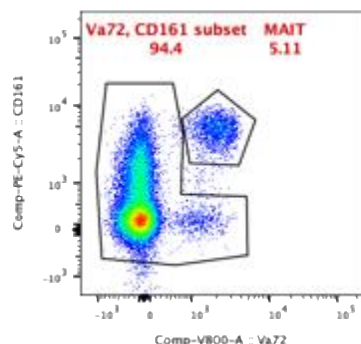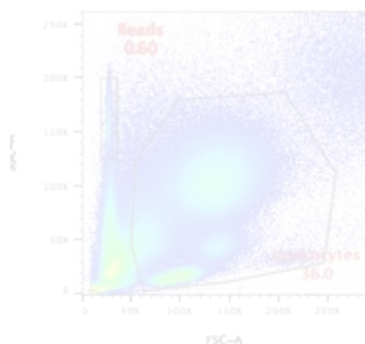
(3) High retention and resolution of rare cell populations (MAIT, $\gamma\delta$T cells and Treg)

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Magnetic depletion of erythrocytes with LW gave clear resolution of cell populations

Parent gate	All events	Single, live CD3$^+$	Va7.2$^-$	$\alpha\beta$ T cells	CD4$^+$ T cells	CD8$^+$ T cells
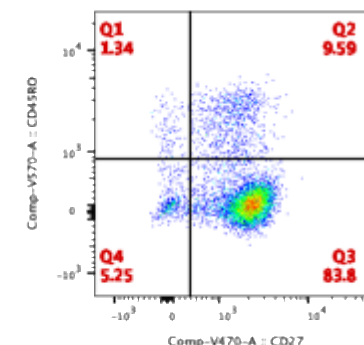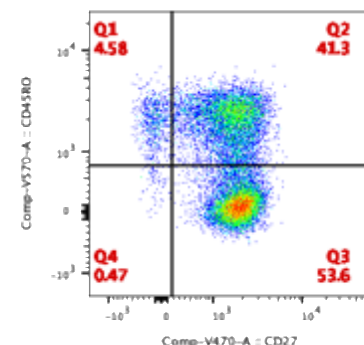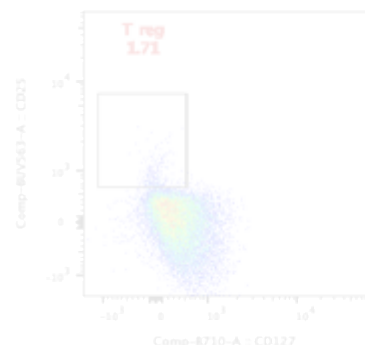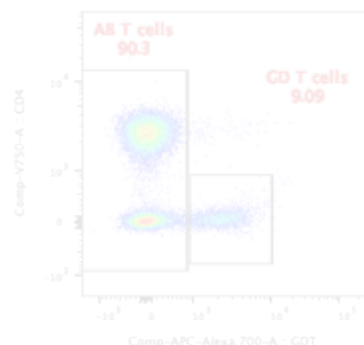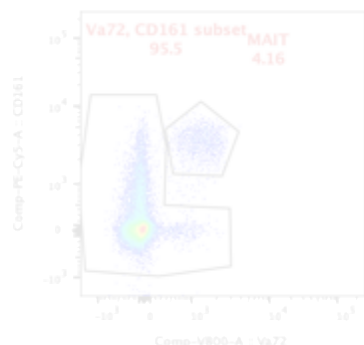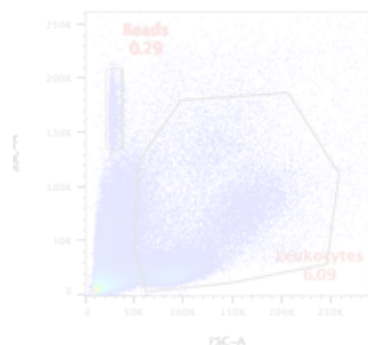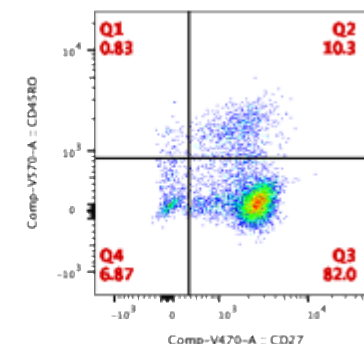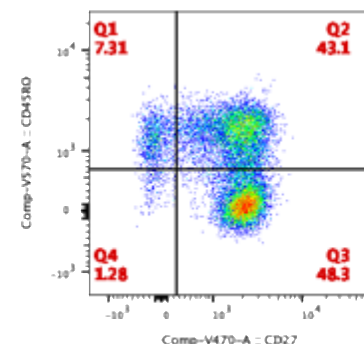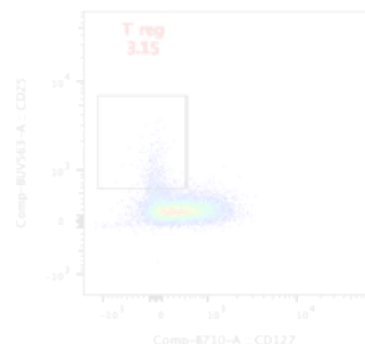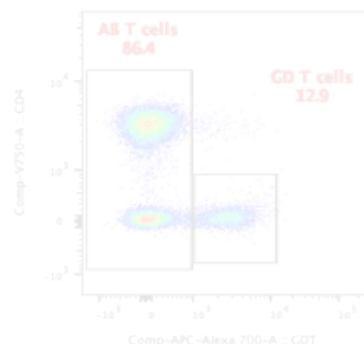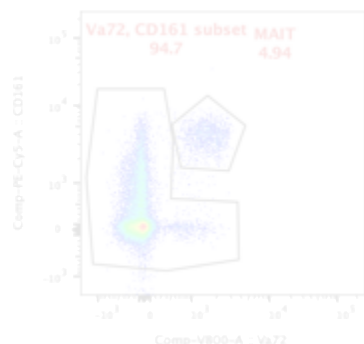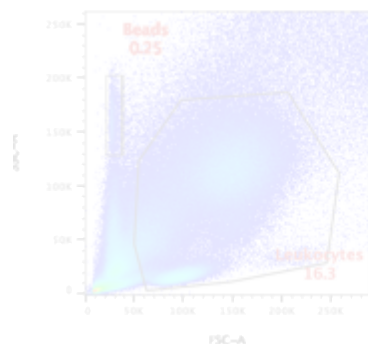
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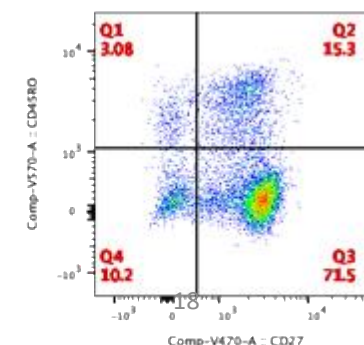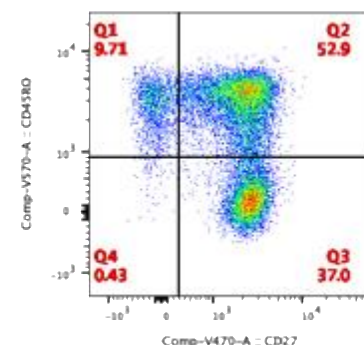
(4) High staining index enables better resolution of activation markers

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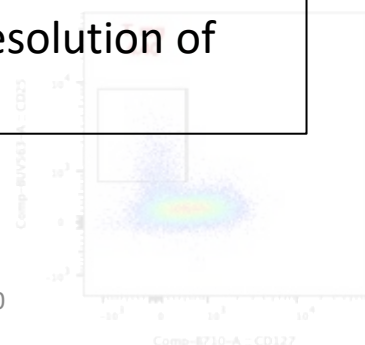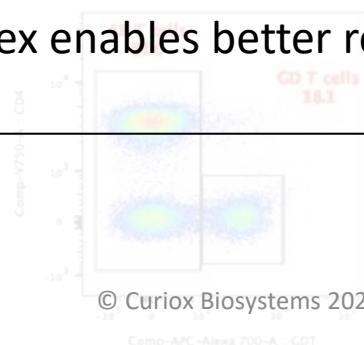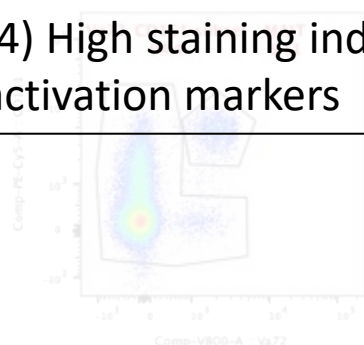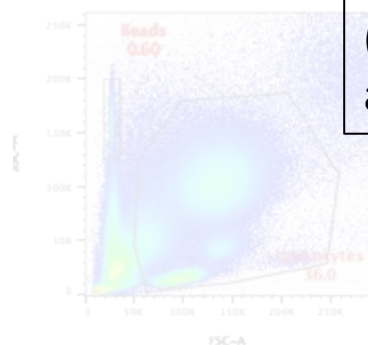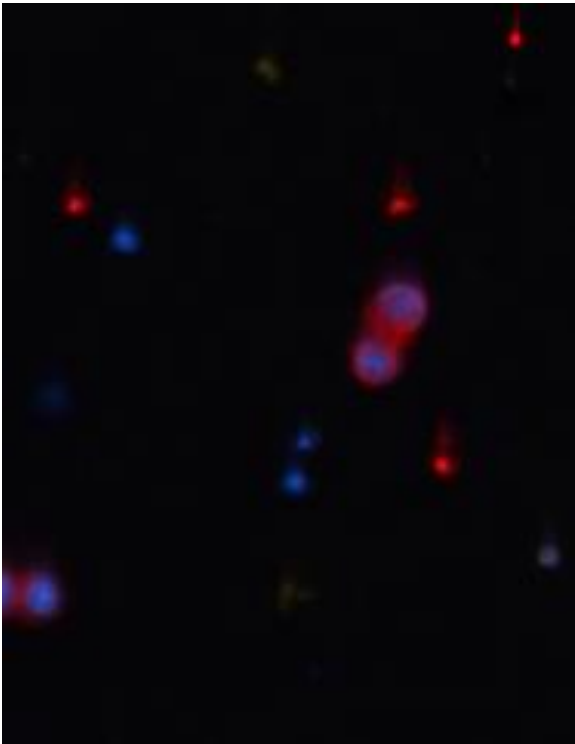# Centrifugation causes Cell Damage and Bias in analysis

Zoom: 20X, CKs/EpCAM/Nuclear Acid Dye
RFP: Light 100 Exp: 150ms   Gain: 0 db (red)
DAPI: Light 100 Exp: 70ms   Gain: 0 db (blue)

Cells processed by centrifugation
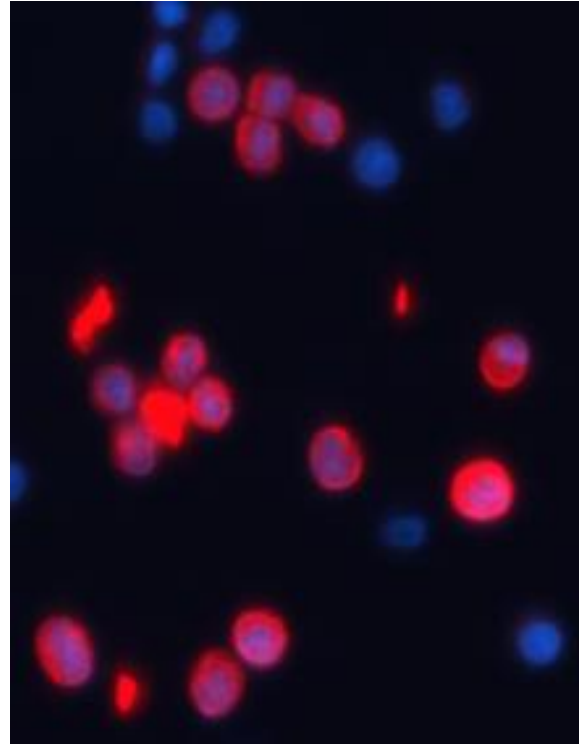
Cells processed by Laminar Wash, no centrifugation

- 50-100 cells per well

- Cells from Laminar Wash method demonstrate much stronger fluorescence signal and intact morphology of cells and fewer debris

- Centrifugation presumably causes significant stress on cells distorting its physiology and protein expression

Data from a biotech company in San Diego

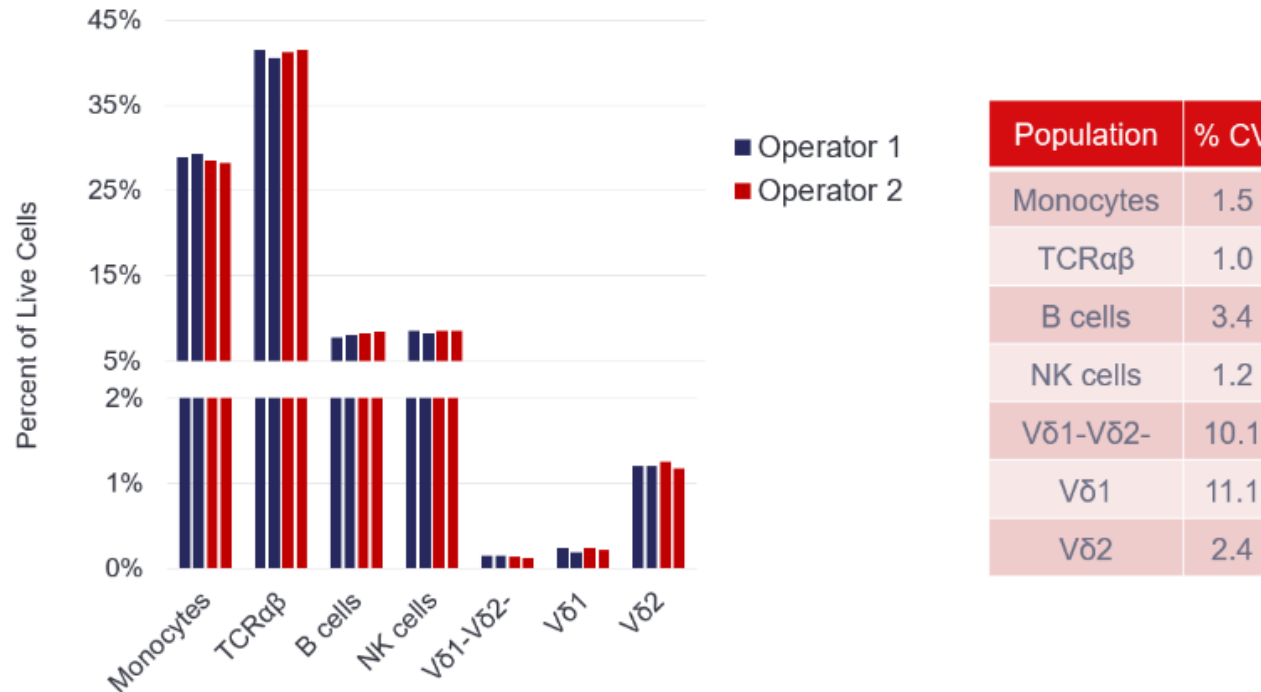# Example of Improving Consistency at Adicet by Automating a wash step only with LW HT

Multiple Operator - Replicates

Population	% CV
Monocytes	1.5
TCRαβ	1.0
B cells	3.4
NK cells	1.2
Vδ1-Vδ2-	10.1
Vδ1	11.1
Vδ2	2.4

"DA-Cell allows consistent analysis of rare cells less than 1 % population over and over"

Adicet is a biotech company developing a novel CAR T cell therapy.

A slide from a webinar by Dr. Arnaud Colantonio, associate director of process development, available at www.curiox.com

Laminar Wash technology <u>does</u> help our Vaccine customers!
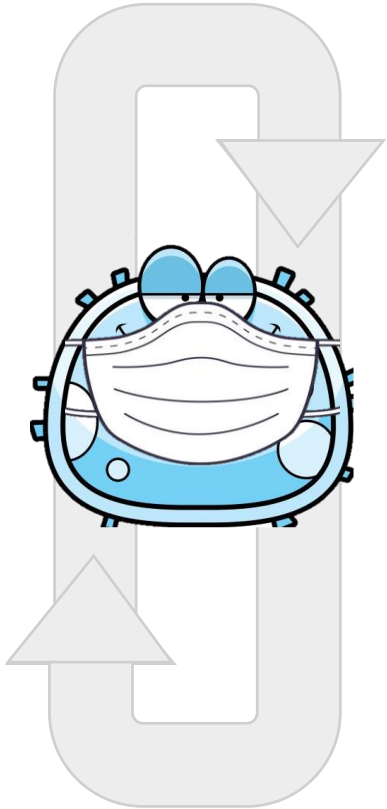
Retain

Biosafety: No centrifugal aerosols and enable safety hood installation.
Control: Eliminate plate flicking and cross contamination.
Sample quality: Cell viability, cell numbers and cell separation.

Reduce

Cell stress: Sample debris, cell clumping and cytometer clogging.
Data variation: And operator inconsistency.
Workflow steps: And time to results.

Repeat

And optimise: For multiple sample types, protocols and applications
And integrate: With laboratory automation.

Thank you!