



**DRUG DEVELOPMENT
SOLUTIONS**
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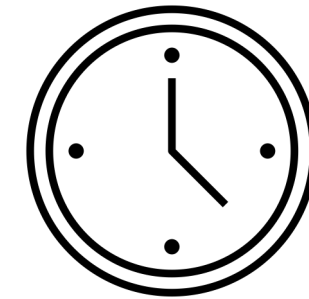
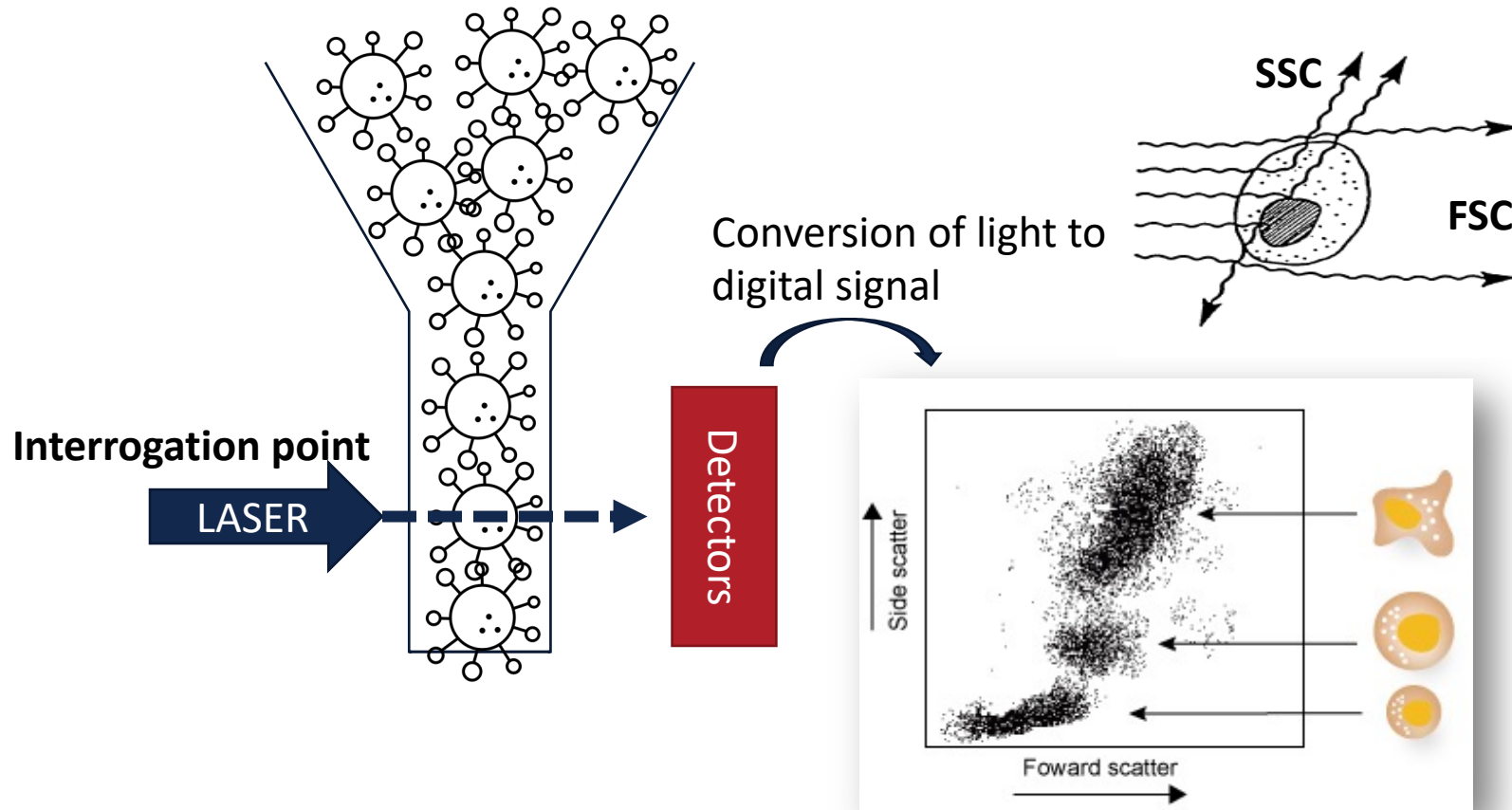
How can we use automation to streamline flow cytometry processes?

By Zoe Georgakopoulou

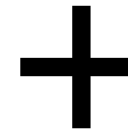


Streamlining flow cytometry

Flow Cytometry - Why is automation needed?



TIME

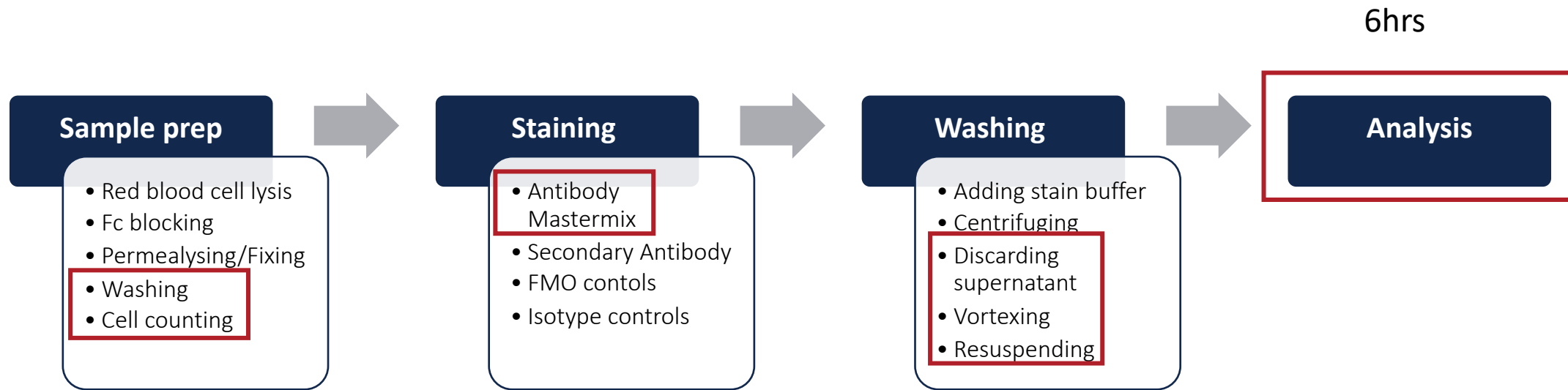


**Inter-analyst
VARIATION**

Typical Workflow



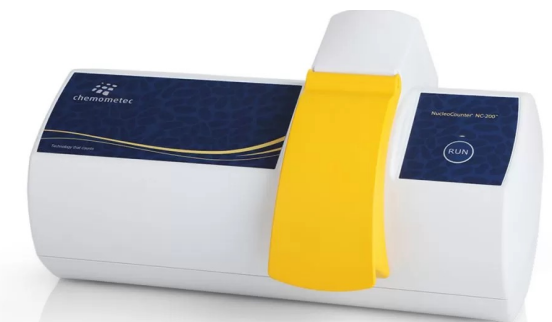
ANALYST VARIATION





1. Automated cell counting

	Manual cell counting	Automated cell counting
Time	>30min	<2min
Accuracy	Subjectivity in defining cells	Objective
Analyst variation	CV>15% calculation errors	Low CV<5%
Viability staining	Trypan blue	DAPI and Acridine orange
Compliance	Manual counts recorded	Able to digitally record and store data
Sample volume	10 μ L	200μL



Automated cell counting – GCP compliant

Technology that counts

chemometec

NucleoCounter® NC-200™ Report

File name: 20220322-0007-c-LGC339240QB10_PBMCI_unstim.cm

Protocol: Viability and Cell Count - Mononuclear Cells (Viability and Cell Count Assay)

Purpose: Cell Count and Viability with AO and DAPI (Viability and Cell Count Assay)

NucleoView NC-200 version 1.3.0.0

Login ID: zoe.georgakopou Operator: zoe.georgakopou @INS-NUCLEO Instrument: 9000201031801895 Time: 2022-03-22 11:44:01

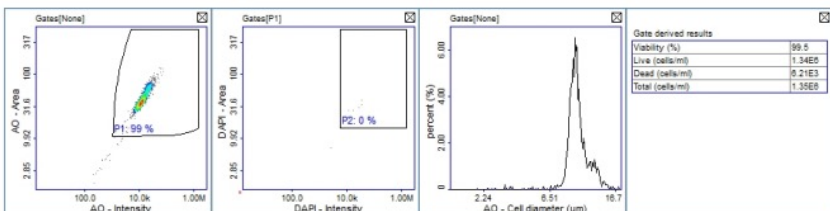
Image 1 of 1: All cells (AO) Dead cells (DAPI)



Viability (%)	99.5
Live (cells/ml)	1.34E6
Dead (cells/ml)	6.21E3
Total (cells/ml)	1.35E6

Sample Volume (ul)	200.0
Dilution Volume (ul)	0.000
Multiplication factor	1.000

Estimated cell diameter (um)	9.5
Cell diameter standard deviation (um)	4.5
(%) of cells in aggregates with five or more cells	1



Audit trail:

- Operator and time
- Protocol used
- Cell counts
- Gating

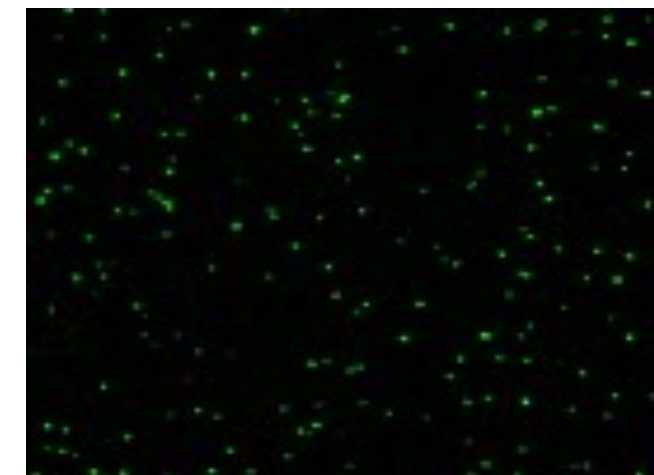


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DAPI stains dead cells



AO stains nucleated cells



2. Plate washer



	Manual pipetting	Plate washer
Time	>5min	<2min
Technical skill	High- must not touch cell pellet	Low – needle height is set
Variation	Variable	Uniform residual volume

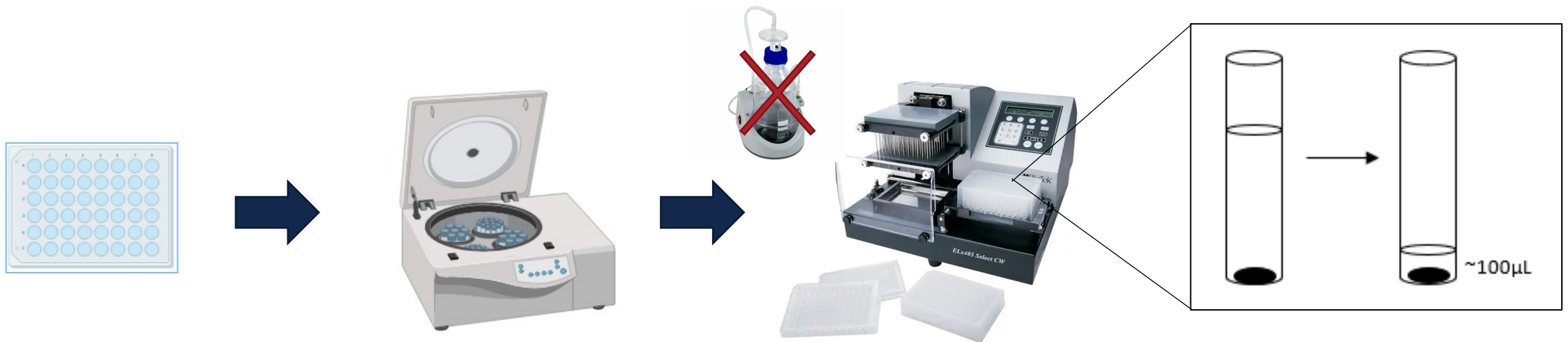
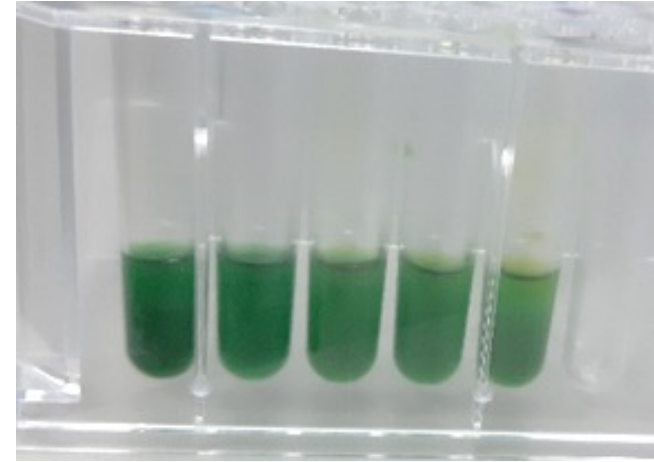


Plate washer - improvement in precision and accuracy



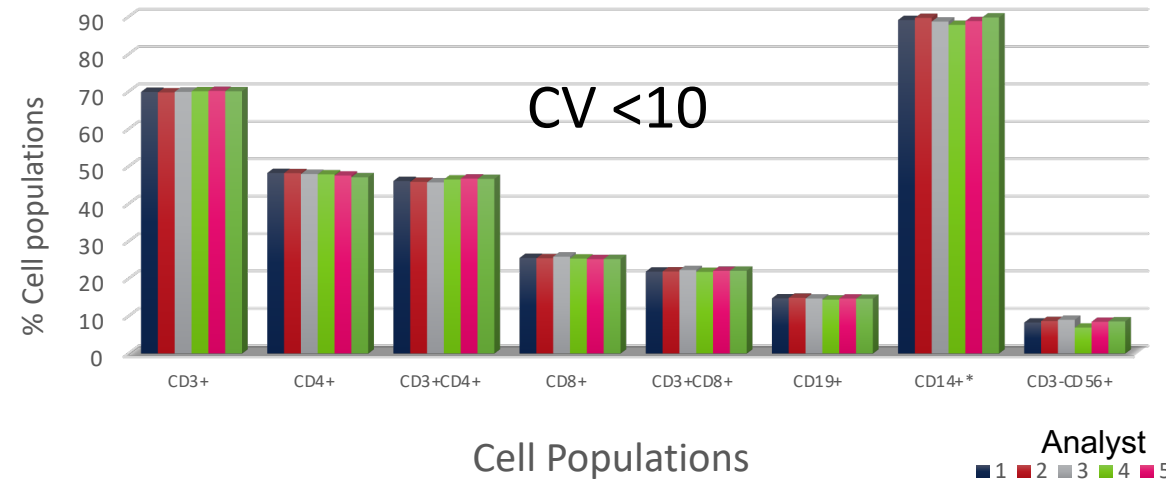
Experiment overview:

- Immunophenotyping method
- Immuno-trol samples
- 6 analysts
- Over a couple of days
- 2 flow cytometers

7587251	LOT	Unit			
2022-09-28	🕒	US		SI 2	
		%+	±	%+	±
CD2+	LY %	76	8	0.76	0.08
CD3+	LY %	70	9	0.70	0.09
CD4+	LY %	44	5	0.44	0.05
CD3+/CD4+	LY %	43	9	0.43	0.09
CD5+	LY %	73	12	0.73	0.12
CD8+	LY %	29	7	0.29	0.07
CD3+/CD8+	LY %	25	6	0.25	0.06
CD14+	MO %	85	20	0.85	0.20
CD19+	LY %	16	5	0.16	0.05
CD45+	LY %				
CD3-/CD56+	LY %	13	4	0.13	0.04

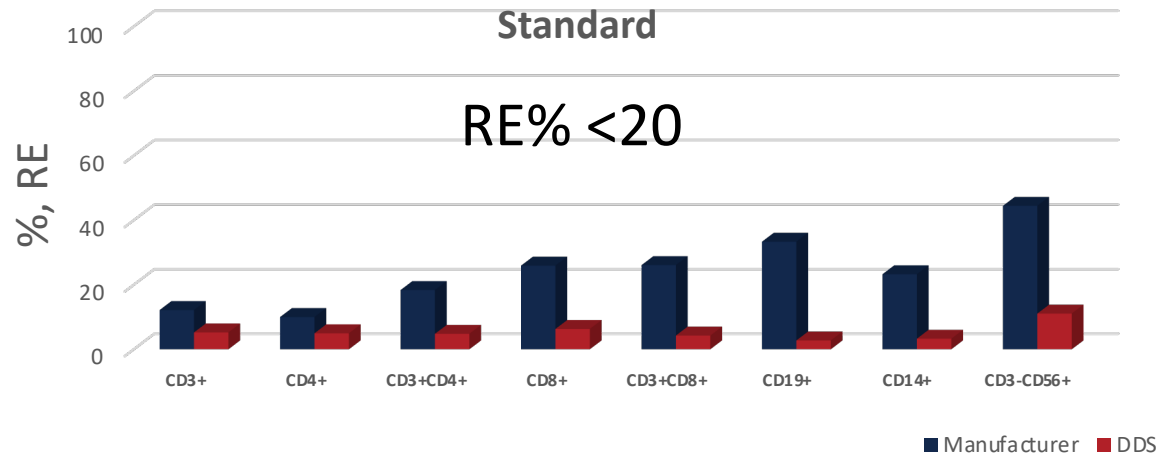
Immuno-trol cells: number of cells in specific populations are within specified ranges

Overall Precision of Cell Populations



Cell Populations

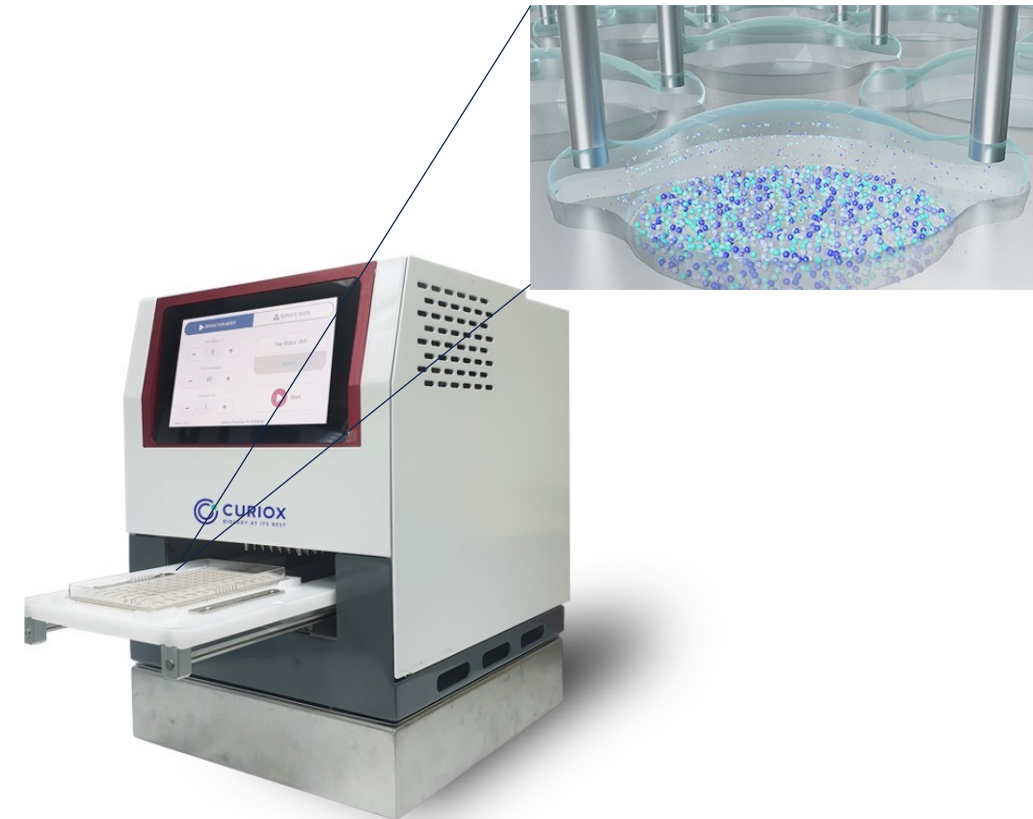
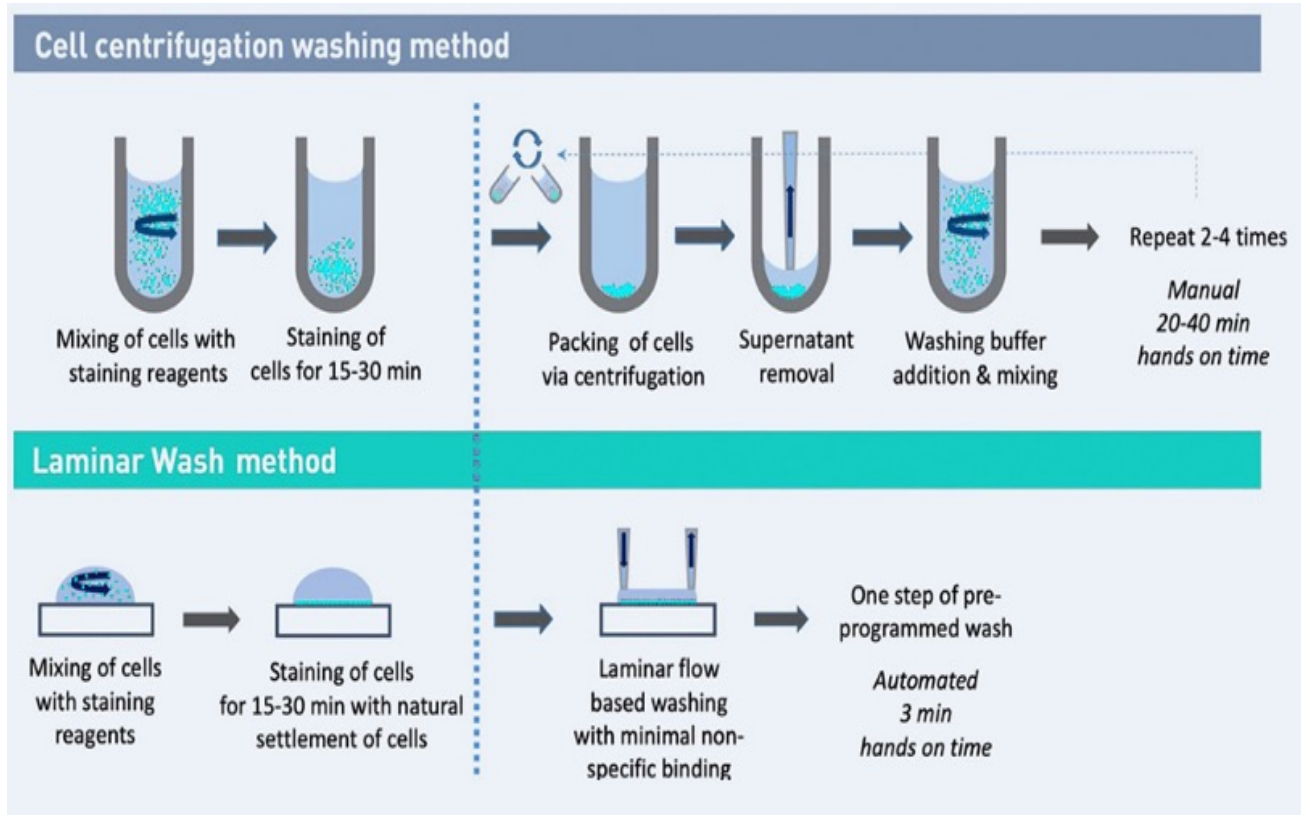
Overall Accuracy compared to Commercial Cell Standard



Can we fully automate washing?



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3. Hamilton – risk-free antibody mastermix

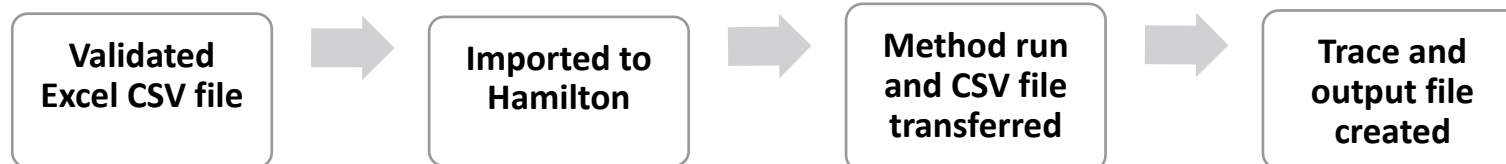


Case study:

- 4 panels
 - 32 antibodies
 - 5 reagents require pre-dilution
- = 3 hrs manually**

62 pipetting steps!

	Manual pipetting	Hamilton
Time	3hrs	20min
Reproducibility concern	High – analyst variation	Low - CV<0.5
Risk of error	High	Low – RE%<1 Use of barcodes
Risk of RSI	High	No risk
Compliance	Pipettes recorded on paperwork	Trace file created (Excel)

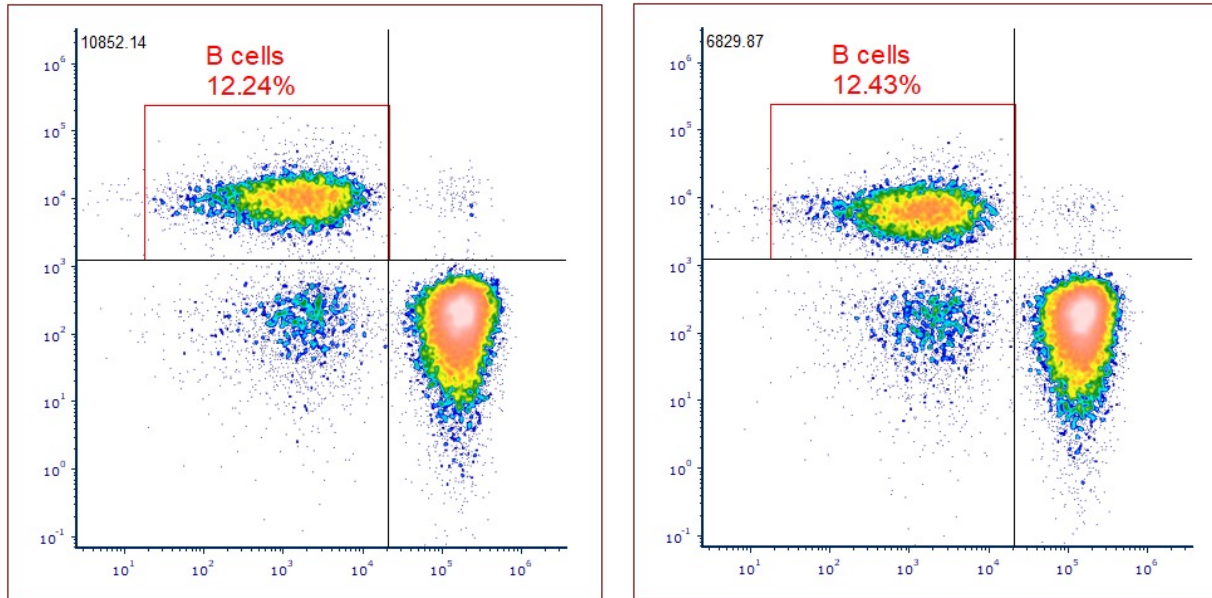


Hamilton data

Manual Antibody MM

Hamilton Antibody MM

APC CD19



BB515 CD3

Comparable results

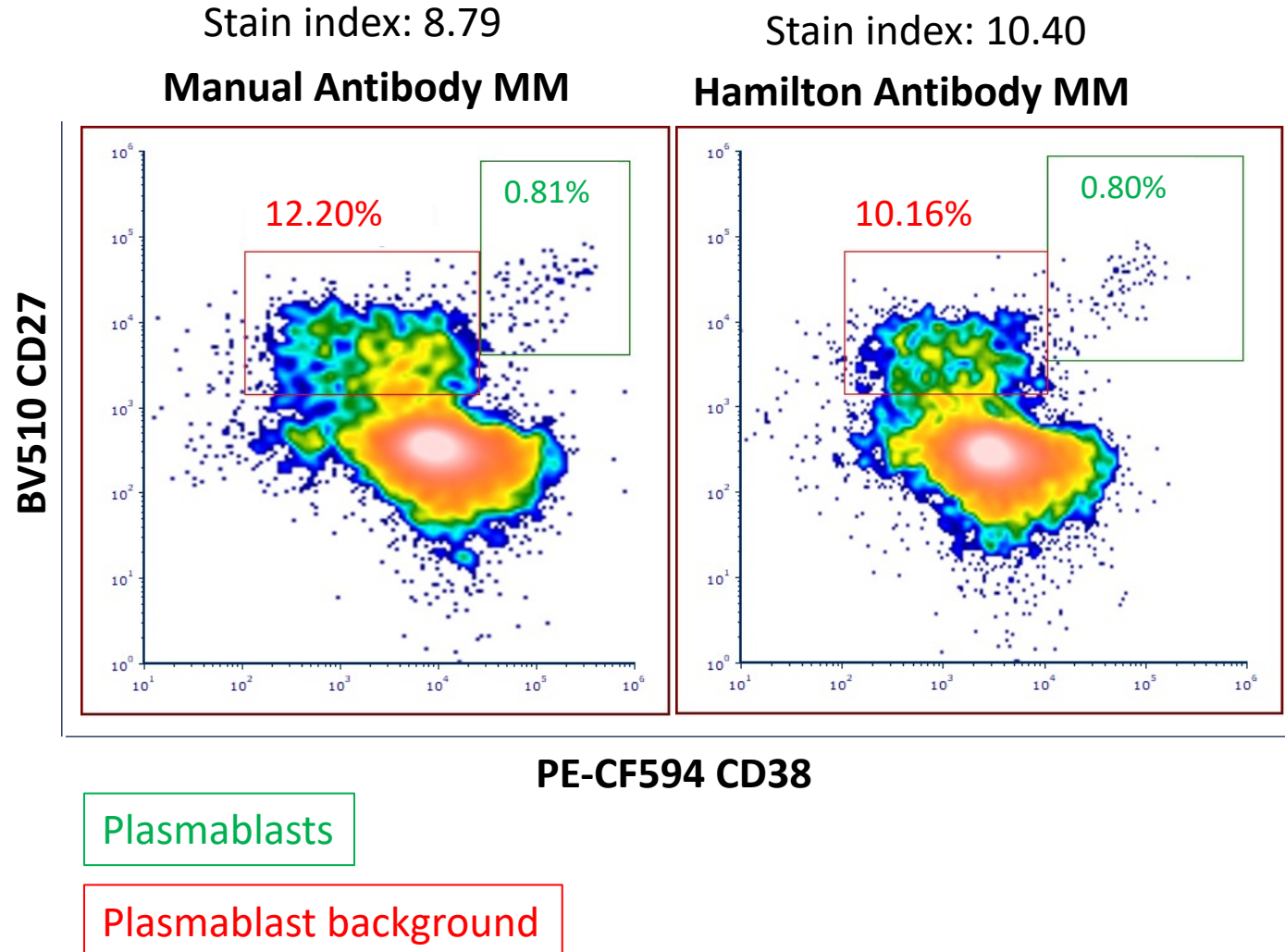
	Manual	Hamilton
Live cells	54.18%	55.45%
Singlets	77.43%	75.98%
B cells	22.10%	22.70%
CD4 cells	42.23%	41.67%
CD8 cells	15.36%	15.60%

Hamilton data

Comparable results even for rarer populations

- Decreased data spread

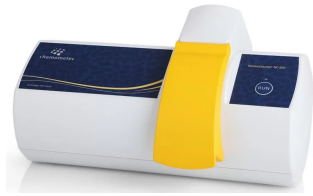
Stain index: how well can we resolve positive from negative
Larger stain index = better separation



Current optimised workflow



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Sample prep

- Red blood cell lysis
- Fc blocking
- Permealysing/Fixing
- Cell counting

- Faster
- Objective counts

Staining

- Antibody Mastermix
- Secondary Antibody
- FMO contols
- Isotype controls

- Fully comparable results
- Saves time
- Low risk

Washing

- Adding stain buffer
- Centrifuging
- Discarding supernatant
- Vortexing
- Resuspending

- Increased assay robustness
- Consistent washing

6hrs → 3hrs

Analysis

lower variability

Future prospects – can flow cytometry be fully automated?



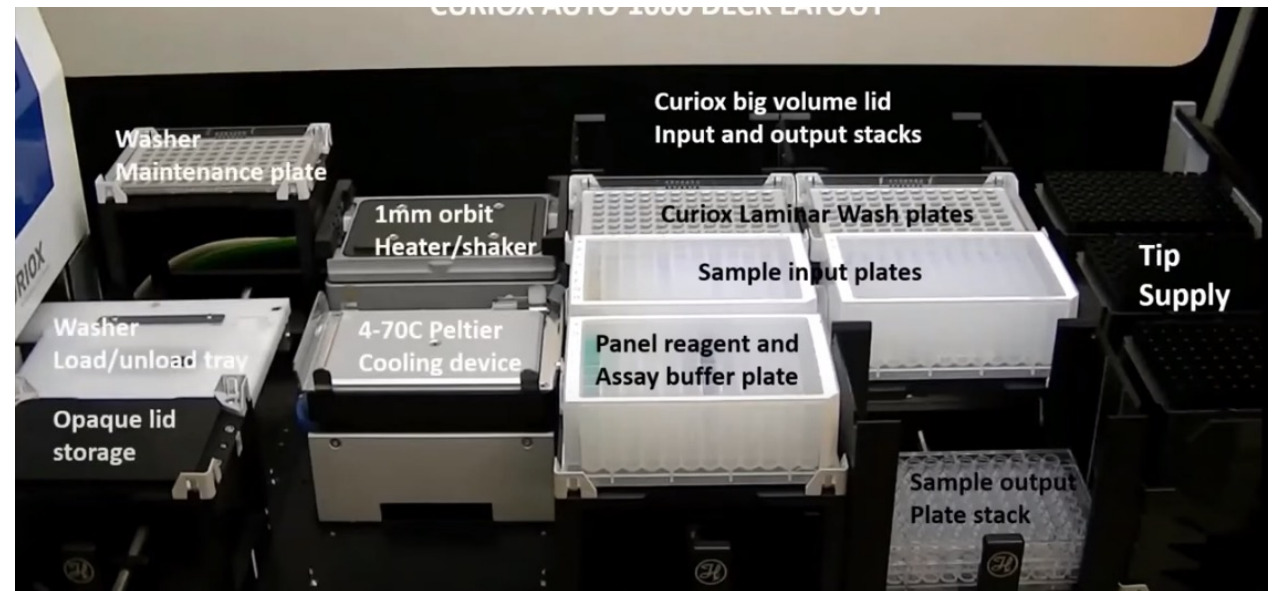
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LAMINAR WASH AUTO 1000

All steps automated:

- Sample addition
- Vortexing
- Live/dead staining
- Incubating
- Washing



Data analysis - can we minimise analyst variation?



FCS Express templates:

- Minimal gating adjustments
- Analysts run an SOP – full gating instructions
- Analysis can be signed and approved by technical specialist

General Information				
LGC project number:	<input type="text"/>	Bioanalytical Method:	<input type="text"/>	Date: 10-Aug-2022
Panel ID:	Panel C	Sample ID:	11212483-07	File ID: Multiple files
Experiment ID	Exp011	Instrument Operator:	Zoe Georgakopoulou	
Instrument ID	BA32234	Plate ID:	01	Analyst: ZGE

Sample ID:
File:
Subject ID and Visit:
Collection Date and Time: 09/08/2022 08:57

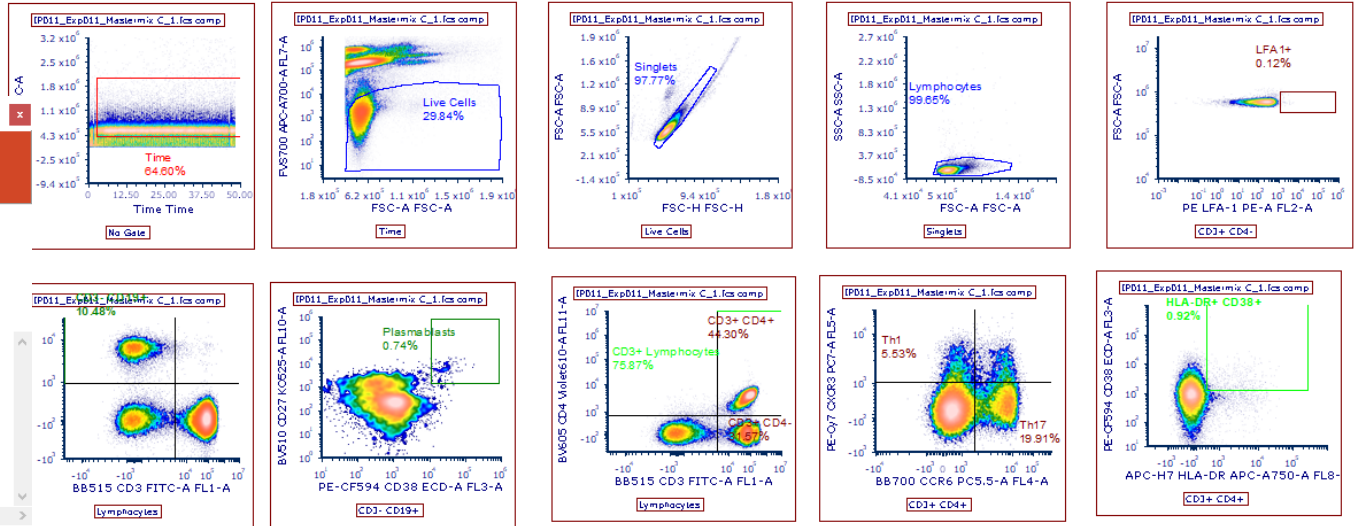
Run SOP

Load data

Description
Load data

Instructions
S:\Health Science\UK\Fordham\F_LabData\LMB\LGC0023_CytoFLEX
Load data one subject per template e.g. IP001 only

The condition for advancing to the next step is satisfied





Conclusion

Integrating automation in your workflow:

- Saves time
- Increases assay robustness
- Decreases risk of errors
- Provides audit trail
- Maximises lab efficiency
- **Increases data quality and reproducibility**





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**Thank you for
your attention**
Any further question?

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