Lessons Learnt:

Establishment of a benchmark cytometric immune phenotyping workflow for multi-centre clinical trials.

Natalie Smith

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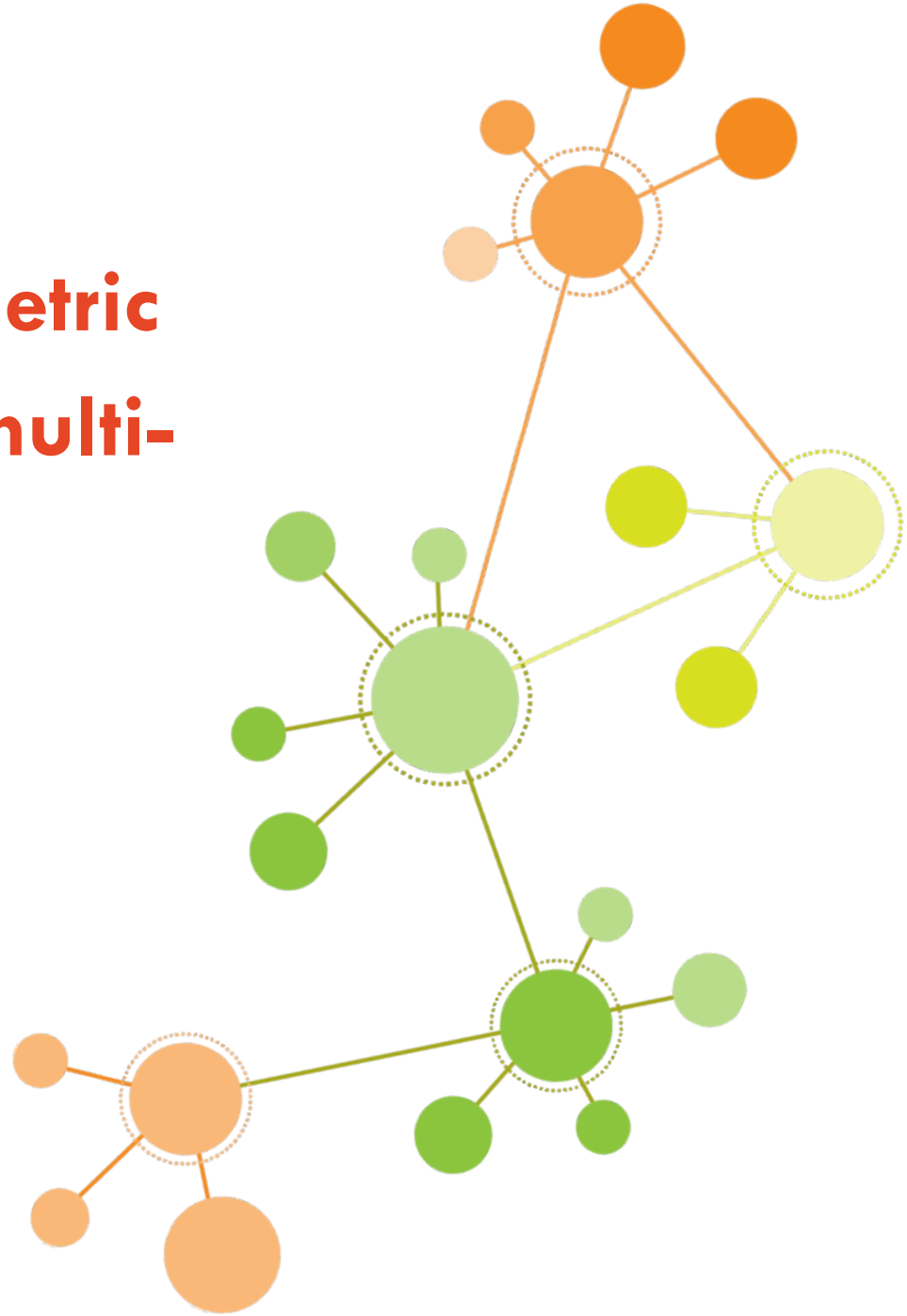
Translational Immunology Group

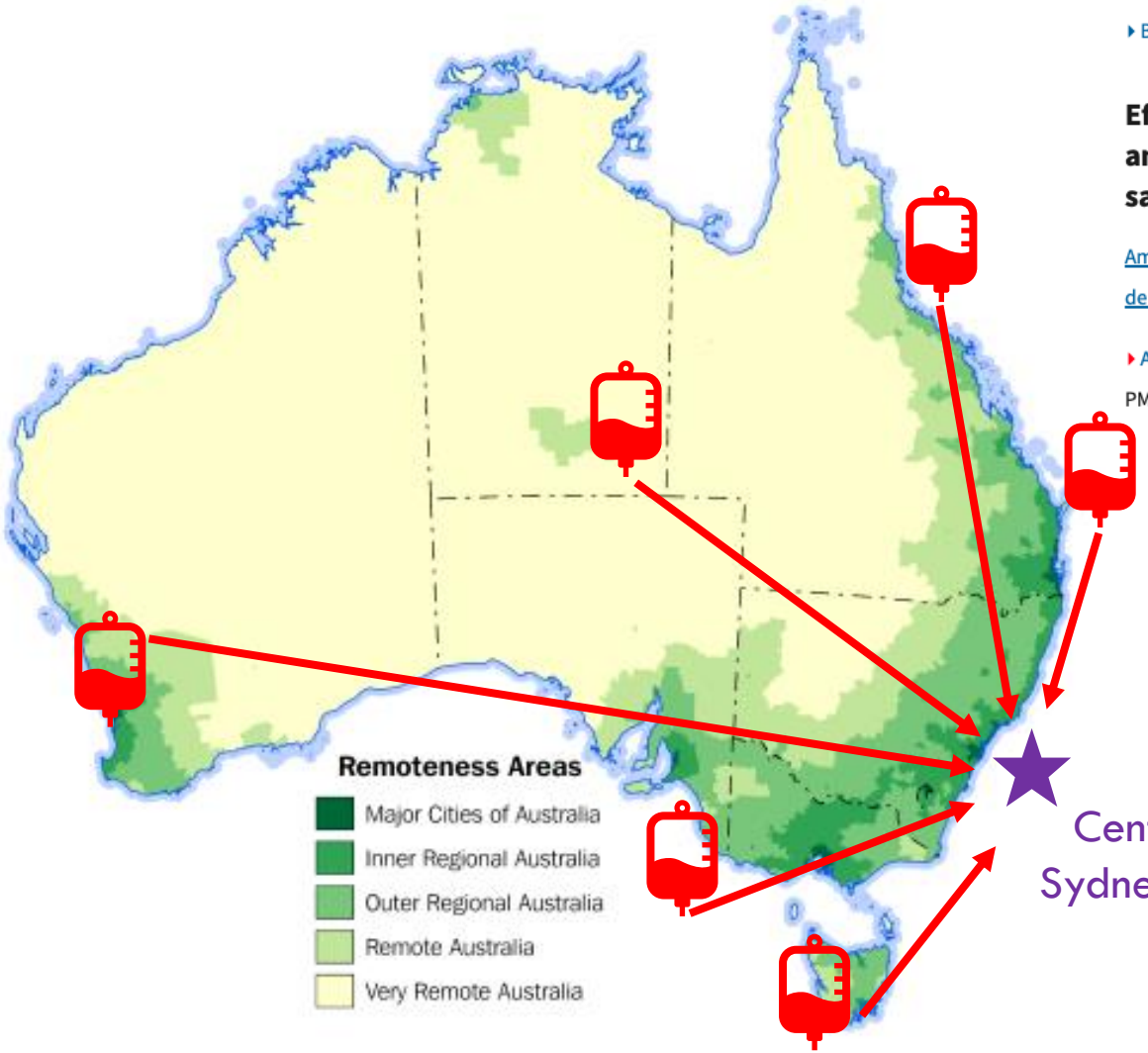
PhD Candidate

Supervised by Dr Helen McGuire

THE UNIVERSITY OF SYDNEY



Typical multi-centre immunophenotyping workflow



Central site at Sydney University

Biosci Rep. 2021 Feb 26;41(2):BSR20203827. doi: 10.1042/BSR20203827

Effects of storage time and temperature on highly multiparametric flow analysis of peripheral blood samples; implications for clinical trial samples

Amelia Jerram [1], Thomas V Guy [2], Lucinda Beutler [1], Bavani Gunasegaran [1], Ronald Sluyter [2,3], Barbara Fazekas de St Groth [1,4,*,✉], Helen M McGuire [1,4,*,✉]

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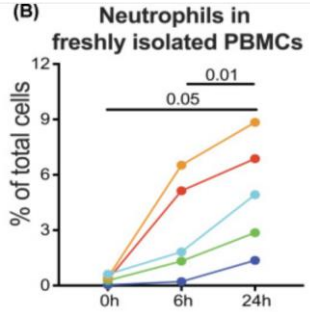
PMCID: PMC7921292 PMID: 33600563

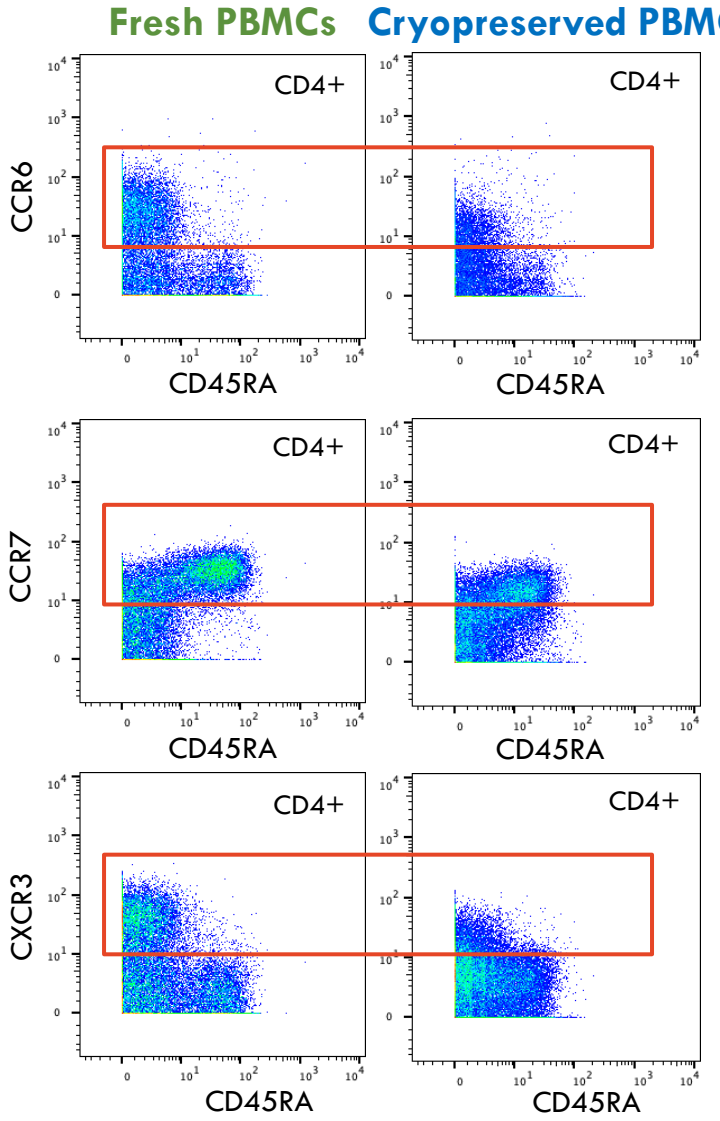


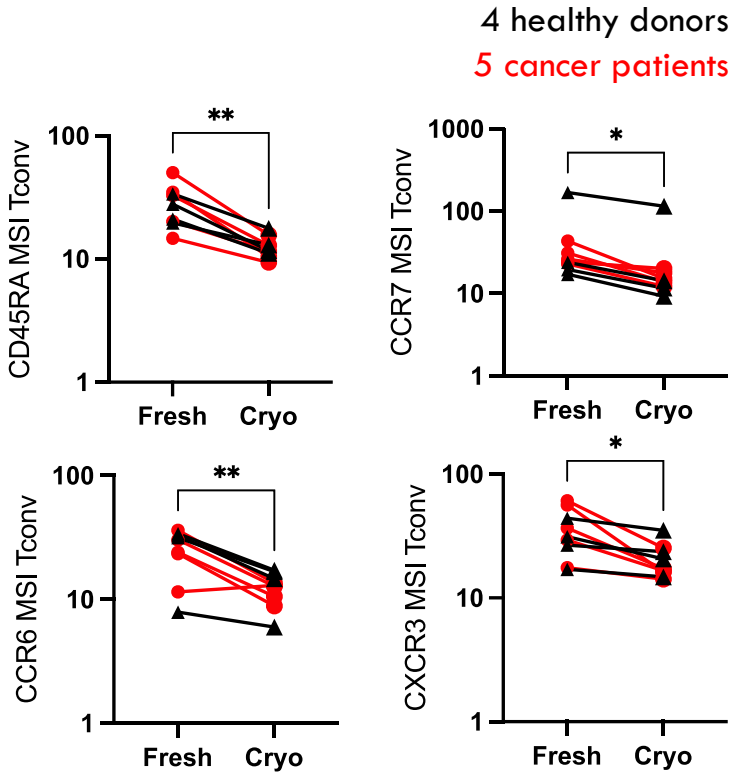
Notes from our previous nation-wide study

Discarded, Blood EDTA tubes came in dry ice, frozen!
RBC contamination
RBC contamination
RBC contamination
Discarded, Blood EDTA tubes came in dry ice, frozen AGAIN!
Received cold with a frozen ice pack
Currently no hand written paper slip, processing details (# cells & tim
Patient commenced steroid treatment 2 days prior to blood draw
Flight delayed, so discarded sample

Our current approach to remote multi-centre clinical trials has room for improvement

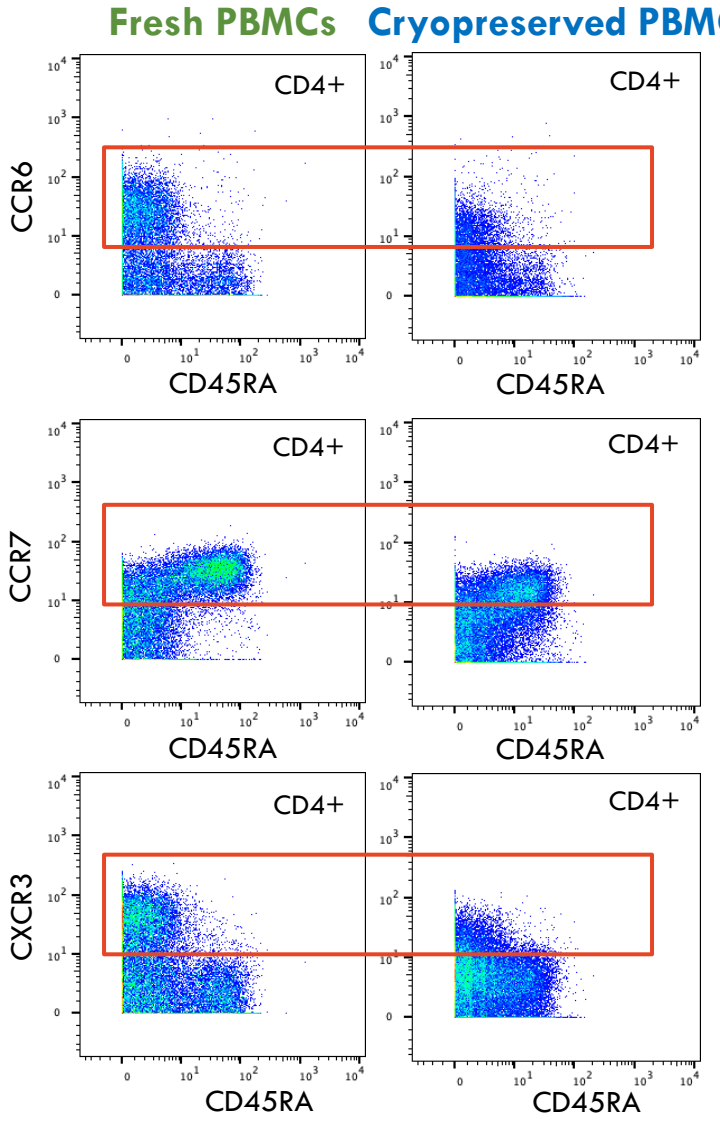
Staining fresh PBMCs gives superior signal

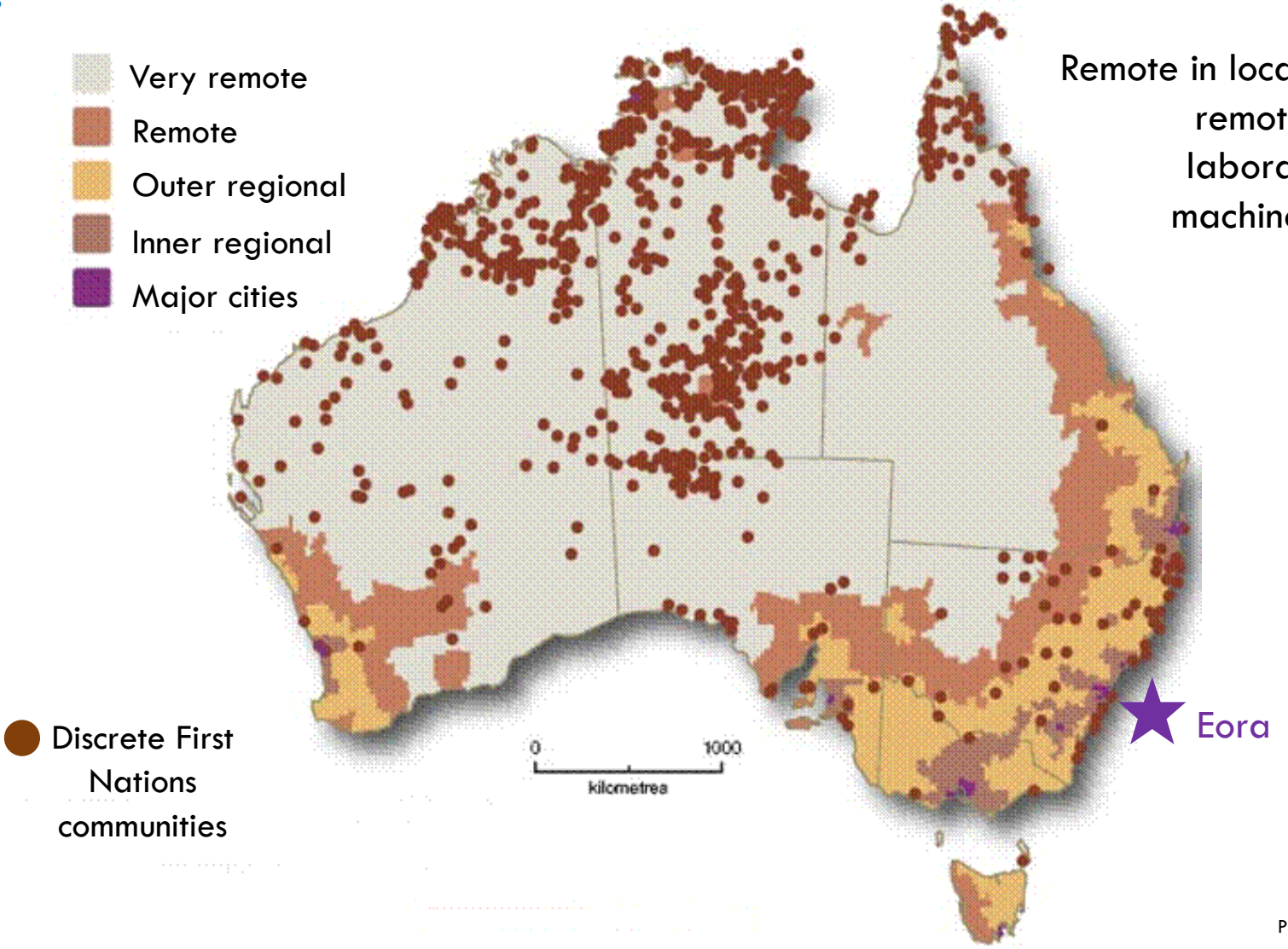




Reduced signal intensity in cryopreserved PBMCs for 10 out of 46 markers

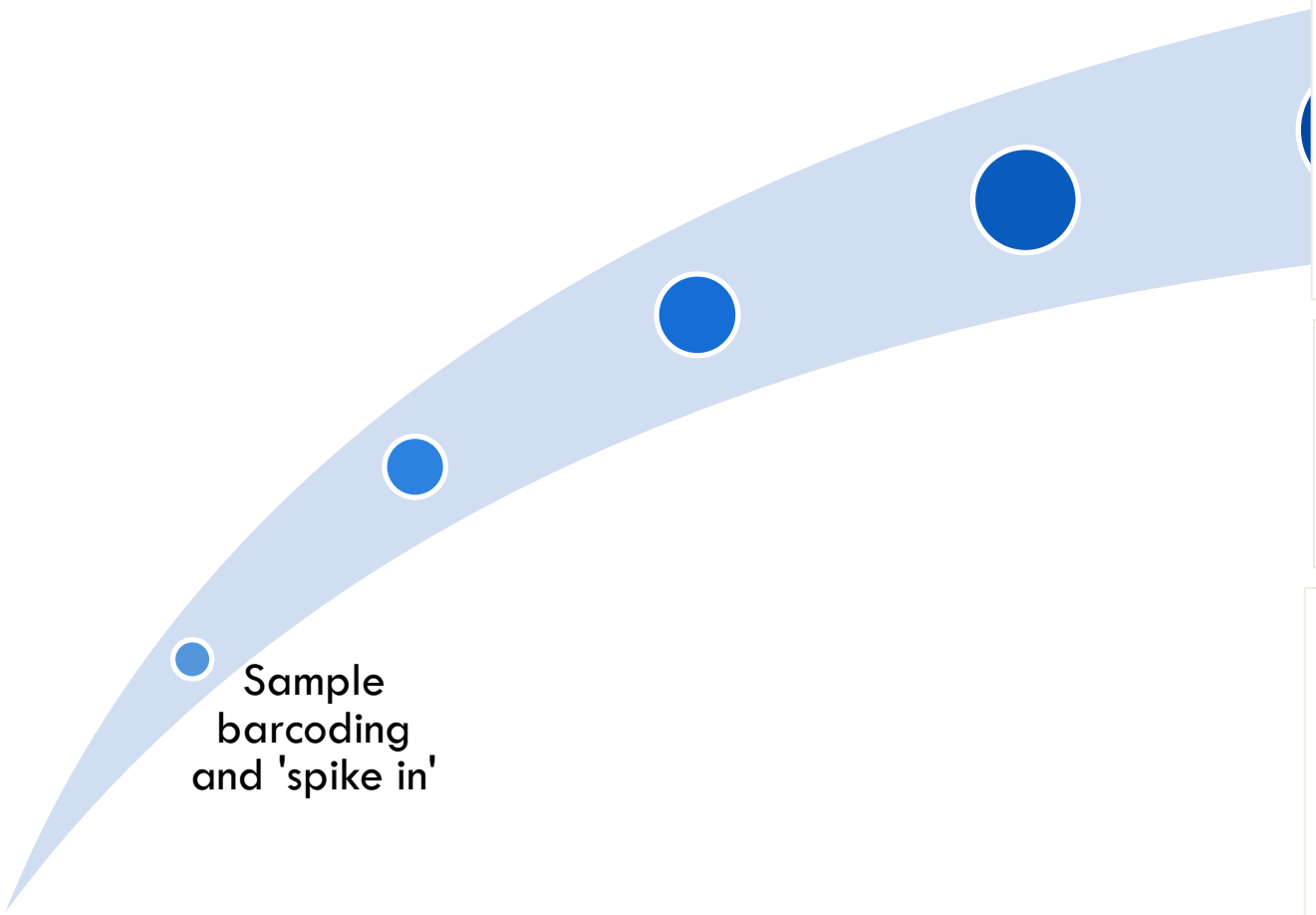
Fresh staining is complicated for remote populations





Remote in location = remote from laboratories, machines, etc.

Mass cytometry enables *enhanced* comprehensive immunophenotyping strategies



Protocol | Published: 22 January 2015

Palladium-based mass tag cell barcoding with a doublet-filtering scheme and single-cell deconvolution algorithm

Eli R Zunder, Rachel Finck, Gregory K Behbehani, El-ad D Amir, Smita Krishnaswamy, Veronica D Gonzalez, Cynthia G Lorang, Zach Bjornson, Matthew H Spitzer, Bernd Bodenmiller, Wendy J Fantl, Dana Pe'er & Garry P Nolan

Nature Protocols **10**, 316–333 (2015) | Cite this article

11k Accesses | **323** Citations | **29** Altmetric | Metrics

J Immunol. 2015 February 15; 194(4): 2022–2031. doi:10.4049/jimmunol.1402661.

Barcoding of live human PBMC for multiplexed mass cytometry

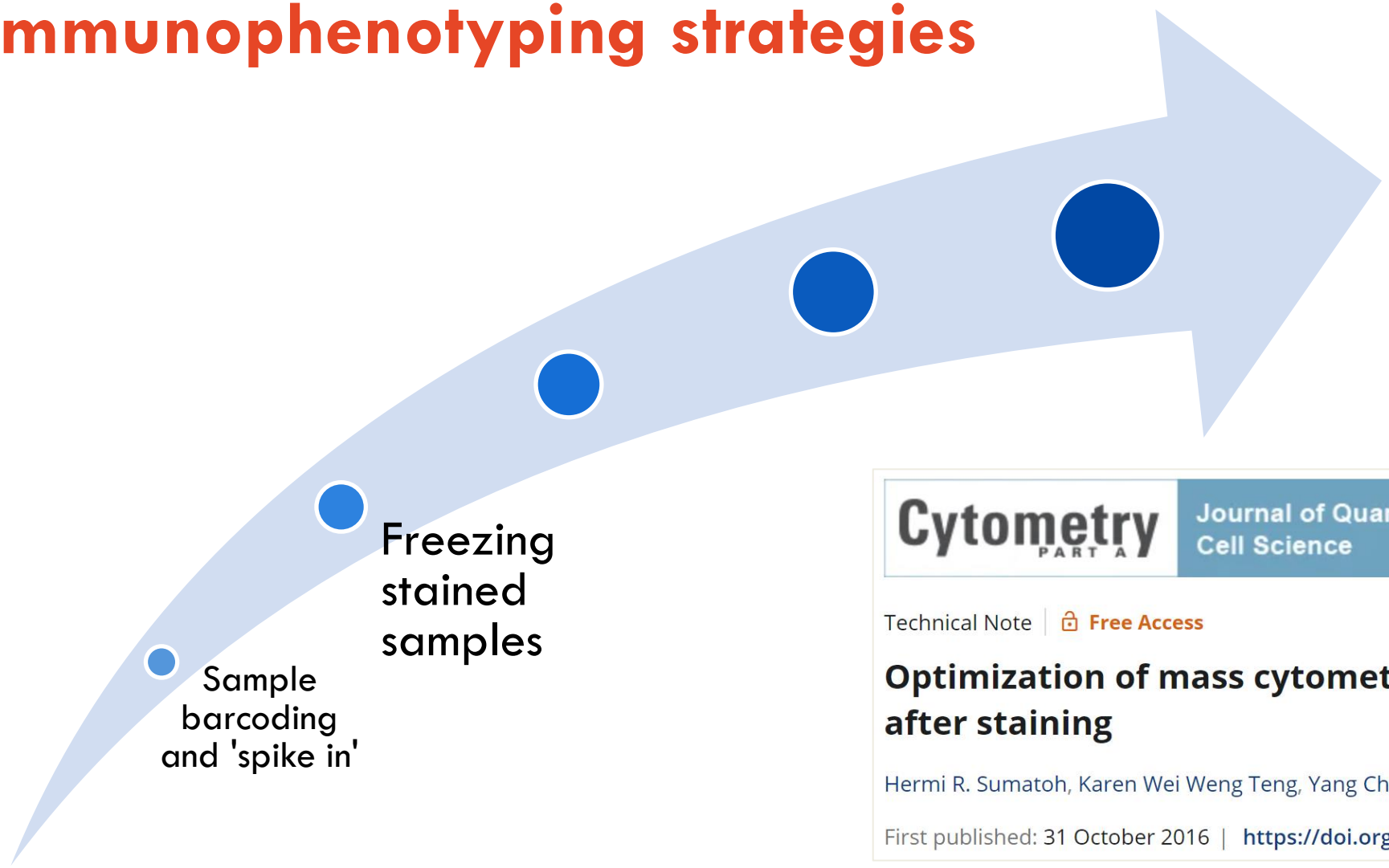
Henrik E. Mei[†], **Michael D. Leipold**[†], **Axel Ronald Schulz**[†,§], **Cariad Chester**[†,‡], and **Holden T. Maecker**[†]

Cytometry A. 2016 October ; 89(10): 903–913. doi:10.1002/cyto.a.22935.

Standardization and Quality Control for High-Dimensional Mass Cytometry Studies of Human Samples

Katja Kleinsteuber[1,2,3], **Björn Corleis**[1], **Narges Rashidi**[1], **Nzuekoh Nchinda**[1,2], **Antonella Lisanti**[1], **Josalyn L. Cho**[4,5], **Benjamin D. Medoff**[4,5], **Douglas Kwon**[1,6], and **Bruce D. Walker**[1,2,6,*]

Mass cytometry enables *enhanced* comprehensive immunophenotyping strategies



Cytometry PART A
Journal of Quantitative Cell Science
ISAC

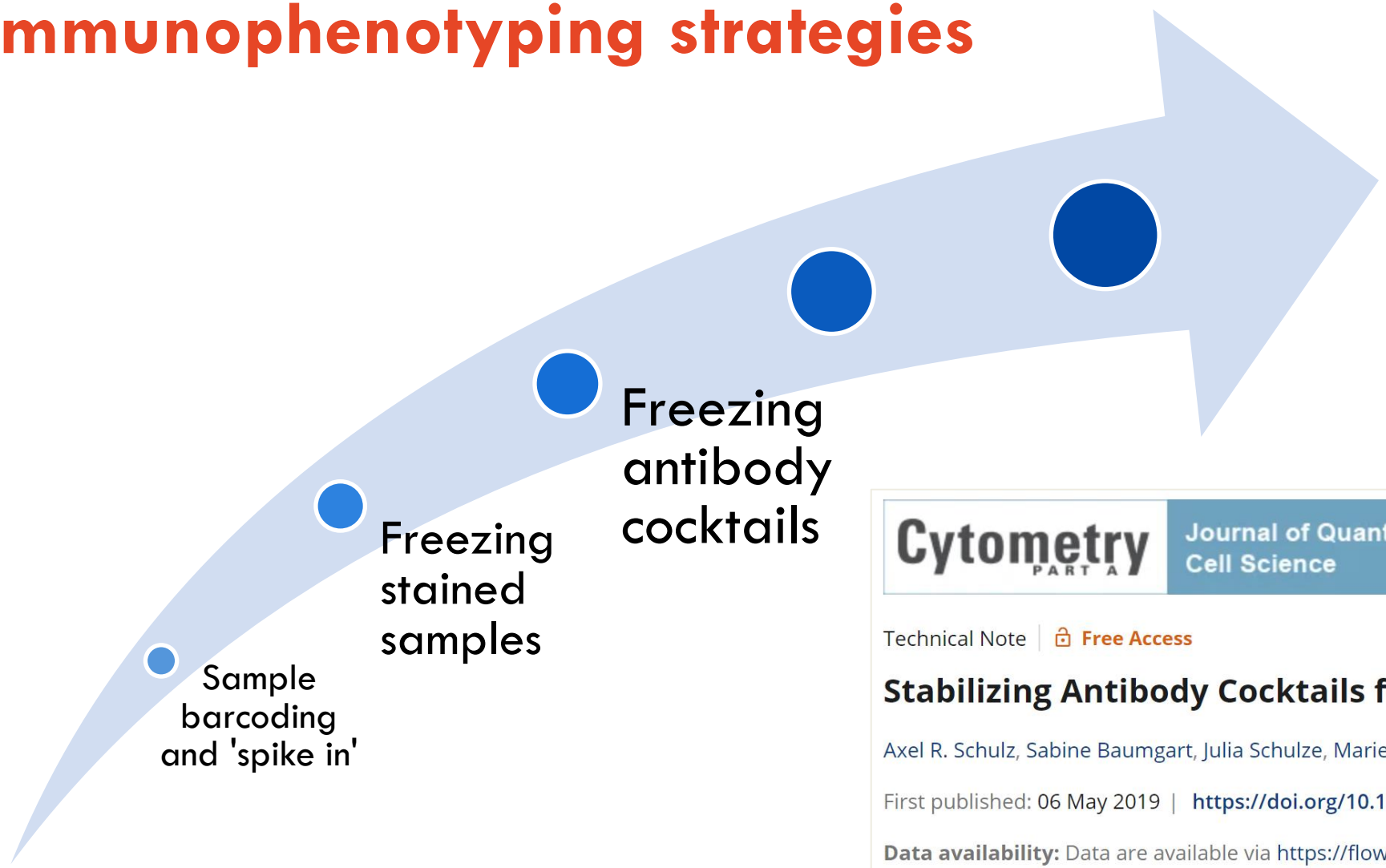
Technical Note | Free Access

Optimization of mass cytometry sample cryopreservation after staining

Hermi R. Sumatoh, Karen Wei Weng Teng, Yang Cheng, Evan W. Newell

First published: 31 October 2016 | https://doi.org/10.1002/cyto.a.23014 | Citations: 39

Mass cytometry enables *enhanced* comprehensive immunophenotyping strategies



Cytometry PART A | Journal of Quantitative Cell Science | ISAC

Technical Note | Free Access

Stabilizing Antibody Cocktails for Mass Cytometry

Axel R. Schulz, Sabine Baumgart, Julia Schulze, Marie Urbicht, Andreas Grützkau, Henrik E. Mei

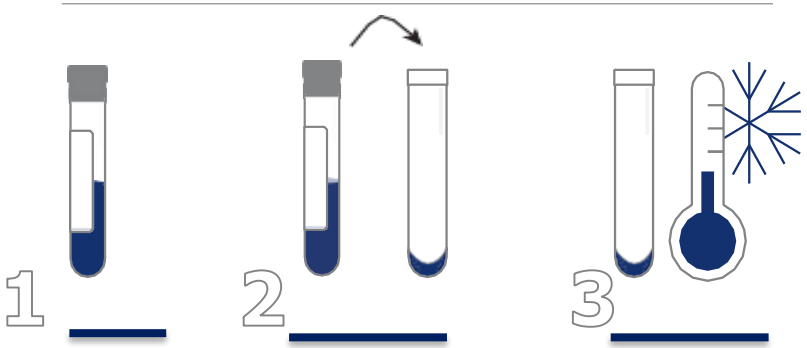
First published: 06 May 2019 | https://doi.org/10.1002/cyto.a.23781 | Citations: 40

Data availability: Data are available via https://flowrepository.org/ (FR-FCM-Z2ZD and FR-FCM-Z2ZF).

Proposed workflow

Remote sites

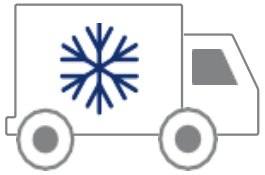
Total upfront processing time: about 40 min



1 **Collect** fresh samples

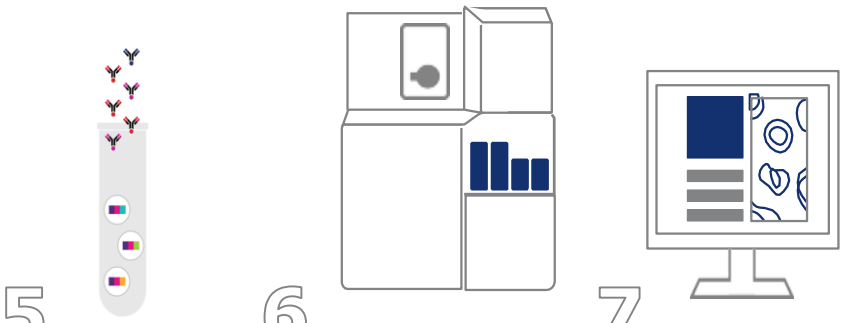
2 **Stain** with ready to use custom CyTOF panel

3 **Freeze** and store



4 **Ship** to central processing site (Charles Perkins Centre)

Sydney (Charles Perkins Centre)



5 **Barcode** and stain samples (intracellular targets)

6 **Acquire** on CyTOF system

7 **Automated report**

Proposed workflow

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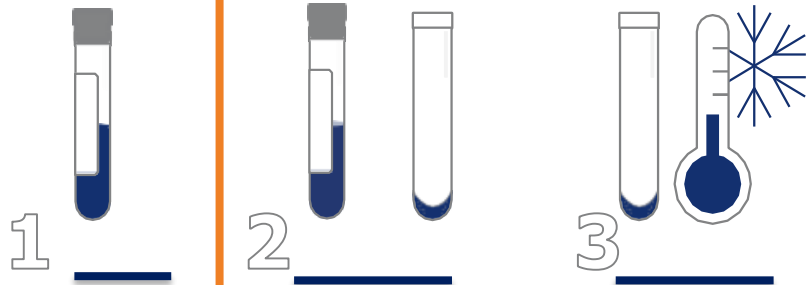

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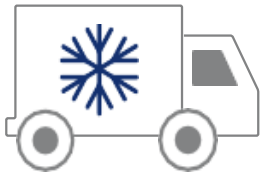

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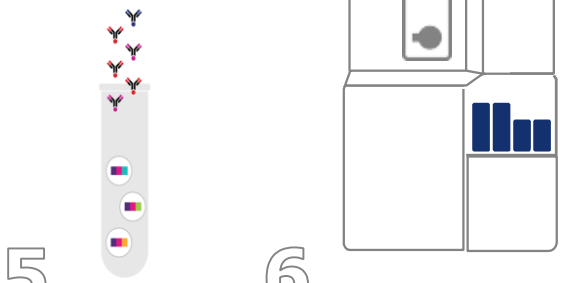

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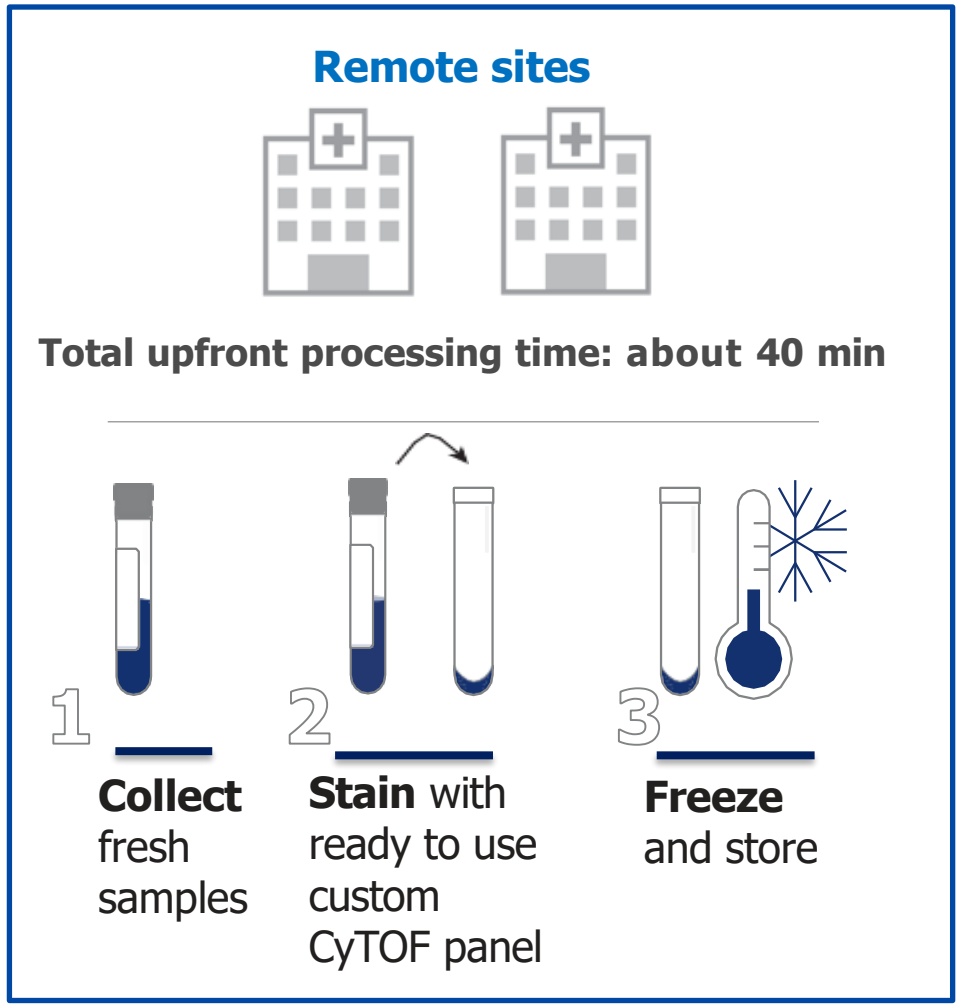
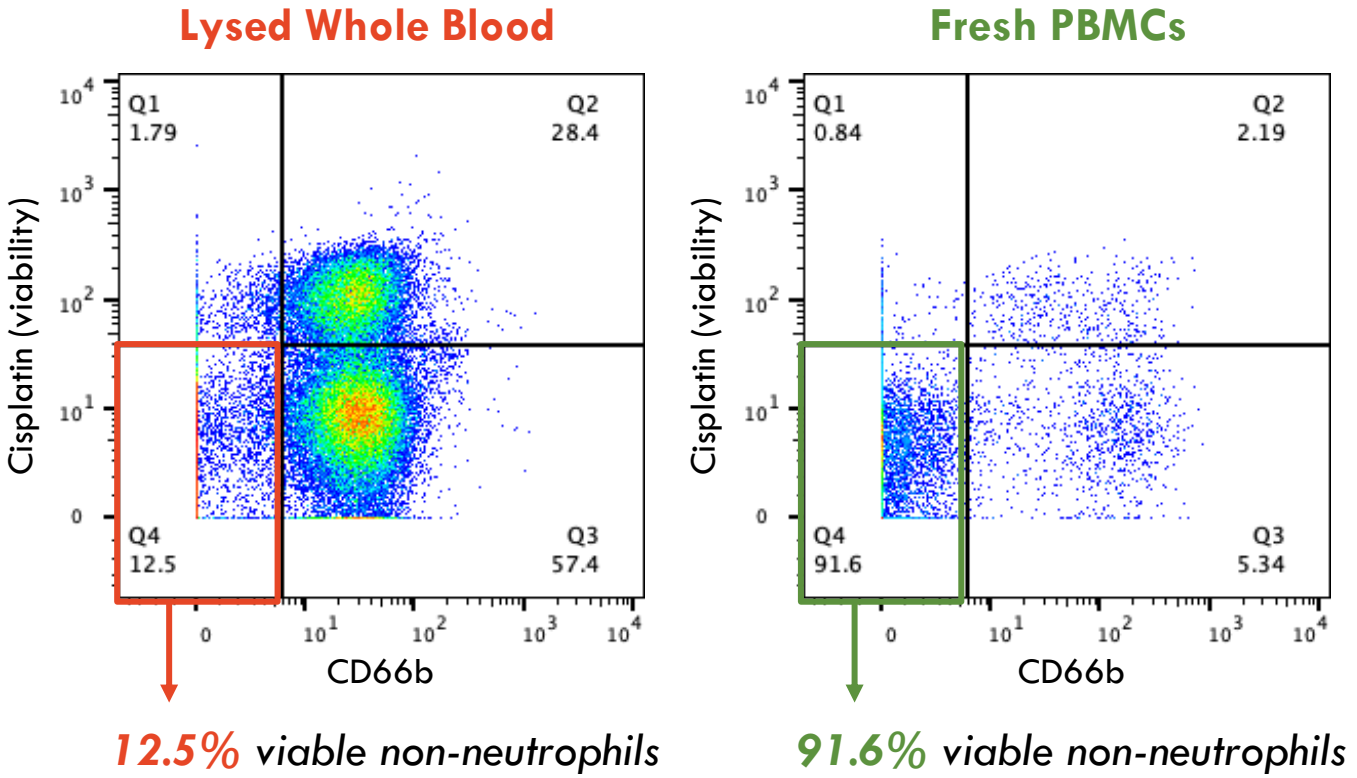
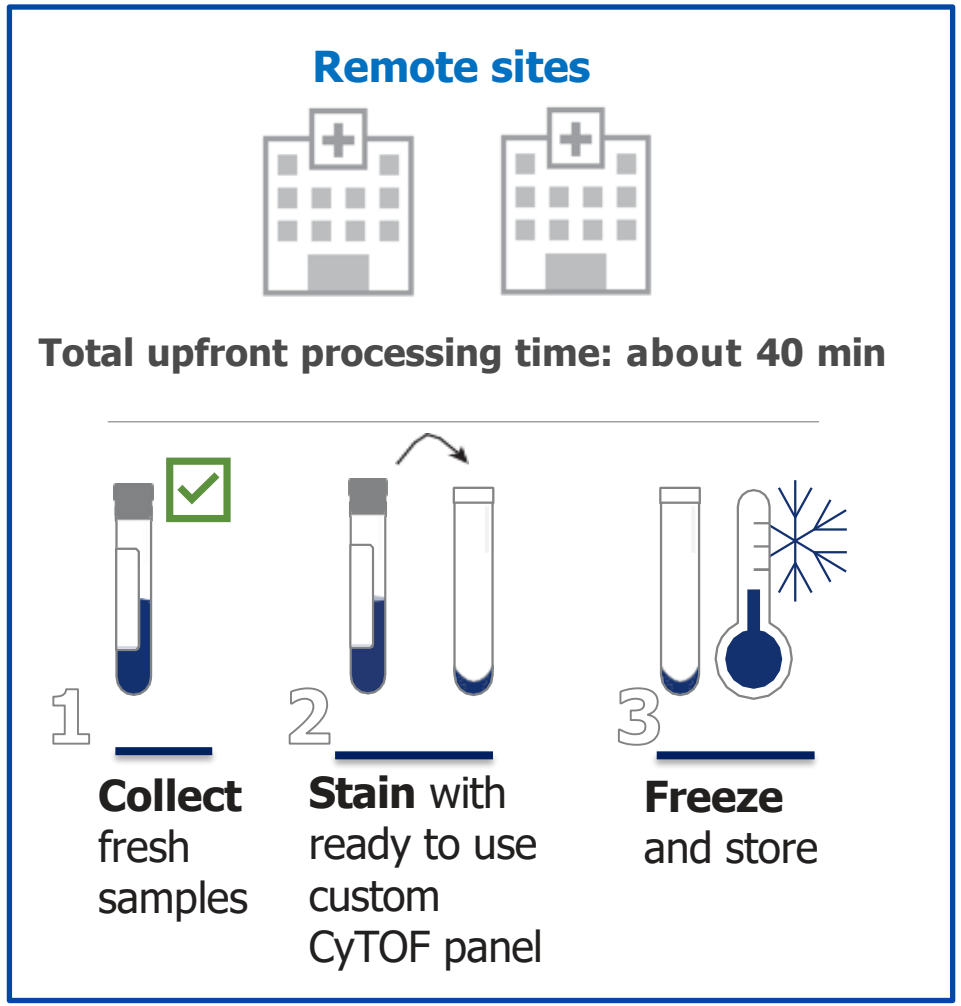


PBMCs or whole blood?



Proposed workflow



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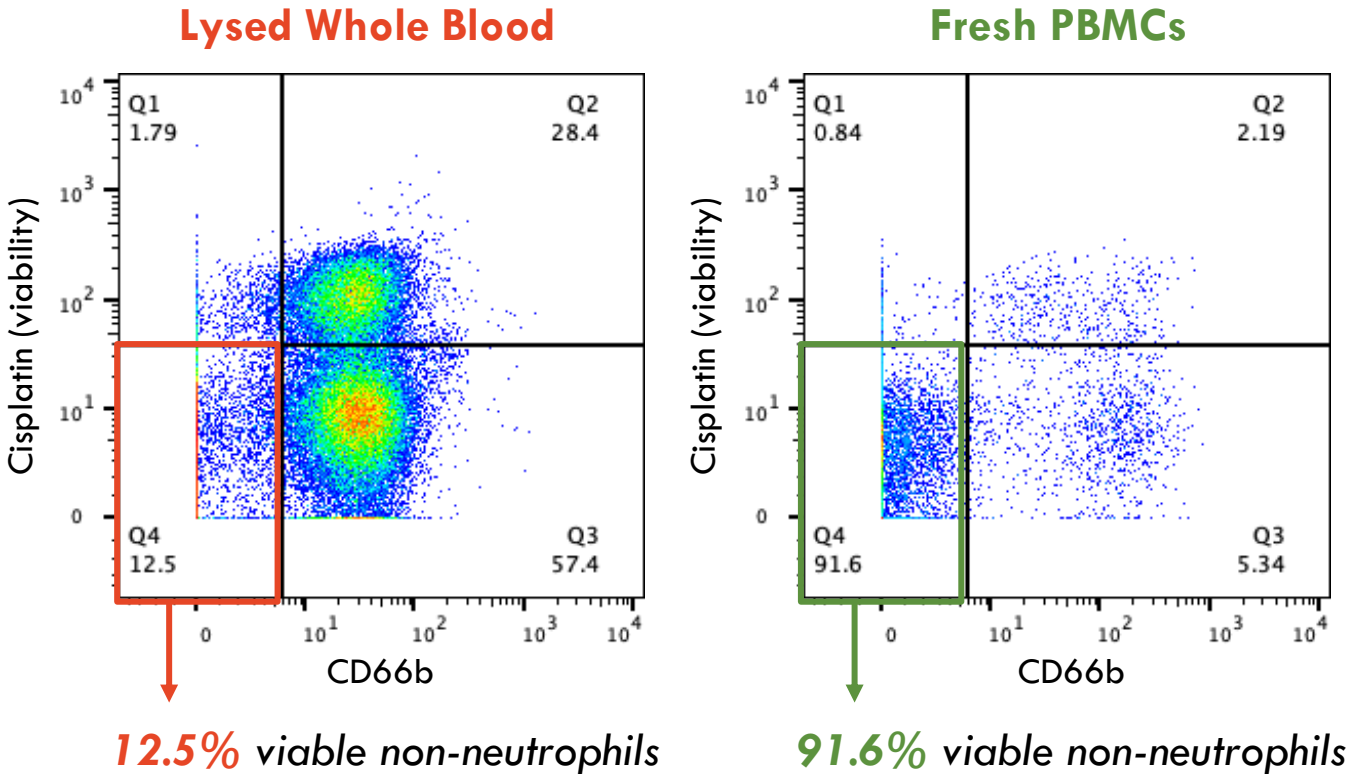
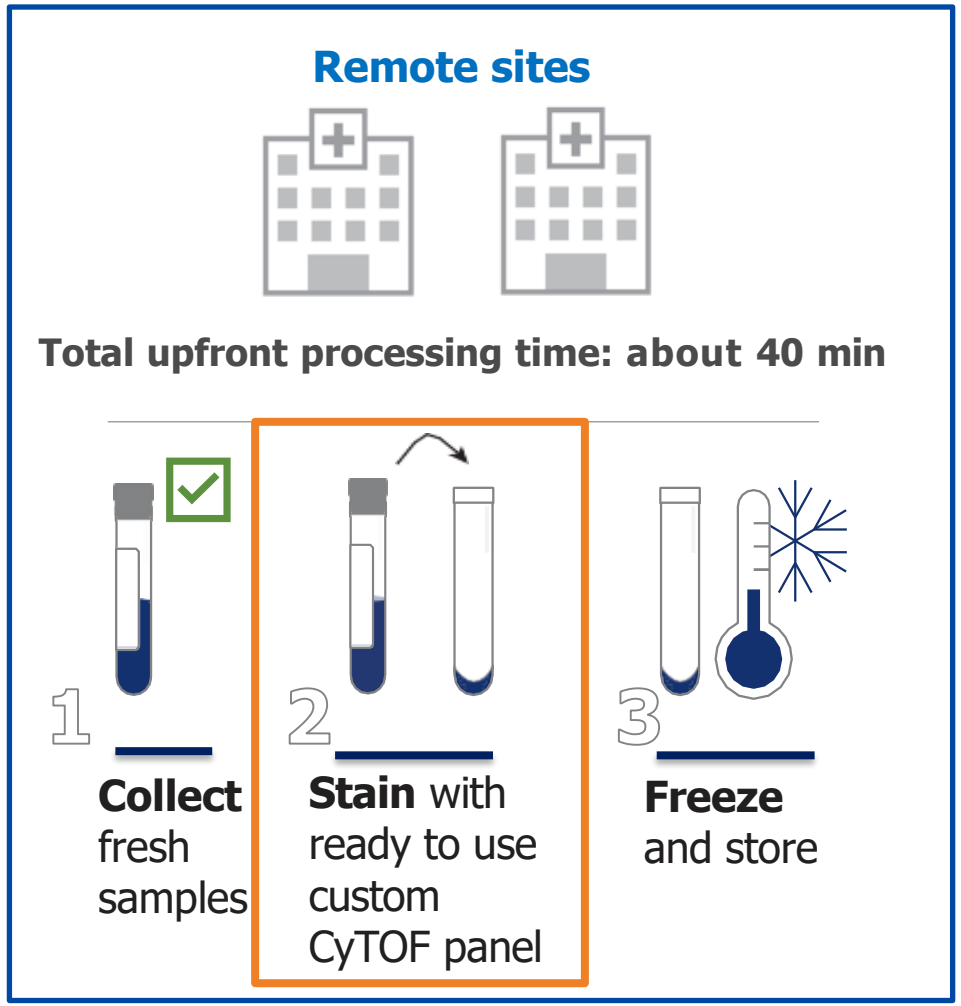


Proposed workflow

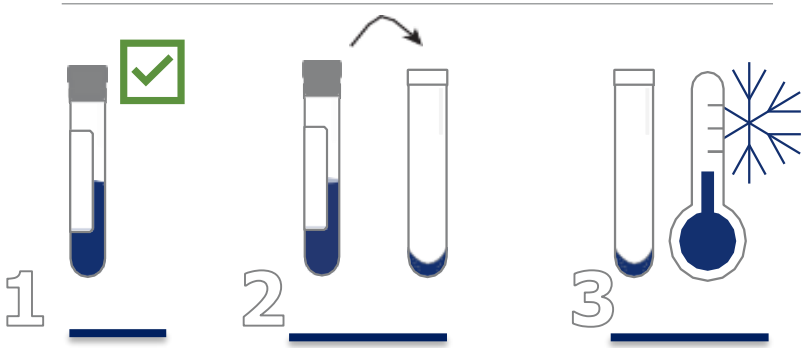


50-plex panel design

Proposed workflow

Remote sites

Total upfront processing time: about 40 min



Collect fresh samples

Stain with ready to use custom CyTOF panel

Freeze and store

50-plex panel design

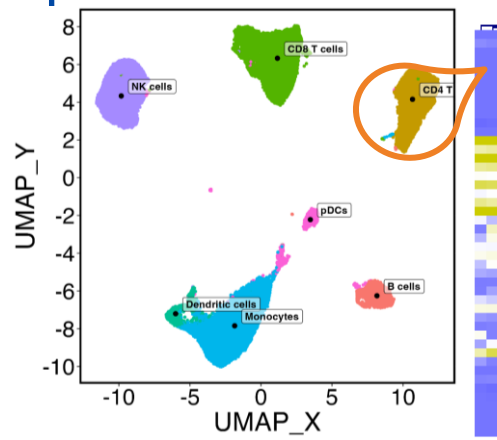
Lineage Markers		
CD45	CD33	CD3
HLA-DR	CD11c	CD19
CD11b	CD8	CD4
CD20	CD66b	CD247
CD16	CD304	FoxP3
CD123	CD56	IgD
CD14	Livedead	

Functional Markers		
Perforin	CD69	CD45RA
CD45RO	CD39	Eomes
CD86	PD-1	CD127
TIGIT	Ki67	Arginase I
CD27	CD25	CD38
Integrin B7	GranzymeB	T-bet

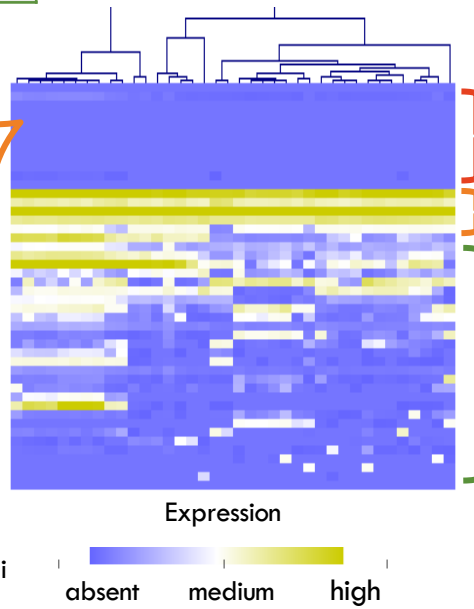
Chemokine Receptors		
CCR2	CCR4	CCR5
CCR6	CCR7	CCR10
CXCR3	CXCR5	

Sample Barcodes

20 plex barcoding achieved through 6 palladium isotopes



Made with Spectre
Ashhurst, Marsh-Wakefield, Putri et al., 2022 (Cytometry A)



Markers absent on CD4+:
CD8, CD14, CD19, CD11b, CD11c, CD20, CD33, CD66b, CD86, CD123, CD304

Markers uniformly present on CD4+:
CD45, CD3, CD4, CD247

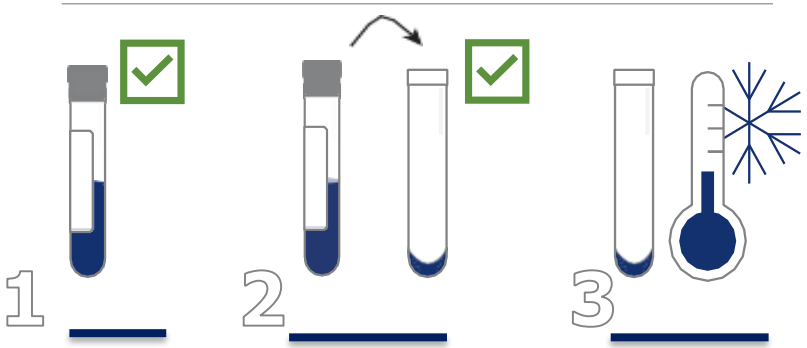
Markers differentially present on CD4 +:
CD45RO, EOMES, CXCR3, Granzyme B, Perforin, CCR5, CD27, CD127, Tbet, FoxP3, TIGIT, CCR4, CCR7, CD38, CD45RA, CCR10, HLADR, CD16, CD56, Ki67, CD25, IntebrinB7, PD1, CCR6, CD39, CXCR5, CCR2, CD69

~30 diverse subsets of CD4+ T cells

Proposed workflow

Remote sites

Total upfront processing time: about 40 min



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Freeze and store

50-plex panel design

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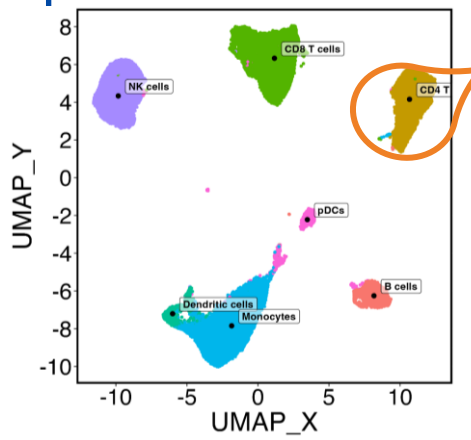
Chemokine Receptors		
CCR2	CCR4	CCR5
CCR6	CCR7	CCR10
CXCR3	CXCR5	

Sample Barcodes

20 plex barcoding achieved through 6 palladium isotopes



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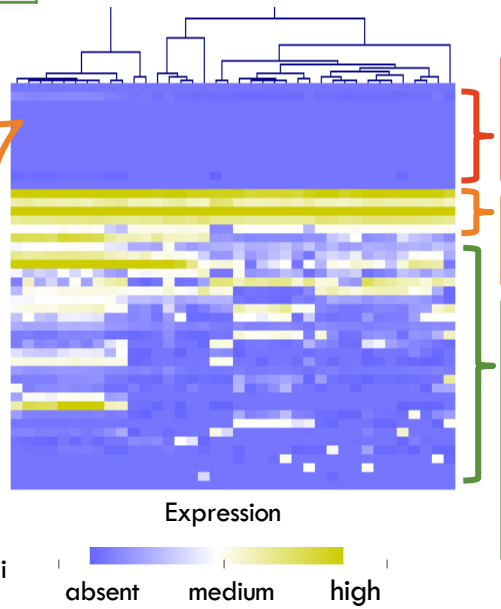

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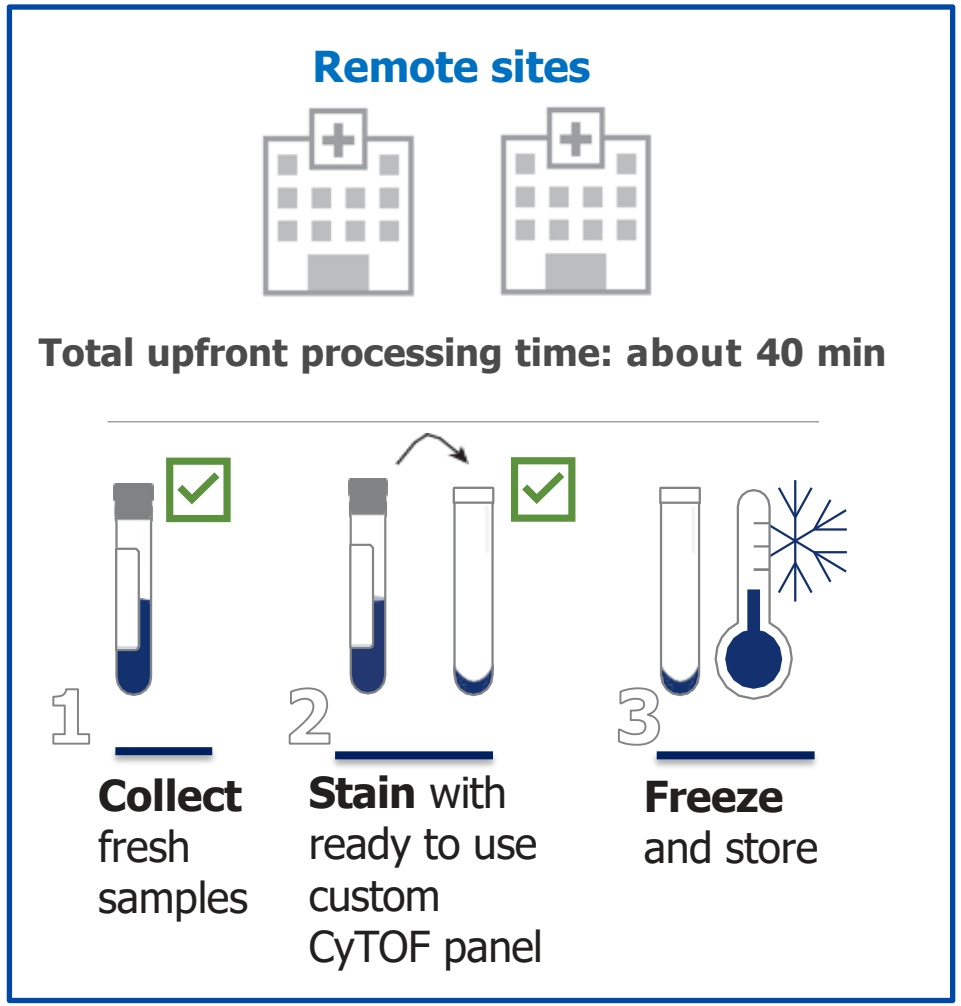
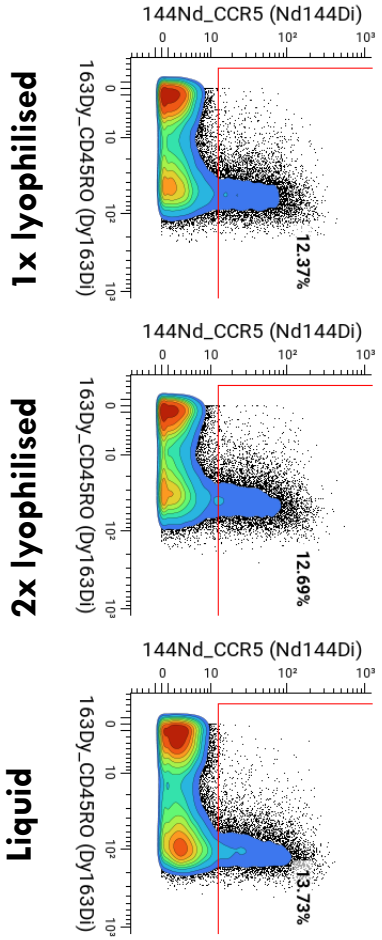
Proposed workflow



Lyophilised antibodies

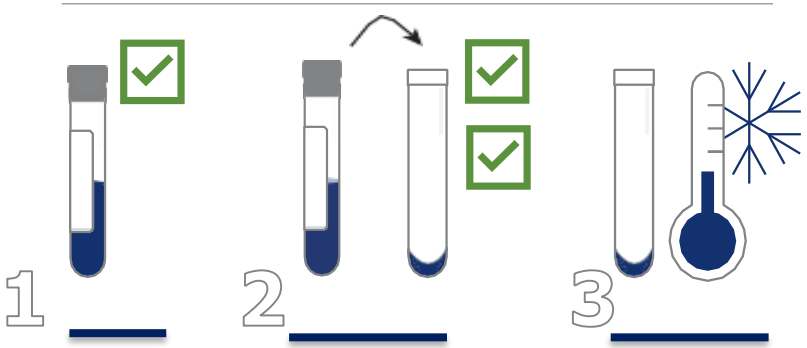


37 surface antibodies & viability stain

Proposed workflow

Remote sites

Total upfront processing time: about 40 min

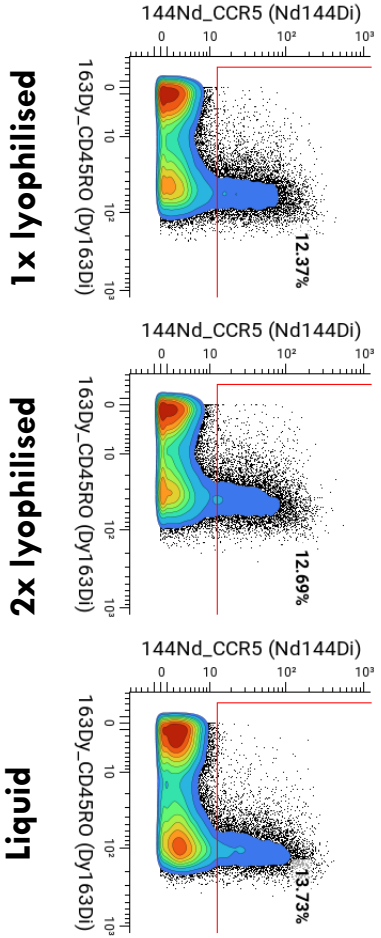


Collect fresh samples

Stain with ready to use custom CyTOF panel

Freeze and store

Lyophilised antibodies



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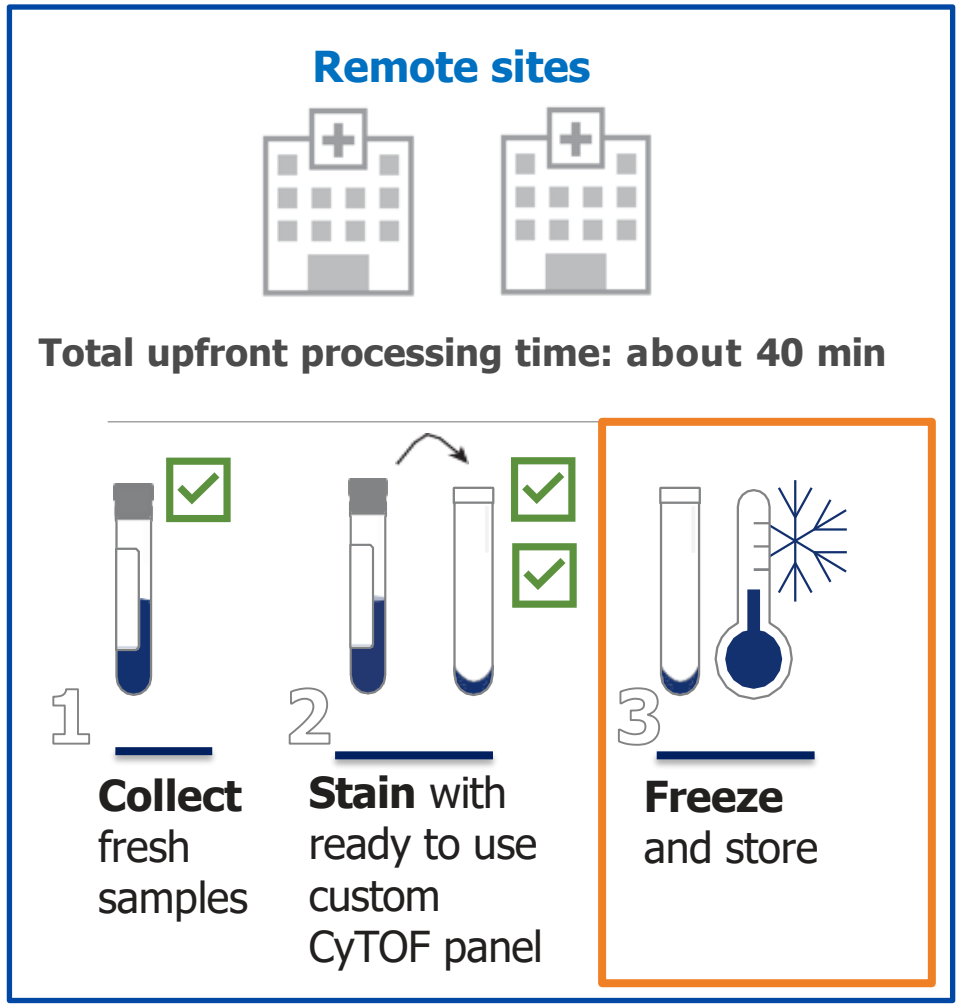
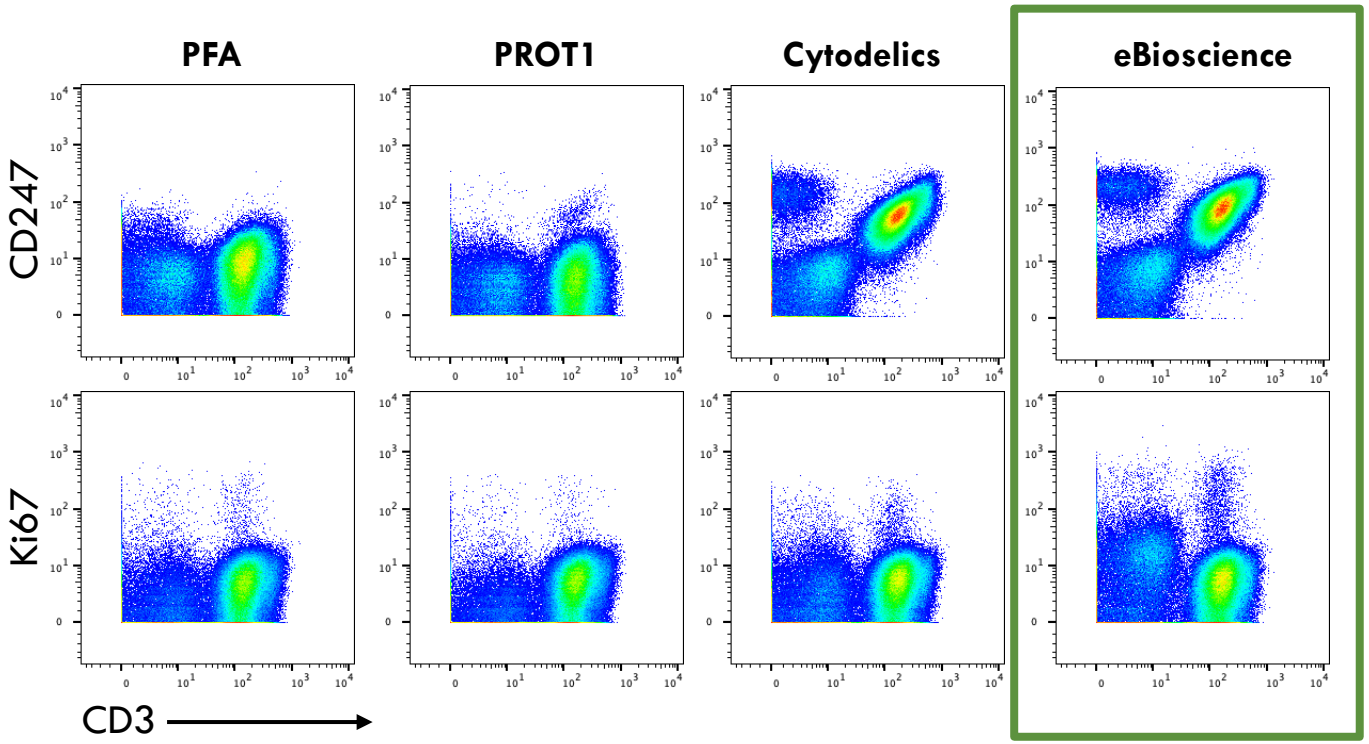


Integrating 'stopping points'

Best fixative & permeabilising agent selected.



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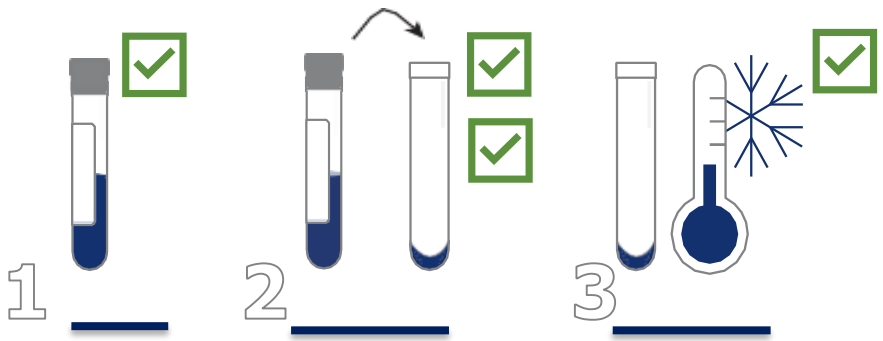

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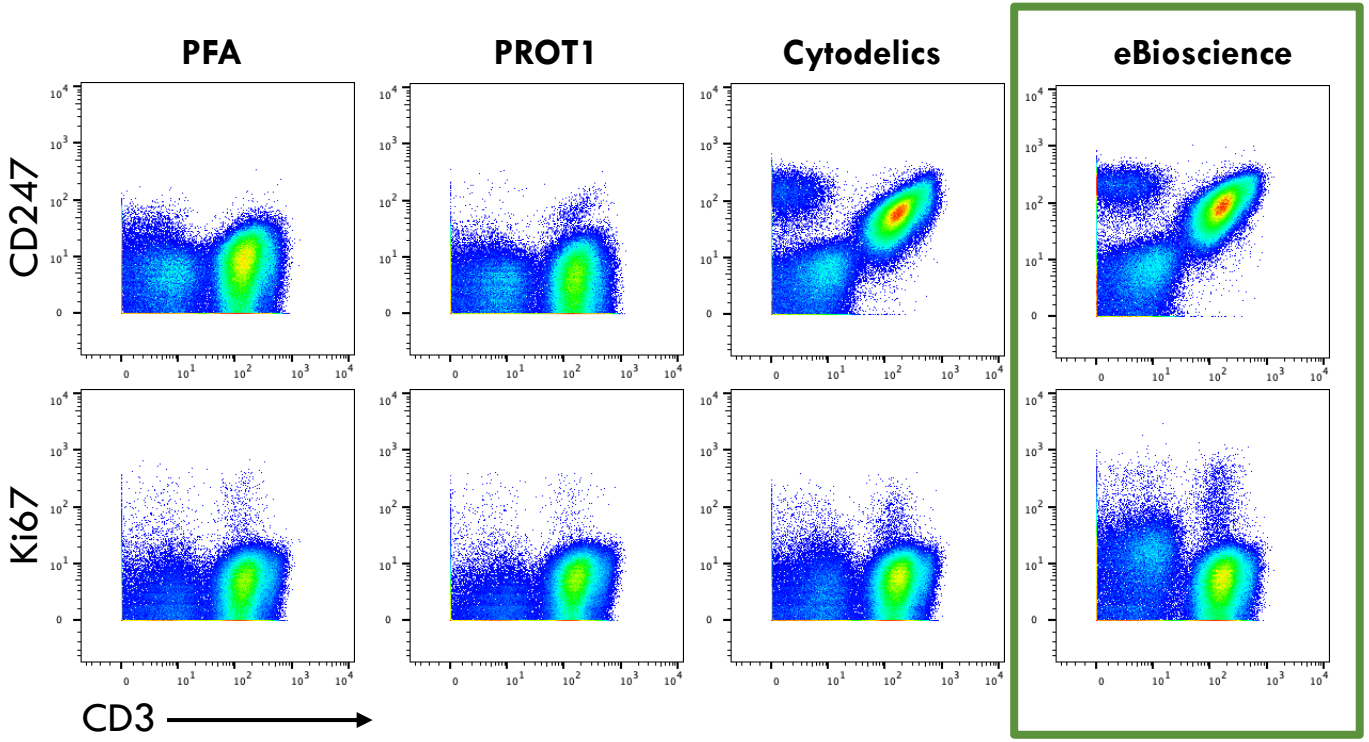
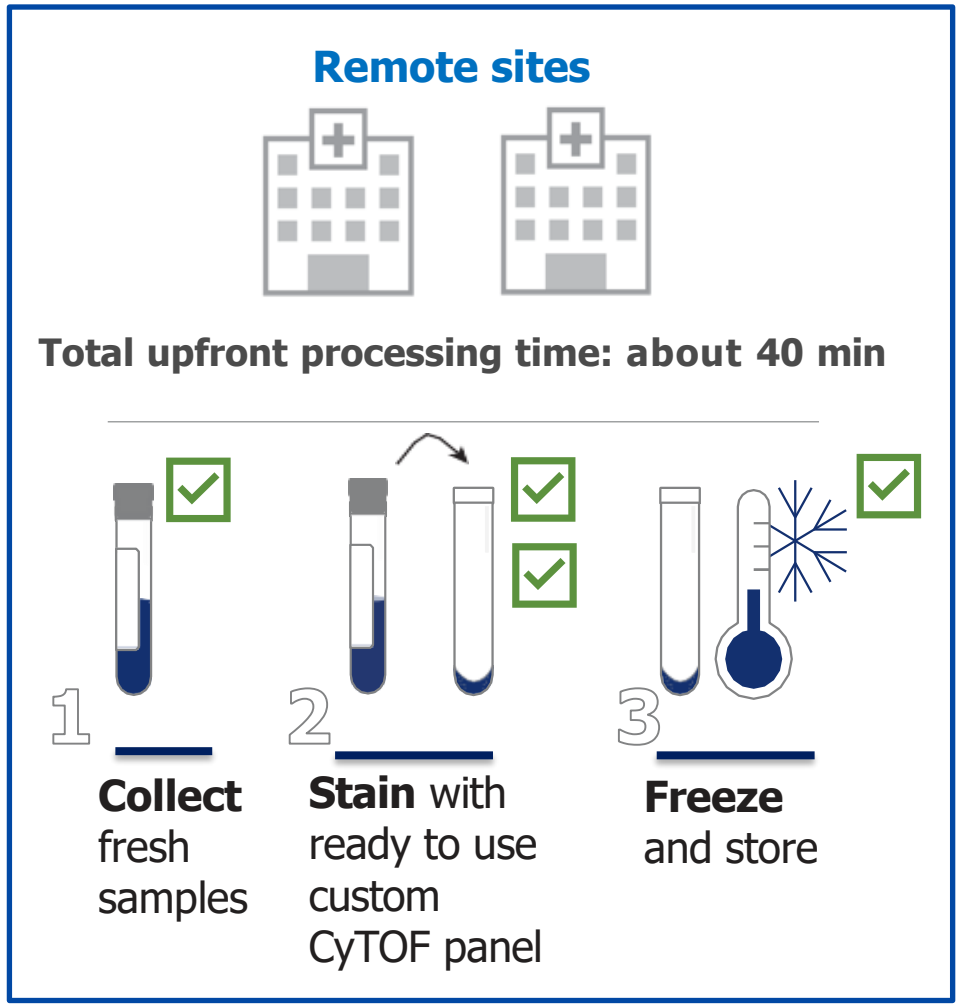


Proposed workflow



Integrating 'stopping points'

When is the best point for fixation/permeabilization?

Proposed workflow

Integrating 'stopping points'

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Remote sites

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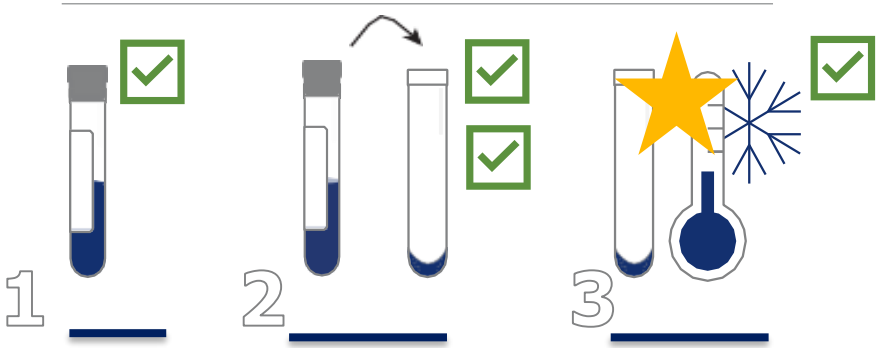

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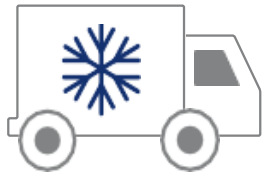

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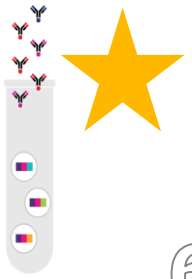

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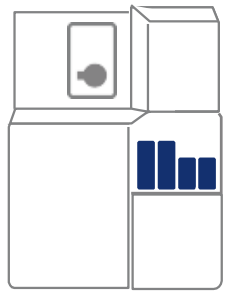
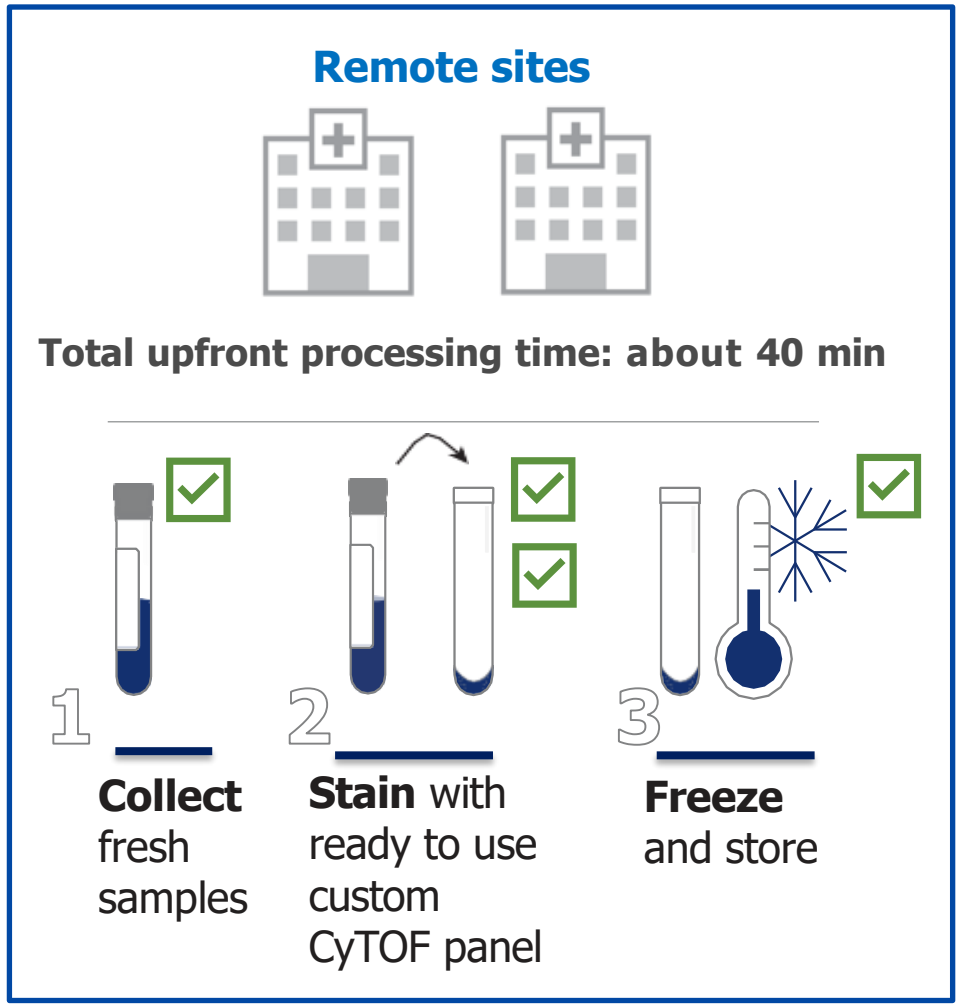


6 **Acquire** on CyTOF system

7 **Automated report**

Proposed workflow

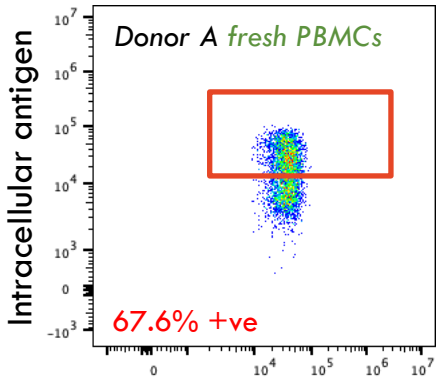


Integrating 'stopping points'

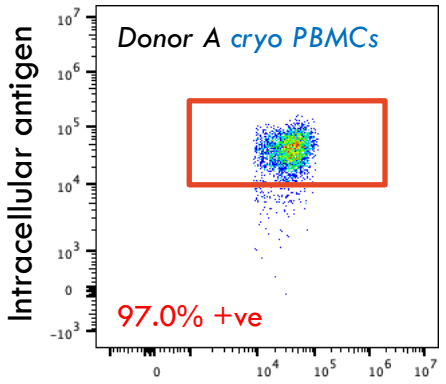
When is the best point for fixation/permeabilization?

Fix before freeze

Samples are stabilised for long-term storage and transport

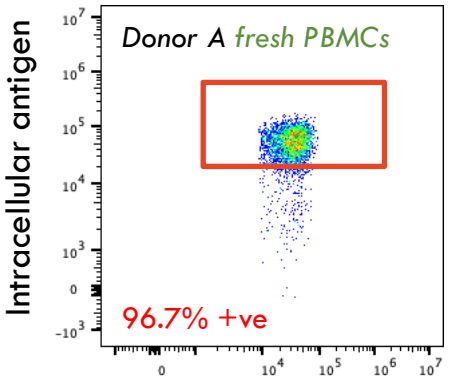


Typical workflow



Fix after thaw

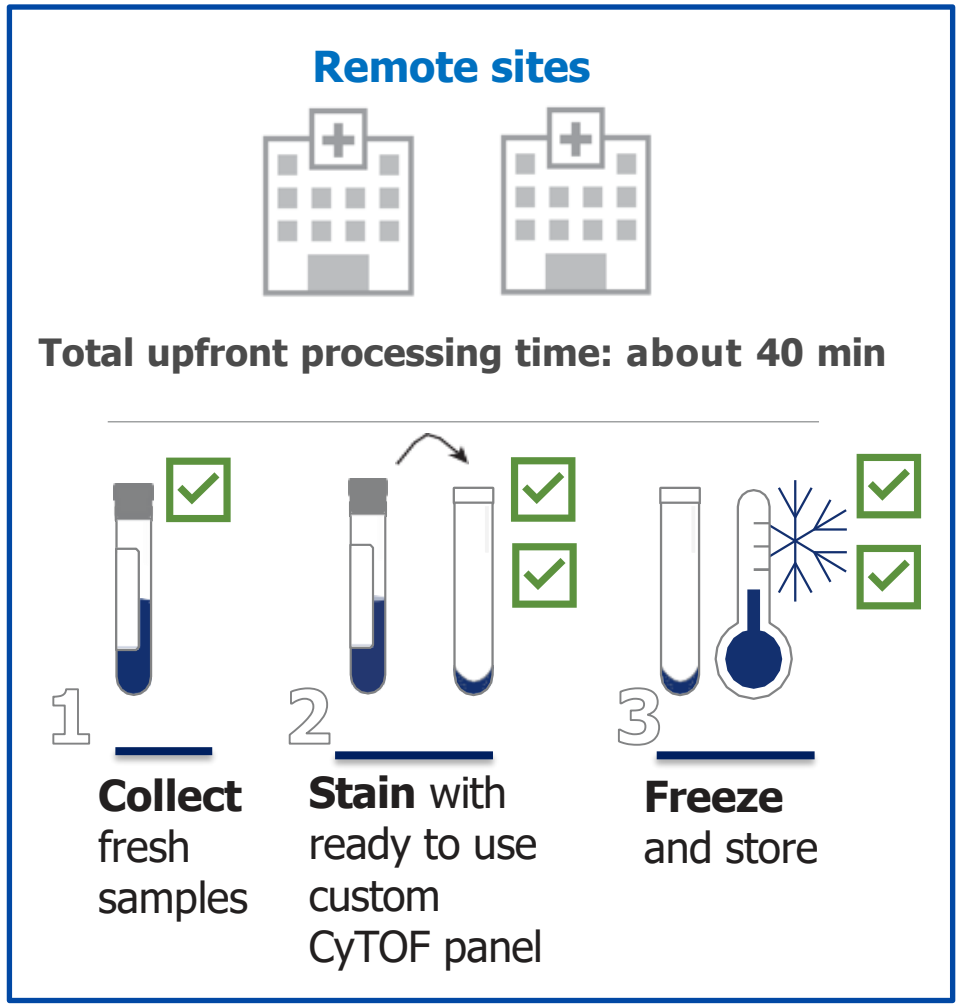
Samples can be stained for further surface antigens later.



Exposure to DMSO during freeze increases cell permeability **ONLY** if cells are not fixed before frozen.

This permeability makes cells permissive to granular staining.

Proposed workflow

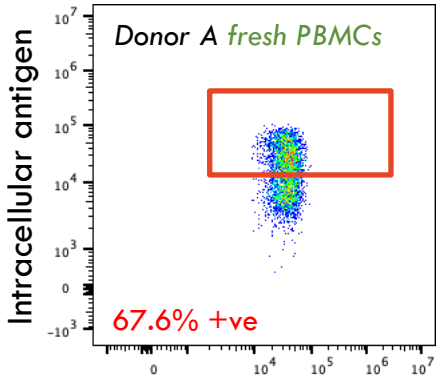


Integrating 'stopping points'

When is the best point for fixation/permeabilization?

Fix before freeze

Samples are stabilised for long-term storage and transport



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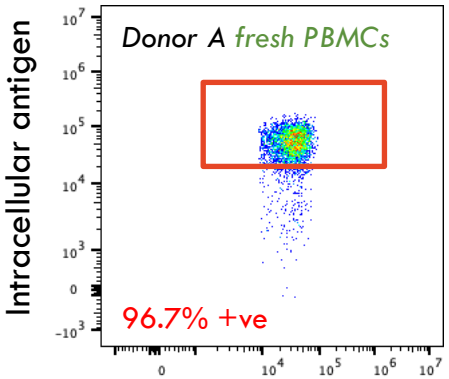
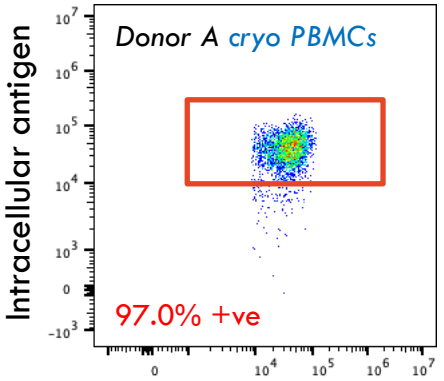


Typical workflow



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~~Proposed~~ implemented workflow

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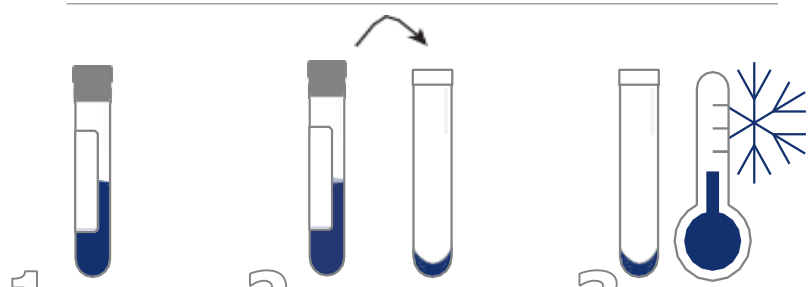
Supported by an ASI postgraduate travel award

University of Otago

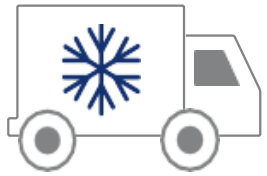
Total upfront processing time: about 40 min



1 **Collect** fresh samples

2 **Stain** with ready to use custom CyTOF panel

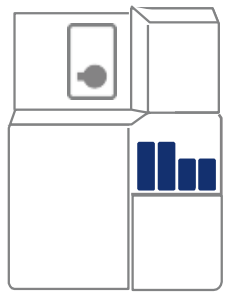
3 **Freeze** and store



4 **Ship** to central processing site (Charles Perkins Centre)

Sydney (Charles Perkins Centre)

5 **Barcode** and stain samples (intracellular targets)



6 **Acquire** on CyTOF system

7 **Automated report**

~~Proposed~~ implemented workflow

Australian and New Zealand SOCIETY FOR IMMUNOLOGY INC.

Supported by an ASI postgraduate travel award

University of Otago

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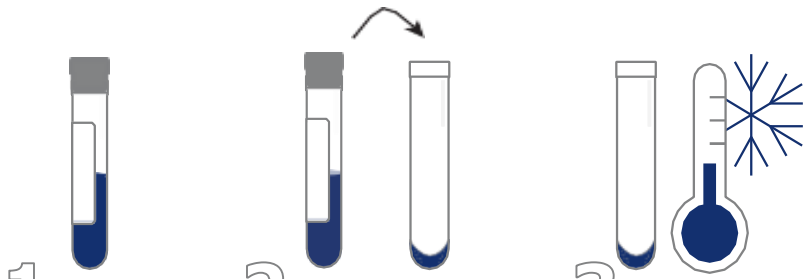

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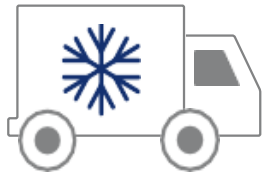

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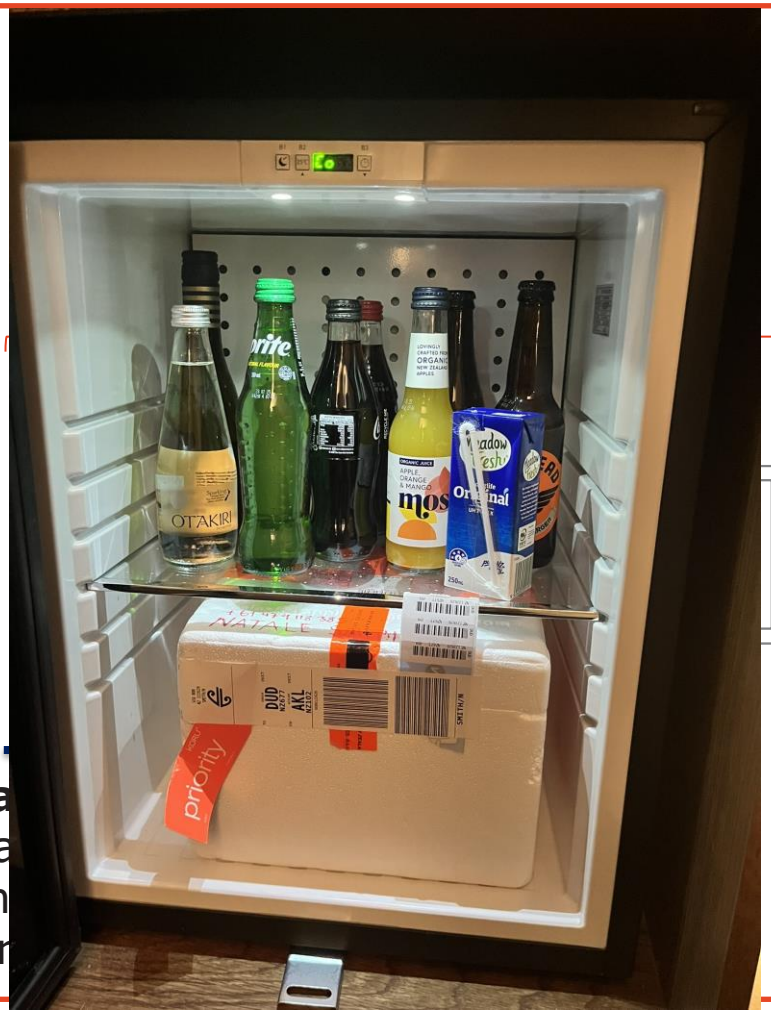


~~Proposed~~ implemented workflow

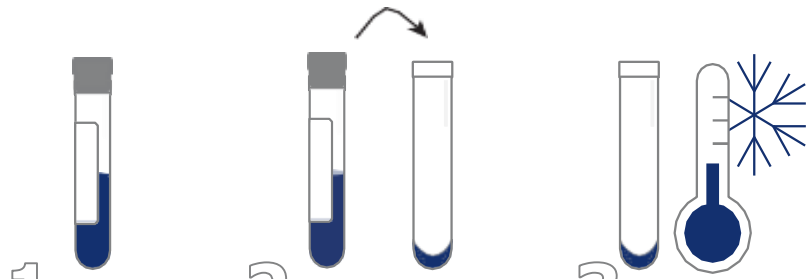
Australian and New Zealand SOCIETY FOR IMMUNOLOGY INC.

Supported by an ASI postgraduate travel award

Brad Devery

University of Otago

Total upfront processing time: about 40 min



1 **Collect** fresh samples

2 **Stain** with ready to use custom CyTOF panel

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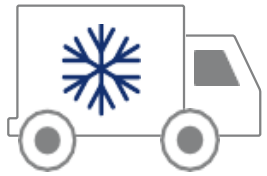

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5 **Ba** sta (in tar **d**

~~Proposed~~ implemented workflow

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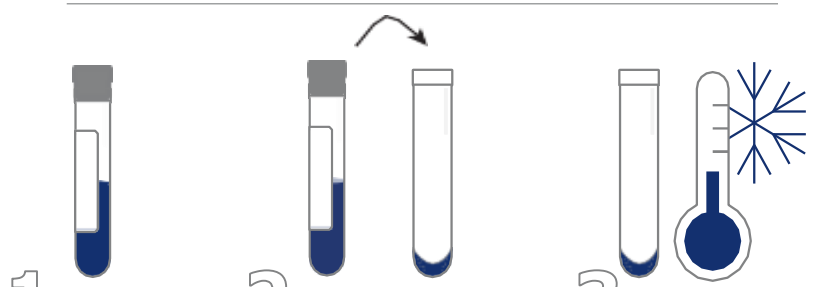
In progress (n = 3 so far!)

University of Otago

Total upfront processing time: about 40 min



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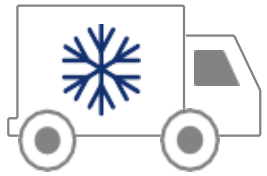
3 **Freeze** and store

Late November

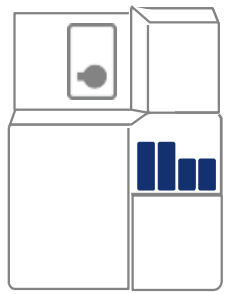


4 **Ship** to central processing site (Charles Perkins Centre)

Complete in early December

Sydney (Charles Perkins Centre)

5 **Barcode** and stain samples (intracellular targets)



6 **Acquire** on CyTOF system

7 **Automated report**

What's next?

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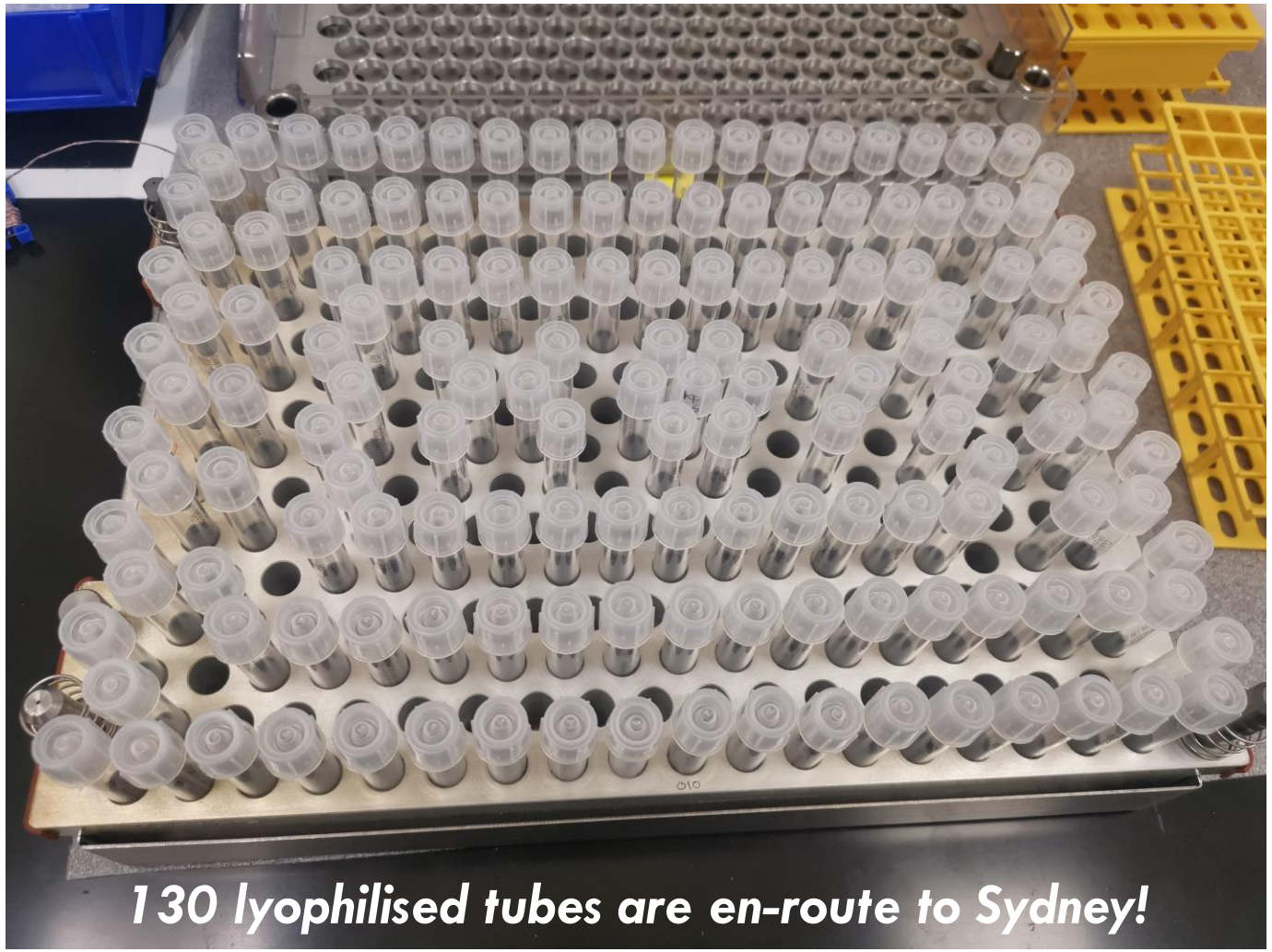
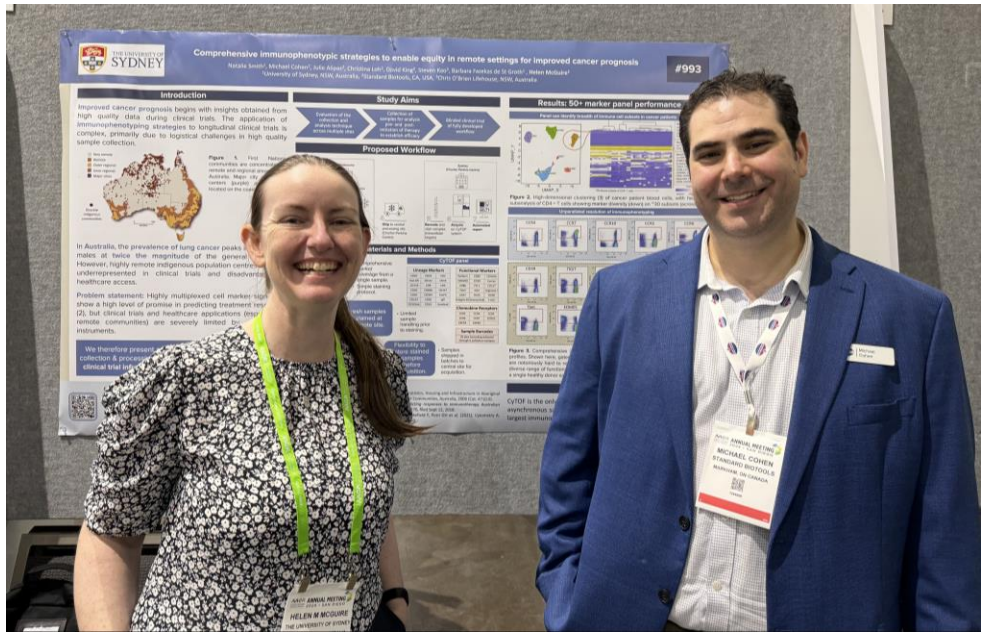


Implementation of our workflow across several sites to ensure the best quality data collection from multi-centre clinical trials.

Establishment of a remote/rural network.

Dr Helen McGuire ***Dr Michael Cohen***



What's next?

Australian and New Zealand SOCIETY FOR IMMUNOLOGY INC.

Supported by an ASI postgraduate travel award

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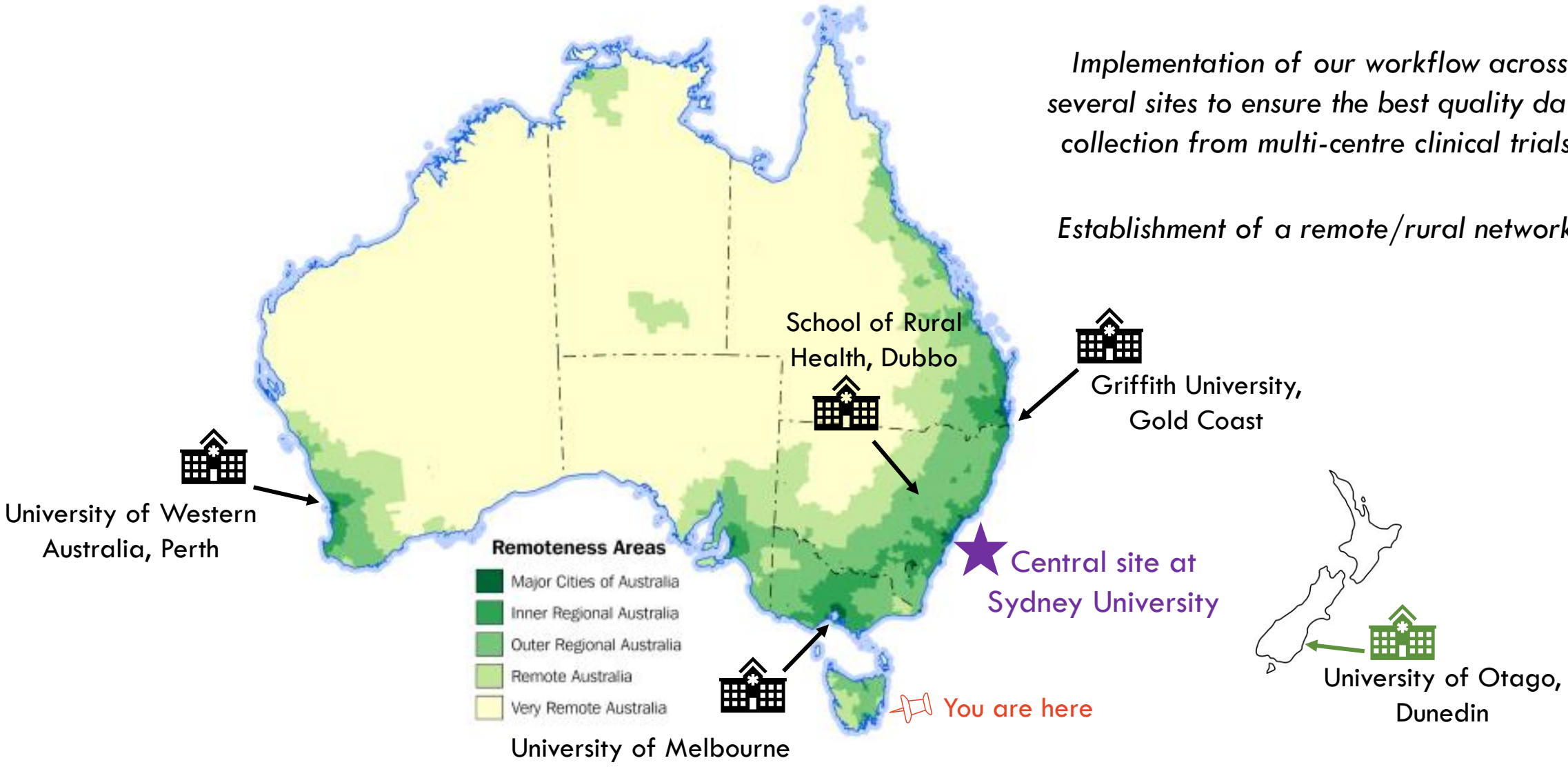
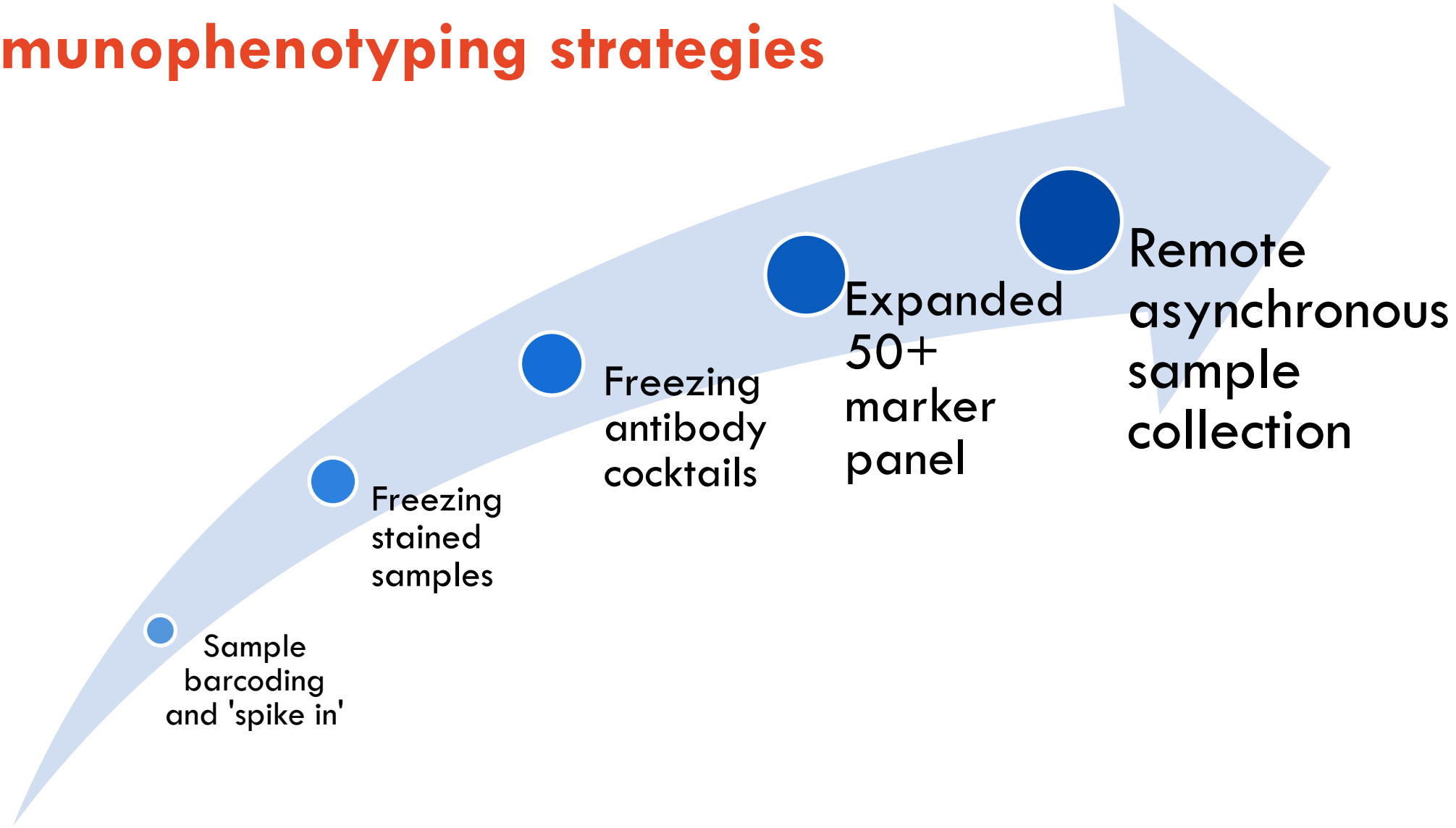


Mass cytometry enables *enhanced* comprehensive immunophenotyping strategies



Acknowledgements:

Thank you to the patients and their families.

USyD Translational Immunology Group

Dr Helen McGuire
Thomas Hueneburg
Annabel Faulkner
Prof. Barbara Fazekas
Dr Navneet Singh
Ash Abe

University of Otago

Prof Roslyn Kemp
Brad Devery

Clinical Collaborators

Chris O'Brien Lifehouse
A/Prof Steven Kao*
Prof Michael Boyer

Woolcock Institute/RPA
Dr Anna McLean*
Prof Maiija Kohonen-Corish

EnRICH – Dr Bea Brown

Australasian Cytometry Society ACS Travel Award

Funding Bodies

NHRMC
Cancer Council NSW
Sydney Cancer Partners
Sydney Health Partners MRFF
Sydney Catalyst
ASI Postgraduate Travel Award

Industry Support

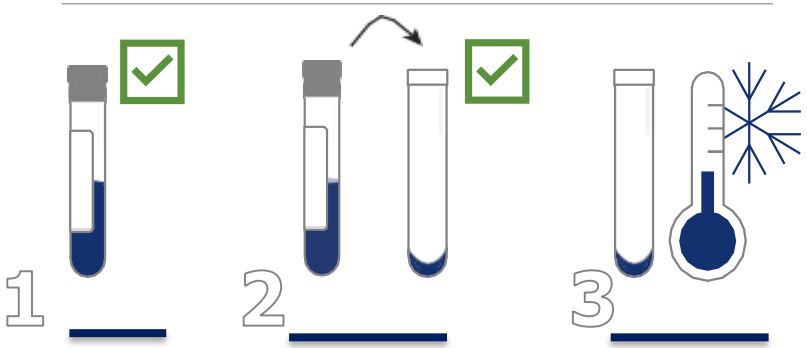
Standard Biotools
ImmuneSignatures Pty Ltd
Beckman Coulter International

nsmi9578@uni.sydney.edu.au

Proposed workflow

Remote sites

Total upfront processing time: about 40 min

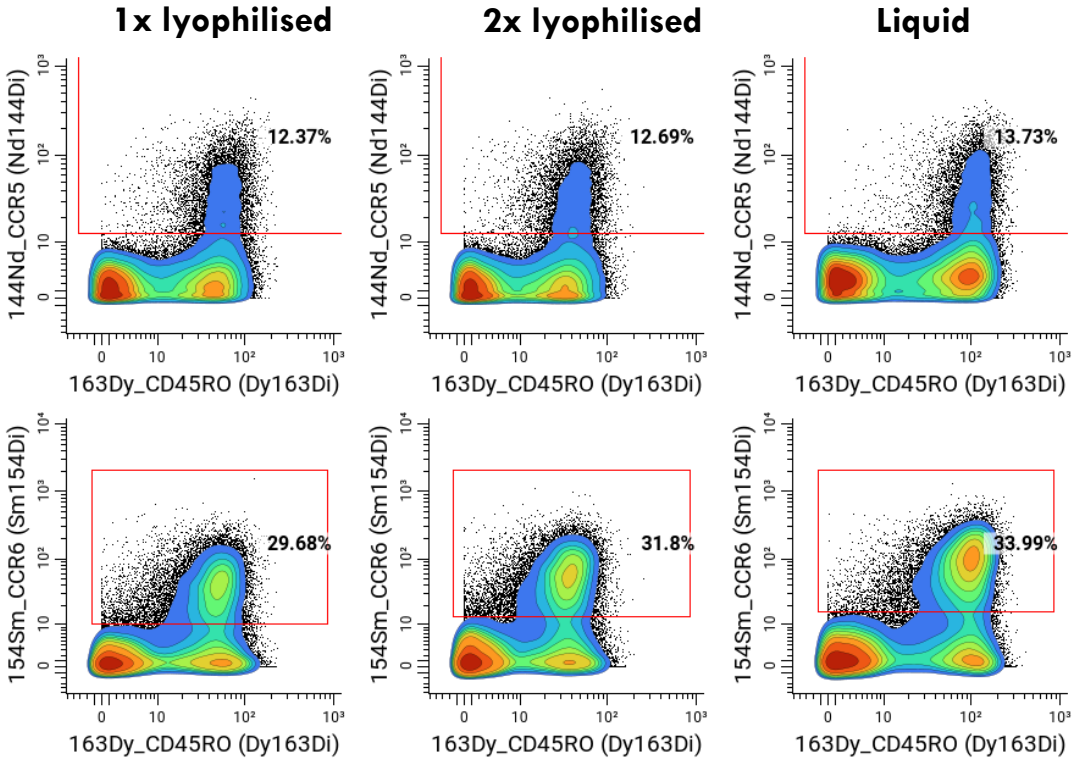


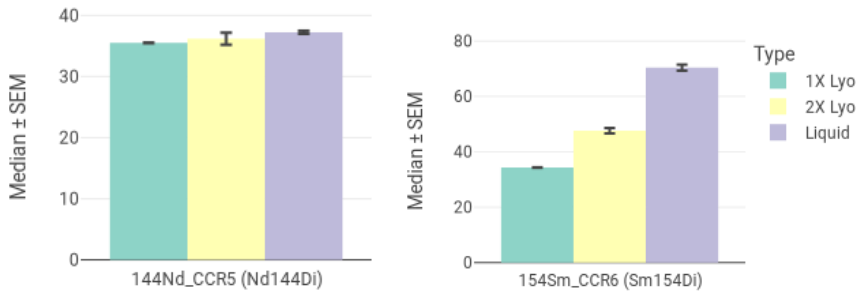
Collect fresh samples

Stain with ready to use custom CyTOF panel

Freeze and store

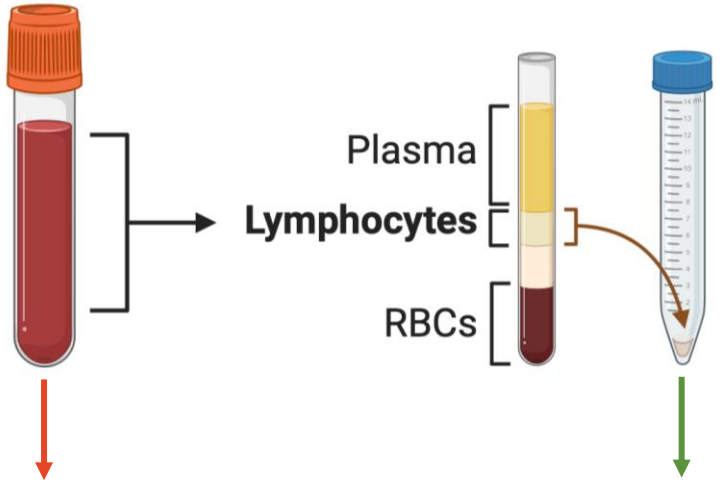
Lyophilised antibodies





Lyophilised formation is stable in the fridge for 12 months

Whole blood vs PBMCs

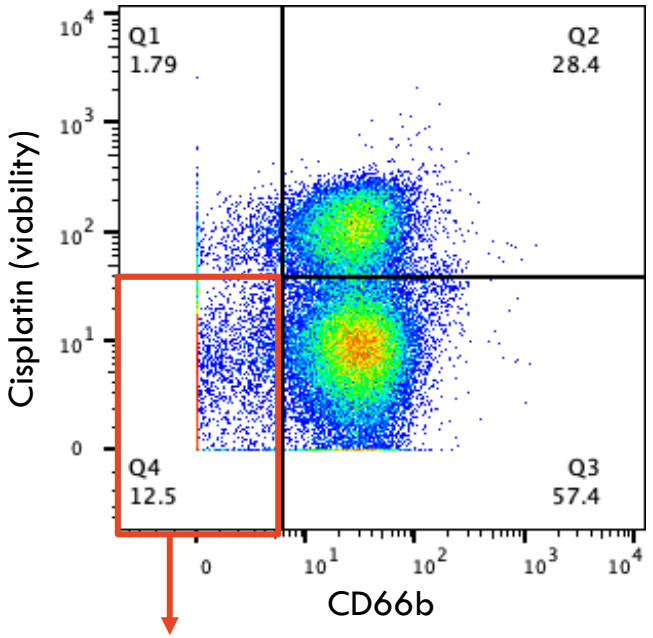


Whole blood and PBMCs from the same donor stained with mass cytometry panel

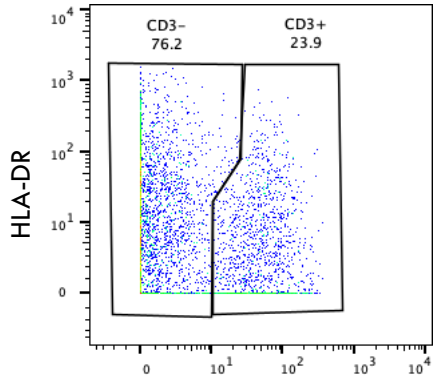
Samples barcoded together for acquisition

Although it adds 30-45 minutes to the protocol, PBMC separation is worthwhile

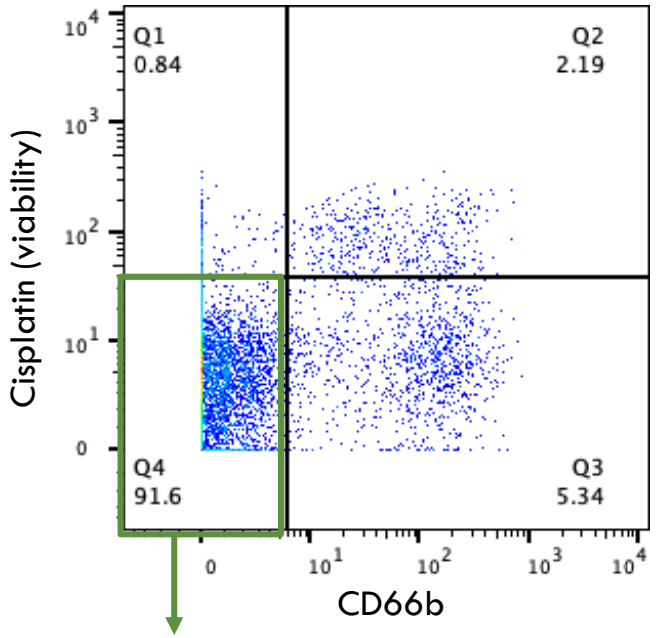
Fresh Whole Blood



12.5% *viable non-neutrophils*



Fresh PBMCs



91.6% *viable non-neutrophils*