Brodin_Lab™

Karolinska Institutet

Minimize technical variation to see more biological signal – an optimized blood sampling methods in Systems Immunology.

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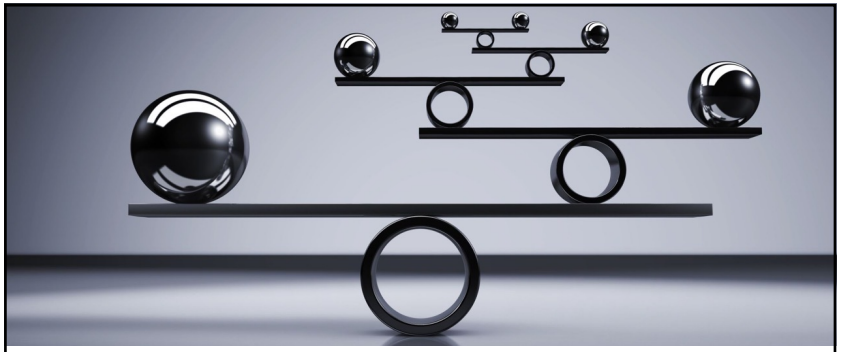
How the immune system works!?

2



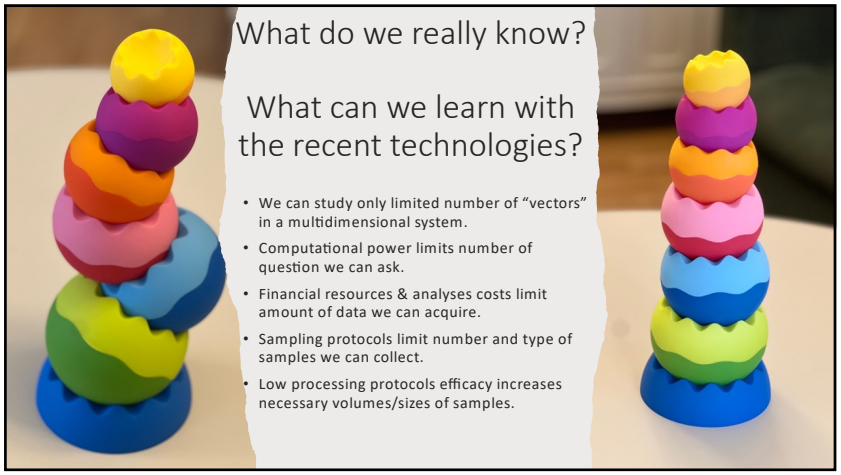
What is our understanding of the immune system functionality and stability?

3

Is this how it works?

Really?

4



6

Our science is as good as our samples

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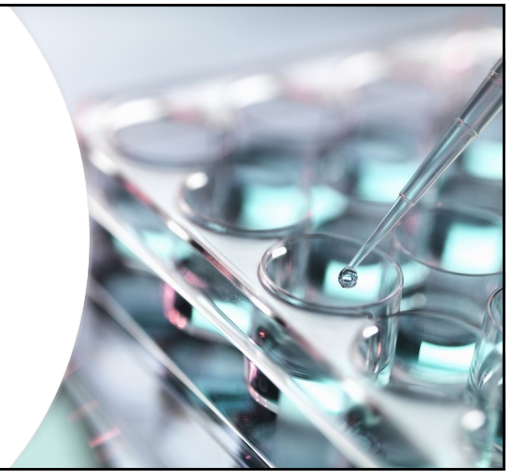
Why do we need high quality samples?



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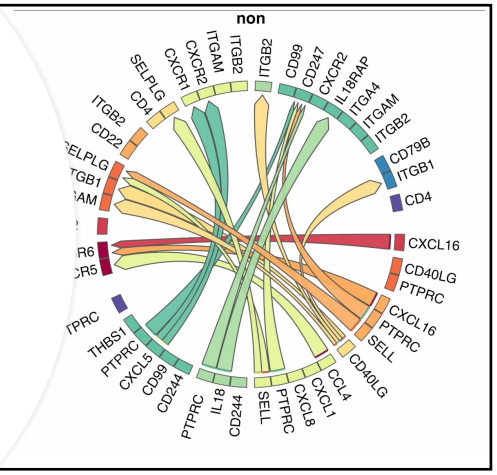
…because we need high-quality data



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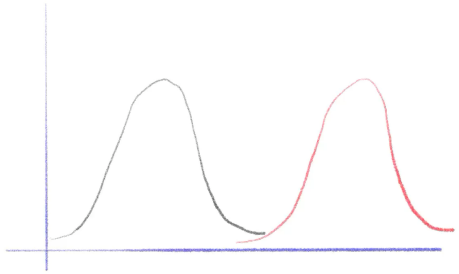
"Signal vs noise ratio"

IDEAL DATA



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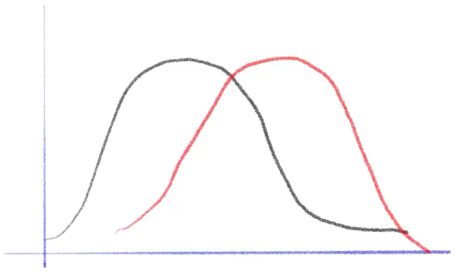
"Signal vs noise ratio"

REAL DATA



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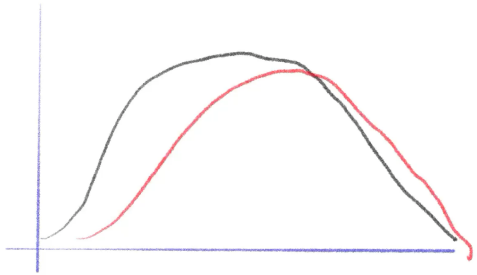
"Signal vs noise ratio"

BAD DATA



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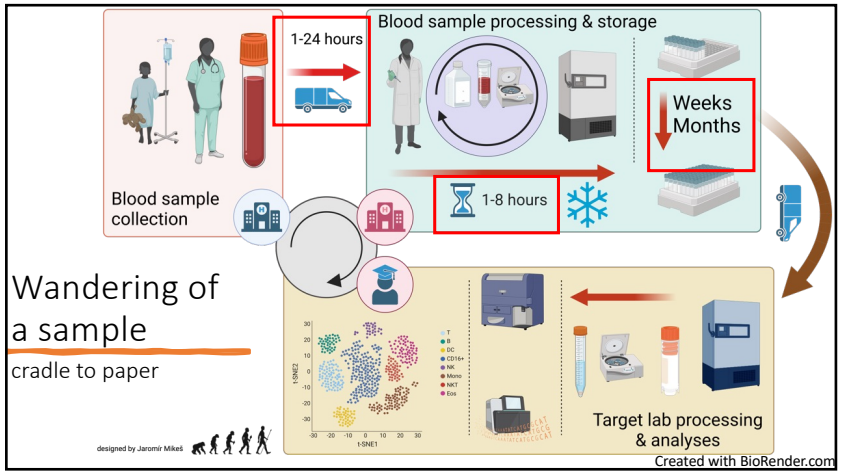

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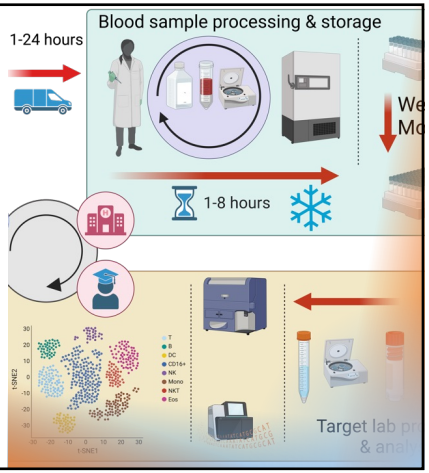
Sources of noise in sampling

- Blood sample collection
  - ⊕ Hospital personnel, nurse (OPERATOR)
  - ⊕ Transportation time and conditions
- Sample processing & storage
  - ⊕ Lab personnel (OPERATOR)
  - ⊕ Lab equipment
  - ⊕ Storage time
- Sample processing and final analyses
  - ± Lab personnel/Automation
  - ⊕Lab equipment
  - ⊕Storage time



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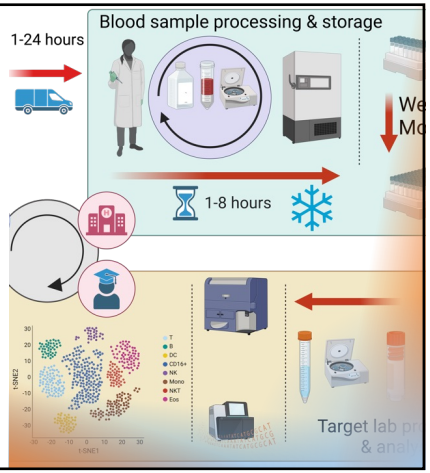

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Identified problems

Sampling & processing protocols are

toooooooooo long

Too many steps and operators

=

LOW THROUGHPUT

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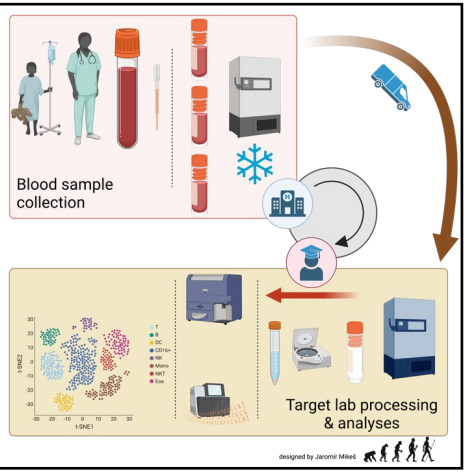
The solution: skip the unnecessary steps

- Minimize time between sampling and cryogenic preservation
- Postpone sample processing for months or 1+ years
- Minimize number of operations
- Minimize human errors
- Increase throughput



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How much blood is really needed?

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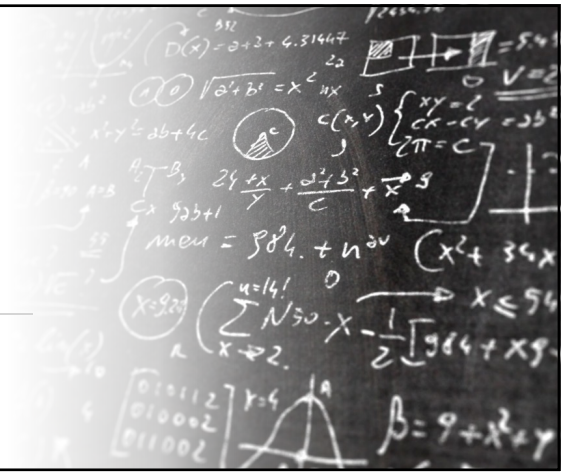
Math homework



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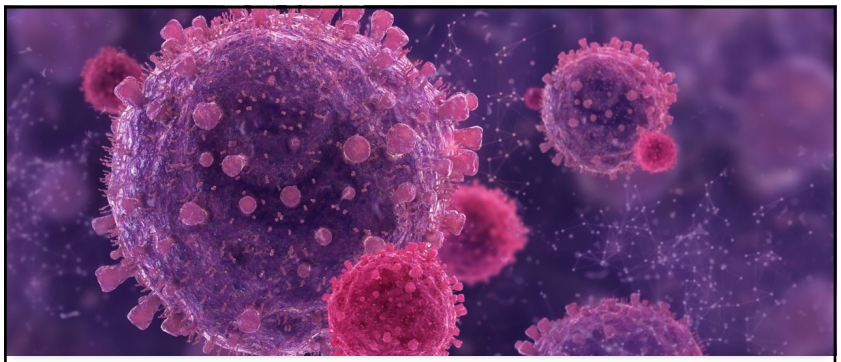
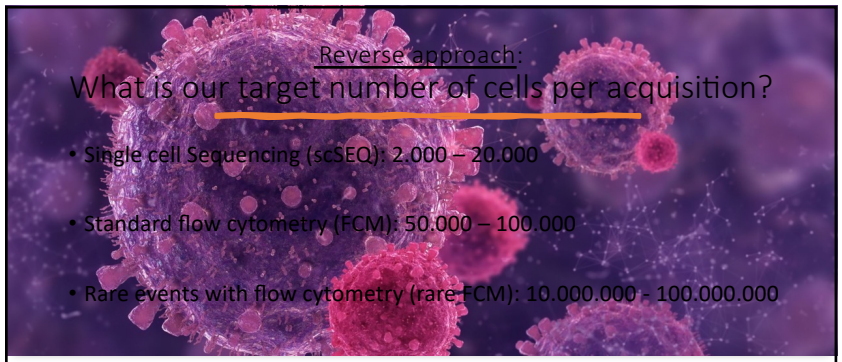


How many cells do we need?

20



How many cells do we need?

21

Modelling: number of cells * efficacy

Expected # cells (Standard blood count)

VOL [µL]		Efficacy 100%	75%	50%	25%	10%
1	Low RANGE	4 000	3 000	2 000	1 000	400
	Upp RANGE	7 000	5 250	3 500	1 750	700
10	Low RANGE	40 000	30 000	20 000	10 000	4 000
	Upp RANGE	70 000	52 500	35 000	17 500	7 000
100	Low RANGE	400 000	300 000	200 000	100 000	40 000
	Upp RANGE	700 000	525 000	350 000	175 000	70 000
1 000	Low RANGE	4 000 000	3 000 000	2 000 000	1 000 000	400 000
	Upp RANGE	7 000 000	5 250 000	3 500 000	1 750 000	700 000

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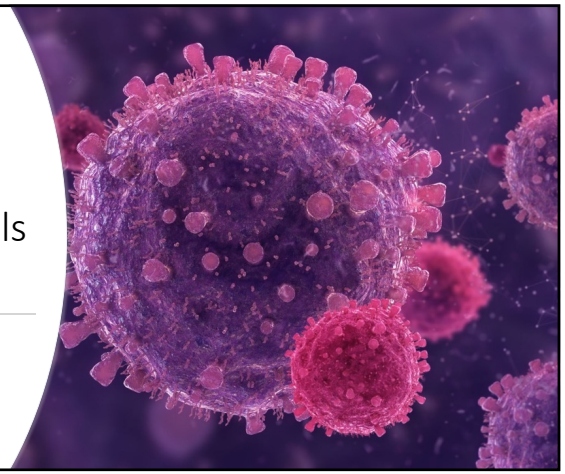
How many cells do we loose?



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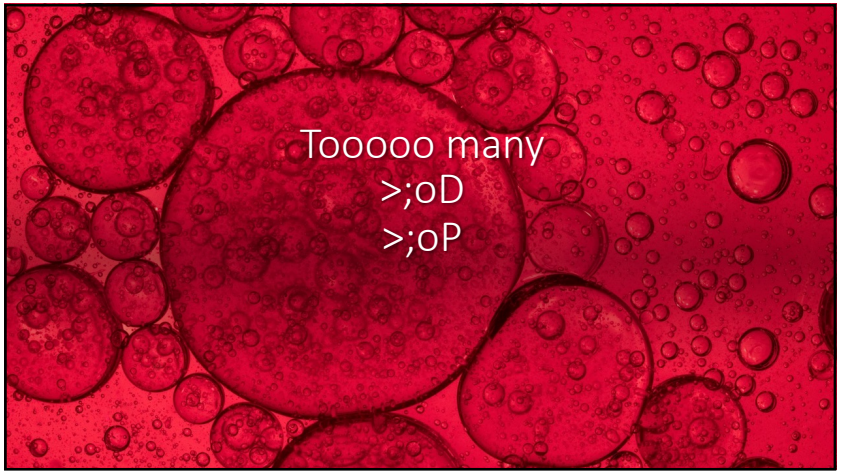

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Cell losses / casualties: factors to consider:

- Centrifugation and washing
  - RCF speed and centrifuge
  - Aspiration vs. decantation
  - Buffers' composition
- Sample's status quo
  - Viability
  - Fixation status
- Cell types composition
- Operator knowledge

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The same RCF is not the same RCF???

VS.

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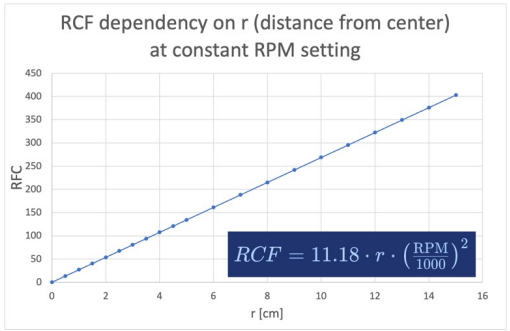
RCF is linear to the distance from the center of a centrifuge



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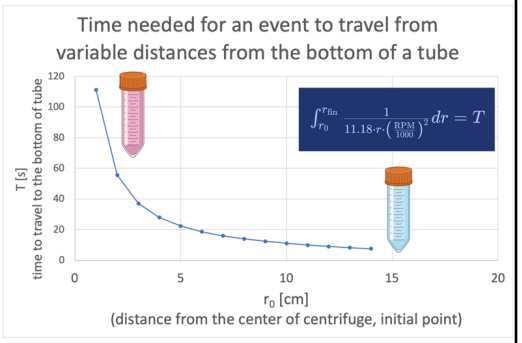
…but the **time to travel** to the bottom of the tube **is NOT**



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Whole blood | **VS** | PBMCs

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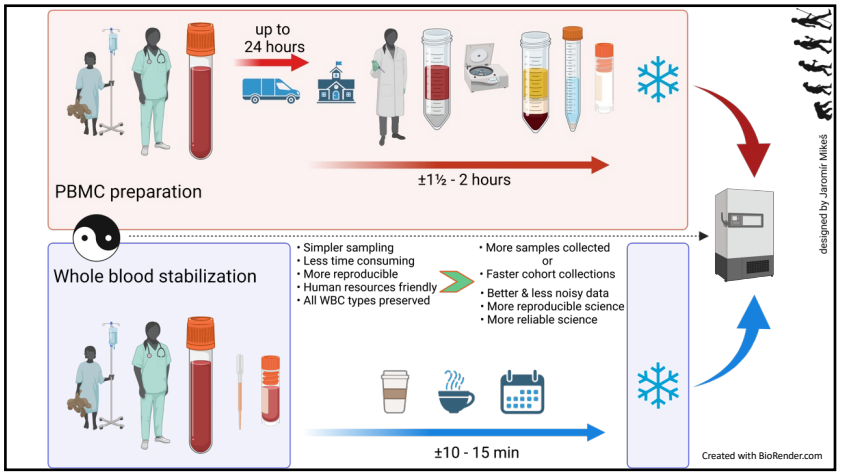
up to
24 hours
PBMC preparation
±1½ - 2 hours
Whole blood stabilization
• Simpler sampling
• Less time consuming
• More reproducible
• Human resources friendly
• All WBC types preserved
• More samples collected
or
• Faster cohort collections
• Better & less noisy data
• More reproducible science
• More reliable science
±10 - 15 min
designed by Jaromir Mikeš
Created with BioRender.com

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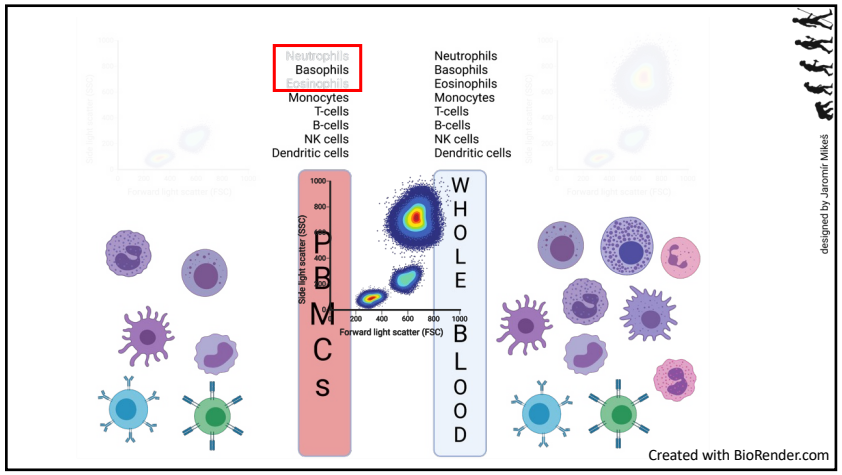
Neutrophils
Basophils
Eosinophils
Monocytes
T-cells
B-cells
NK cells
Dendritic cells
PBMCs
WHOLE BLOOD
Side light scatter (SSC)
Forward light scatter (FSC)
designed by Jaromir Mikeš
Created with BioRender.com

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High-
throughput
sampling

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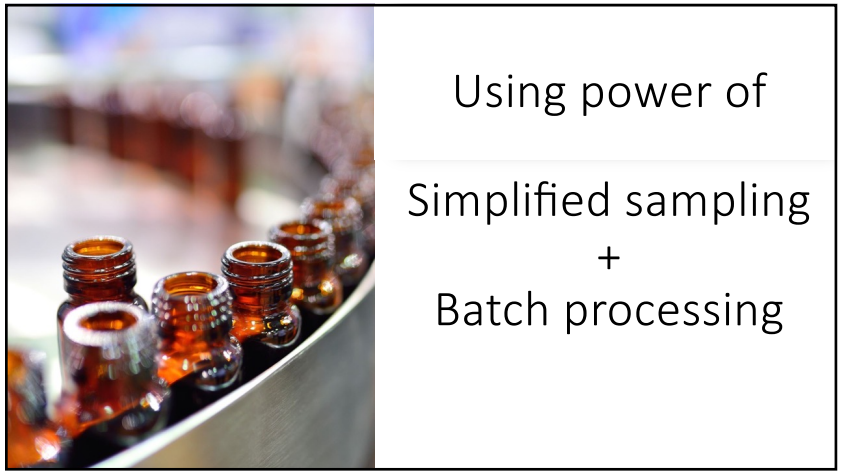
Using power of
Simplified sampling
+
Batch processing

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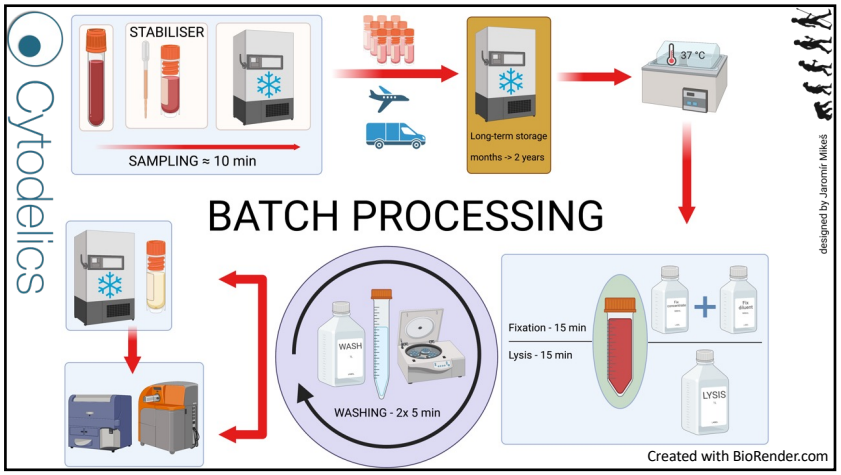

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How many samples can you collect per day?

Scalable *in-vitro* immunostimulation of human blood for in-depth profiling of acute immune response effects

Stefan Markus Reitzner[1,2], Petter Brodin[2,3], Jaromír Mikeš[2]
[1]Department of Physiology & Pharmacology, Karolinska Institutet, Stockholm, Sweden
[2]Department for Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden
[3]Department of Immunology and Inflammation, Imperial College London, London, United Kingdom

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900 samples

- Location: Bosön, Lidingö, Sweden
- Subjects: 12 (martial arts)
- Time: 1 day

Subjects #	12					
			After exercise			
	Time point	Baseline	0 h	0.5 h	1 h	2 h
CTRL	PAXgene	1.0	1.0	1.0	1.0	1.0
	Cytodelics Stabiliser	0.5	0.5	0.5	0.5	0.5
	Plasma	2	2	2	2	2
Stimulation	PAXgene	0.9	0.9	0.9	0.9	0.9
CTRL	Cytodelics Stabiliser	0.1	0.1	0.1	0.1	0.1
	Plasma	≈0.5	≈0.5	≈0.5	≈0.5	≈0.5
Stimulation	PAXgene	0.9	0.9	0.9	0.9	0.9
LPS	Cytodelics Stabiliser	0.1	0.1	0.1	0.1	0.1
	Plasma	≈0.5	≈0.5	≈0.5	≈0.5	≈0.5
Stimulation	PAXgene	0.9	0.9	0.9	0.9	0.9
IAV	Cytodelics Stabiliser	0.1	0.1	0.1	0.1	0.1
	Plasma	≈0.5	≈0.5	≈0.5	≈0.5	≈0.5
Stimulation	PAXgene	0.9	0.9	0.9	0.9	0.9
BCG	Cytodelics Stabiliser	0.1	0.1	0.1	0.1	0.1
	Plasma	≈0.5	≈0.5	≈0.5	≈0.5	≈0.5

SUM/subject = 75
SUM/Total = 900

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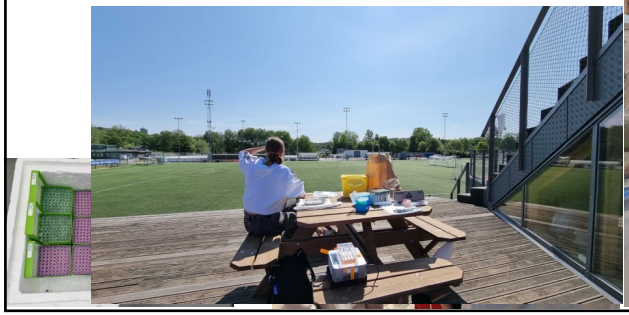
1500 samples

- Location: Kaunas, Lithuania
- Subjects: 20
  - endurance: marathon, long distance, pentathlon, swimming, biking
  - strength: olympic weightlifting, powerlifting, bodybuilding
- Time: 2 days



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What are the keystones?

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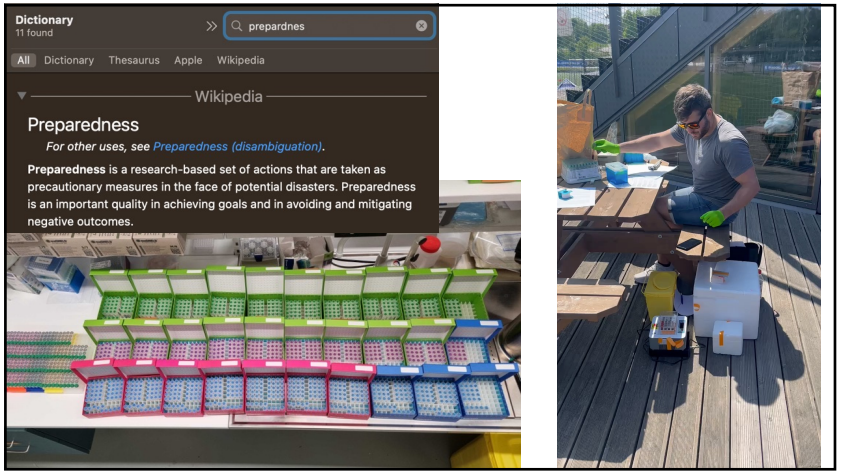

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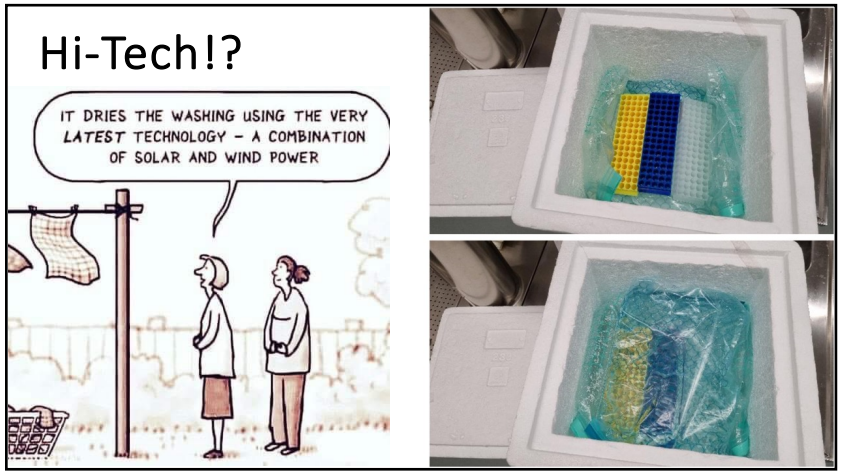

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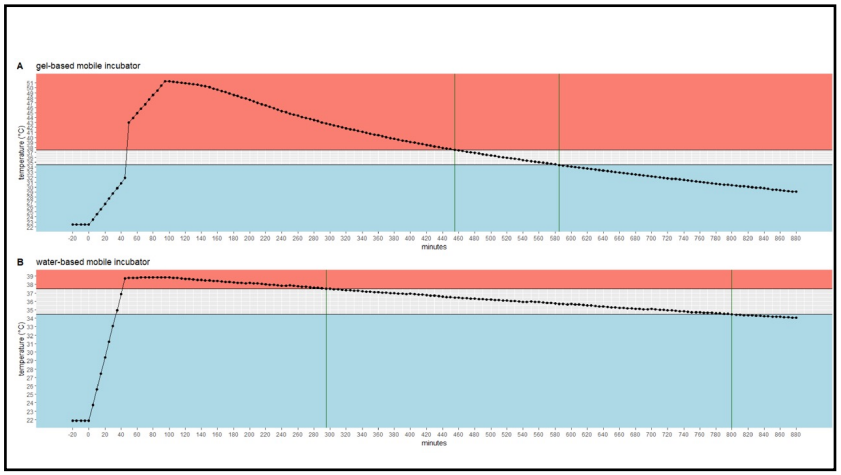

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Dictionary

dedication | ˌdɛdɪˈkeɪʃn |

noun *[mass noun]*

1 the quality of being dedicated or committed to a task or purpose: *his* ***dedication to*** *his duties.*

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Dictionary

sacrifice | ˈsakrɪfʌɪs |

noun

1 an act of slaughtering an animal or person or surrendering a possession as an offering to a deity: *they offer sacrifices to the spirits* | *[mass noun] : the ancient laws of animal sacrifice.*
- an animal, person, or object offered in the act of sacrifice: *a flat cake offered by the Romans as a sacrifice to their gods.*

2 *Christian Church* Christ's offering of himself in the Crucifixion.
- the Eucharist regarded either (in Catholic terms) as a propitiatory offering of the body and blood of Christ or (in Protestant terms) as an act of thanksgiving.

3 an act of giving up something valued for the sake of something else regarded as more important or worthy: *we must all be prepared to* ***make sacrifices.***
- *Chess* a move intended to allow the opponent to win a pawn or piece, for strategic or tactical reasons.
- (also sacrifice bunt or sacrifice fly) *Baseball* a bunted or fly ball which puts the batter out but allows a base runner to advance.
- (also sacrifice bid) *Bridge* a bid made in the belief that it will be less costly to be defeated in the contract than to allow the opponents to make a contract.

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Where are we now?

Sample volumes [ml]		
	Adults	babies
PAXgene	1.0	0.2
Stabiliser	0.5	0.1
PBMCs	1.5-2.0	0.2
Plasma	2x 0.5	4x 0.025

- Standardized blood volumes for each type of sample
- No leftovers: PAXgene or Stabiliser samples can be frozen from pellets after plasma extraction.

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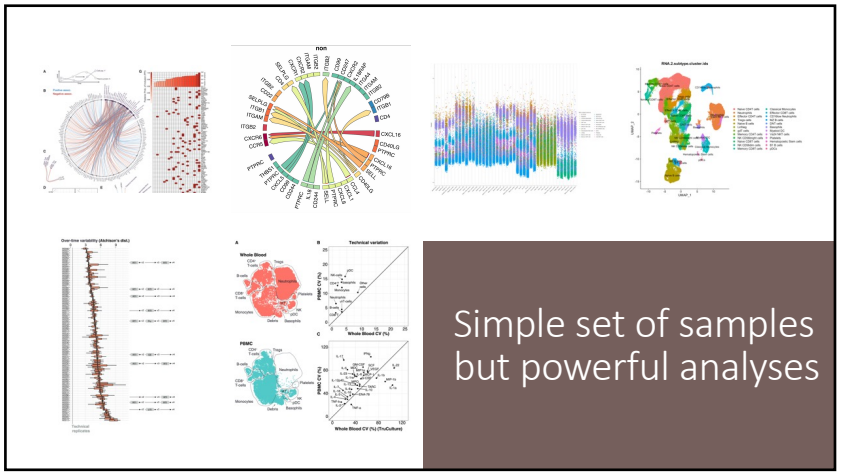

Simple set of samples but powerful analyses

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IMMUNE CELL FUNCTIONAL DEVELOPMENT

B/T lymphocytes – Naïve! (T cell memory cells at adult frequency by 18-24 months).

Cord blood Neutrophils – impaired chemotaxis and acute granulopoiesis. Adult level ROS and phagocytosis.
Carr R. Br J Hematology, 2000

Cord blood Monocytes – Impaired cytokine responses and antigen presentation
Krow-Lucal et al, Blood, 2014 & De Kleer et al, Frontiers in Immunology, 2014

Cord blood DCs – Impaired cytokine responses and antigen presentation
De Kleer et al, Frontiers in Immunology, 2014

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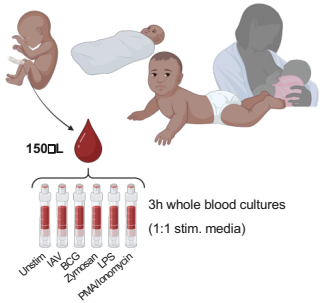
IMMUNE CELL FUNCTIONAL DEVELOPMENT



designed by Petter Brodin

Method development: Jaromir Mikes, Tadepally Lakshmikanth, Jun Wang

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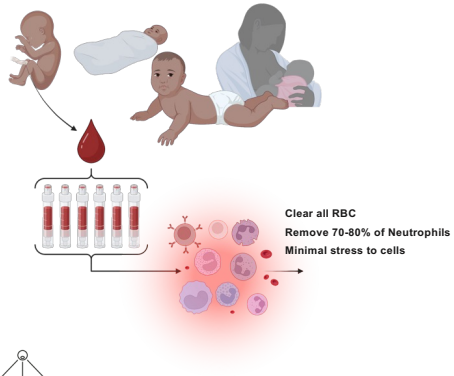
IMMUNE CELL FUNCTIONAL DEVELOPMENT



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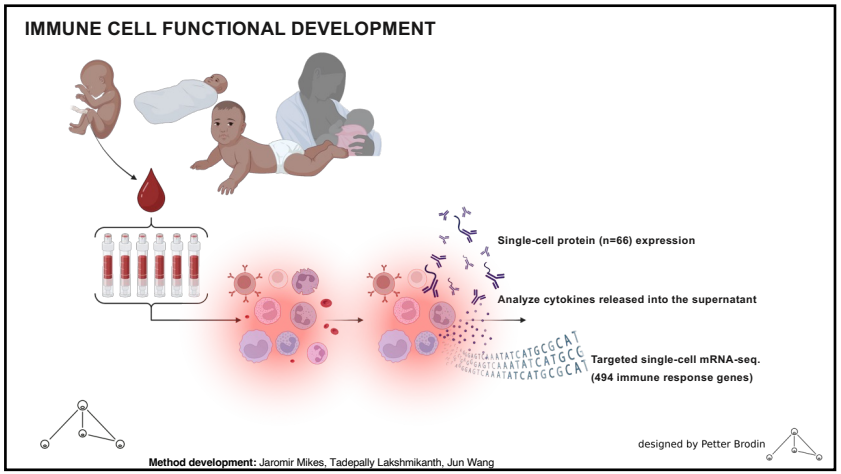

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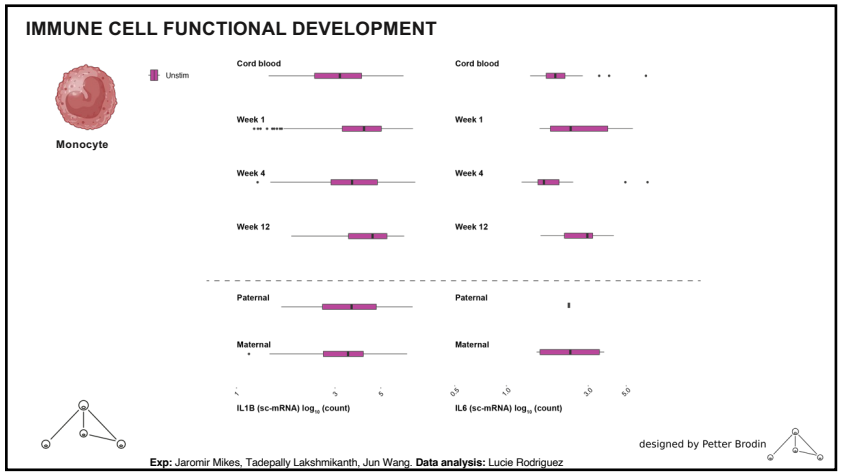

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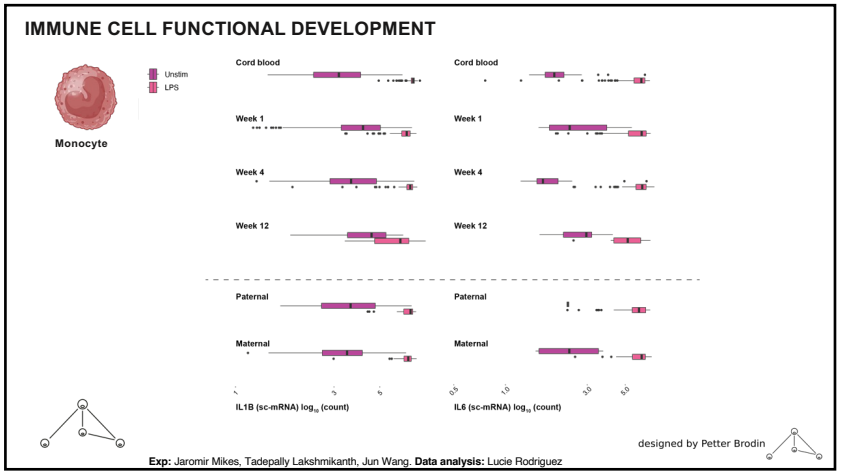

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Why do we do it?

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…because timely, precise, and sophisticated analyses can make the difference between death and life

https://www.theacquiregroup.com.au/sense-of-purpose-happy-life/

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Acknowledgement

Cytodelics

I'm a co-cofounder and CRO of Cytodelics AB

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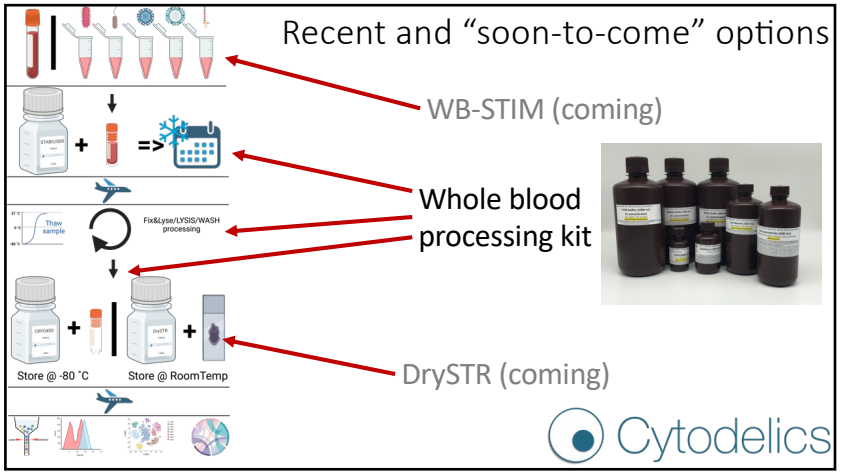

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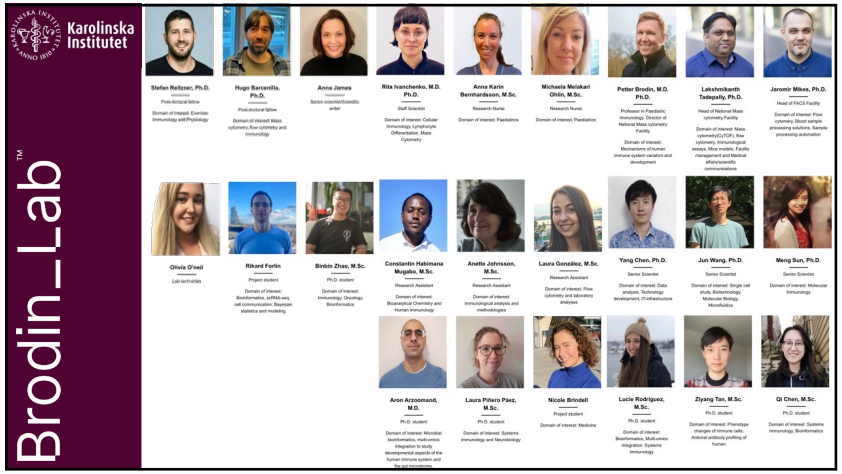

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