


Brodin_Lab™



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

Jaromír Mikeš

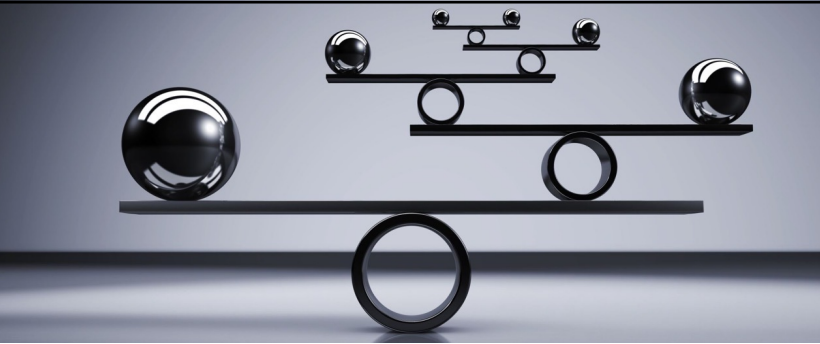
Department for Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

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How the immune system works!?

2



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

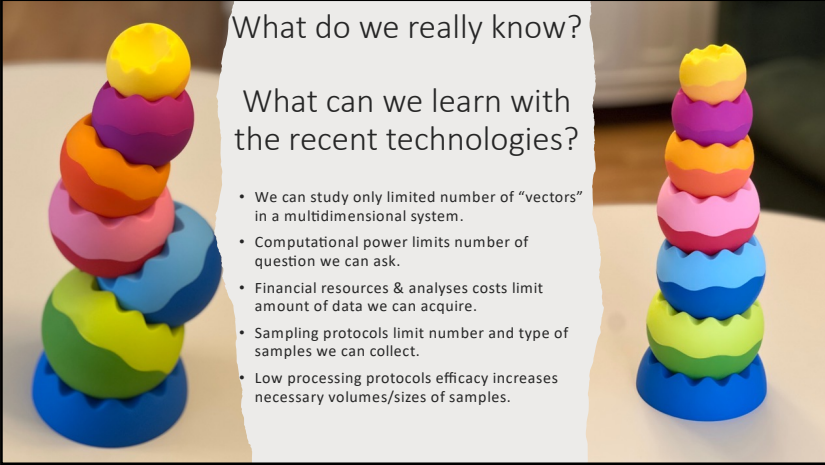
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Is this how it works?

Really?

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What do we really know?
What can we learn with the recent technologies?

- We can study only limited number of “vectors” in a multidimensional system.
- Computational power limits number of question we can ask.
- Financial resources & analyses costs limit amount of data we can acquire.
- Sampling protocols limit number and type of samples we can collect.
- Low processing protocols efficacy increases necessary volumes/sizes of samples.

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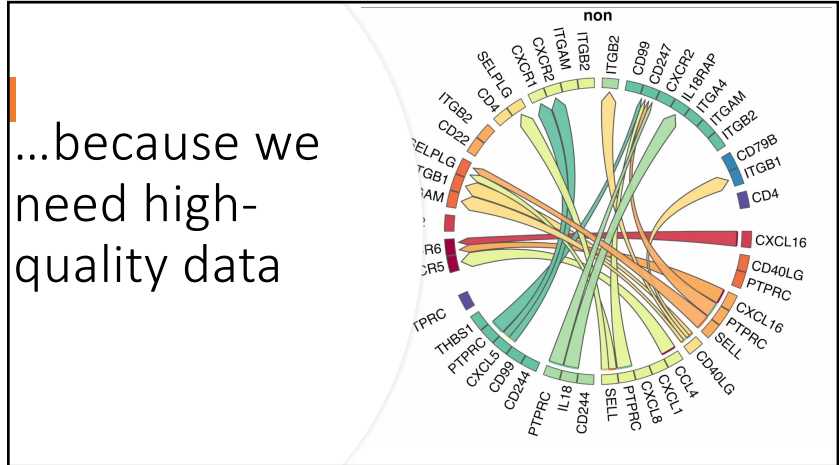
Our science is as good as our samples

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Why do we need high quality samples?

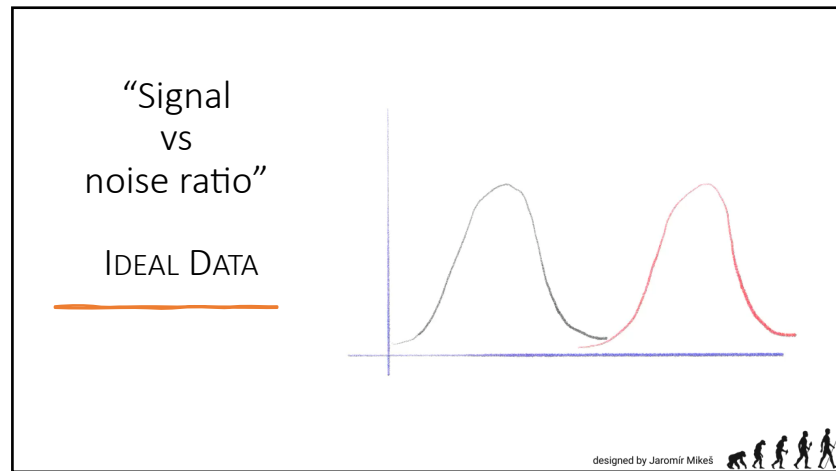
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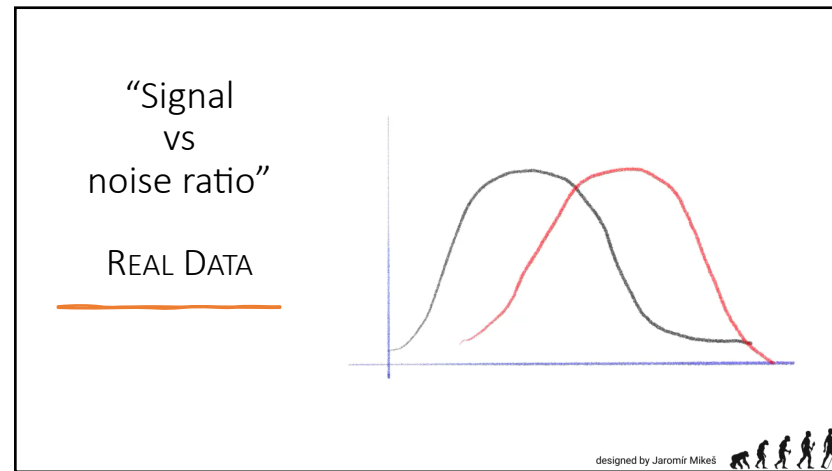
...because we need high-quality data

The diagram is a circular chord diagram with various cell surface markers and receptors labeled around the perimeter. The labels include: ITGB2, CD22, SELPLG, ITGB1, TAM, R6, R5, PTPRC, THBS1, PTPRC, CXCL5, CD98, CD244, IL18, CD244, SELL, CXCR1, CXCR2, CXCR4, CXCR6, CXCR1, CXCR2, CXCR4, CXCR6, CXCL1, CXCL5, CD4, ITGB2, ITGB2, CD99, CD247, CXCR2, IL18R1p, ITGB4, ITGB4, ITGB2, CD79B, ITGB1, CD4, CXCL16, CD40LG, PTPRC, CXCL16, SELL, CD40LG, CD4, ITGB2, ITGB2, CD99, CD247, CXCR2, IL18R1p, ITGB4, ITGB4, ITGB2, CD79B, ITGB1, CD4, CXCL16, CD40LG, PTPRC, CXCL16, SELL, CD40LG, CD4, ITGB2, ITGB2, CD99, CD247, CXCR2, IL18R1p, ITGB4, ITGB4, ITGB2, CD79B, ITGB1, CD4, CXCL16, CD40LG, PTPRC, CXCL16, SELL, CD40LG, CD4.

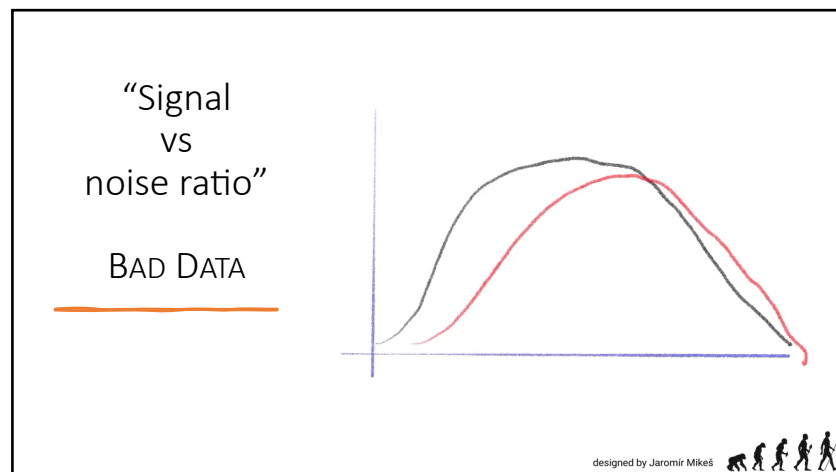
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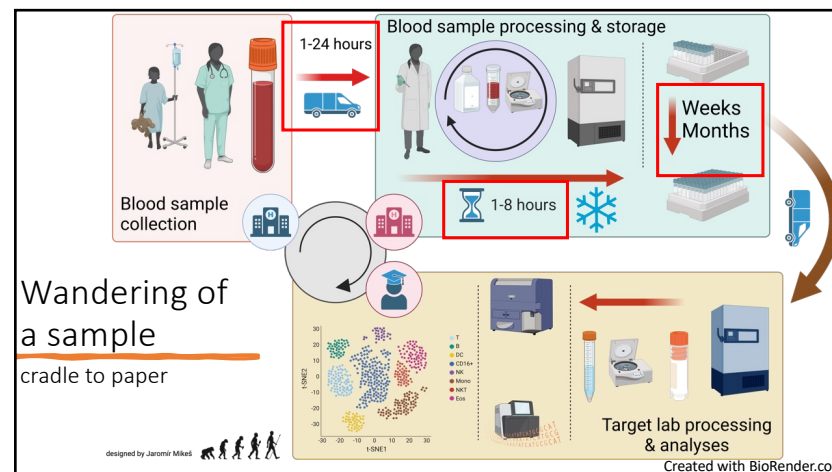
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Sources of noise in sampling

- 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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Identified problems

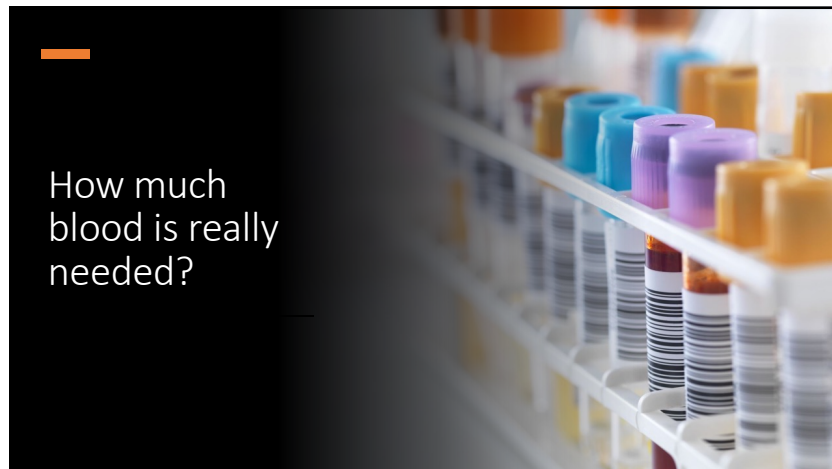
- Sampling & processing protocols are tooooooo long
- Too many steps and operators = LOW THROUGHPUT

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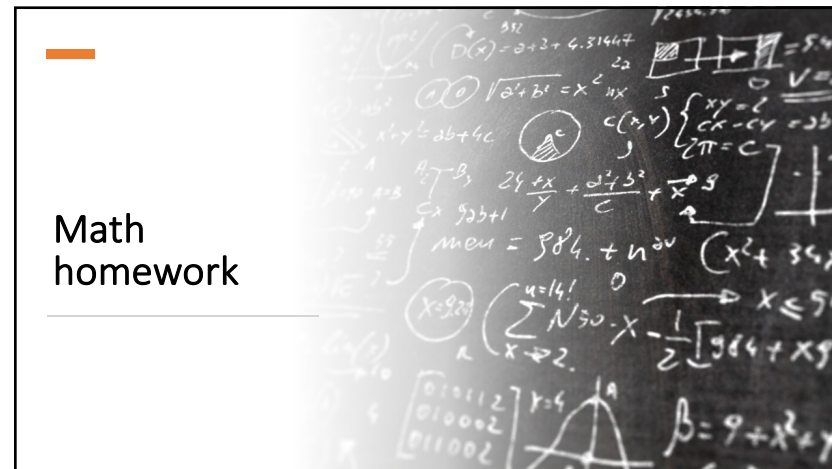
The solution: skip the unnecessary steps

- 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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Reverse approach:
What is our target number of cells per acquisition?

- Single cell Sequencing (scSEQ): 2,000 – 20,000
- Standard flow cytometry (FCM): 50,000 – 100,000
- Rare events with flow cytometry (rare-FCM): 10,000,000 - 100,000,000

How many cells do we need?

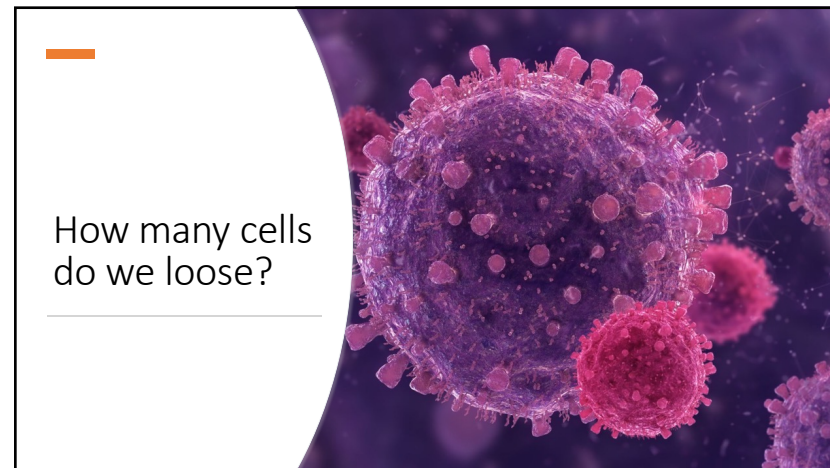
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Modelling: number of cells * efficacy

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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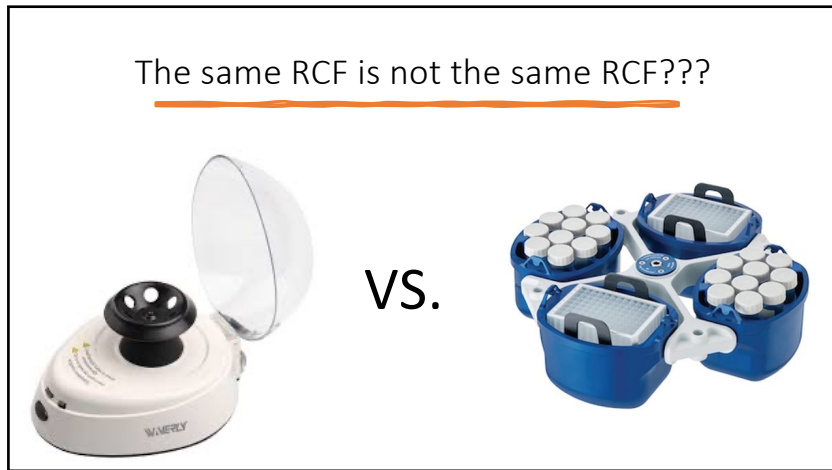


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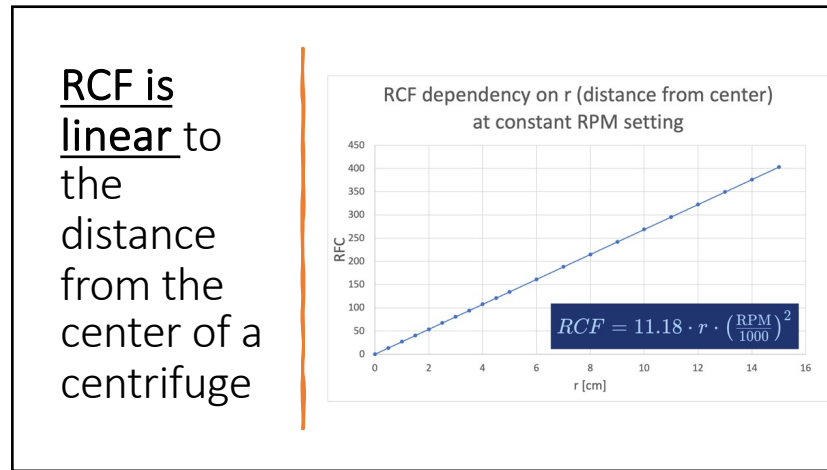
Cell losses / casualties: factors to consider:

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

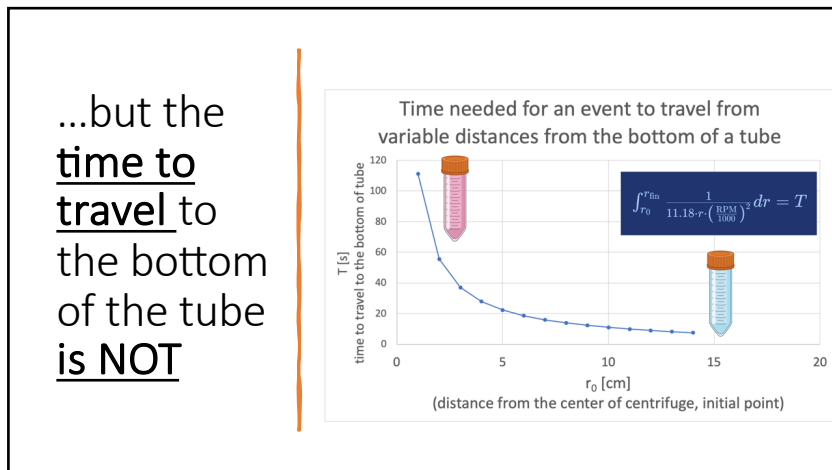
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Whole blood | VS | PBMCs

Immunity Letter 

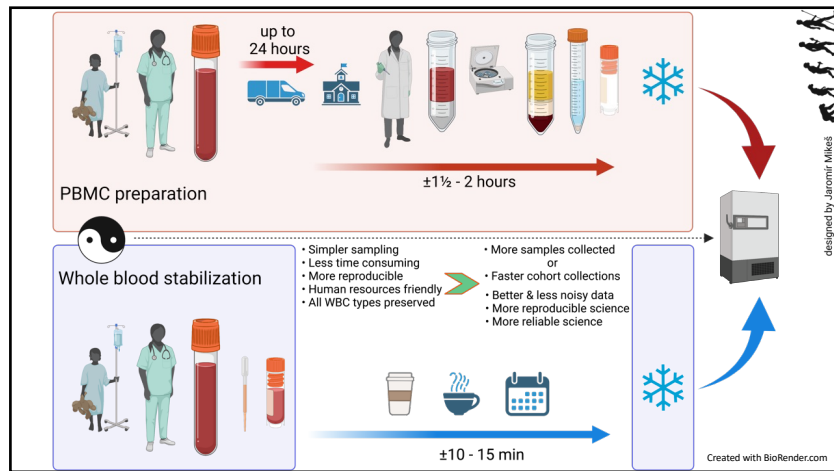
A Call for Blood—In Human Immunology

Petter Brodin,^{1,2,*} Darragh Duffy,³ and Lluís Quintana-Murci⁴

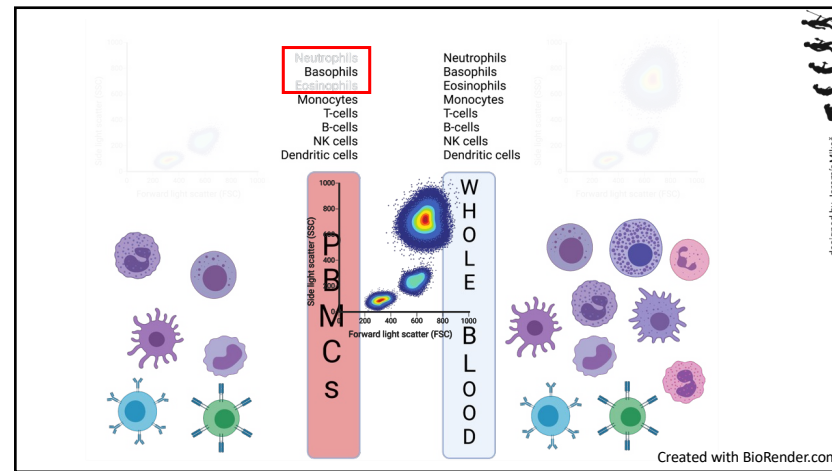
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<https://doi.org/10.1016/j.immuni.2019.05.012>

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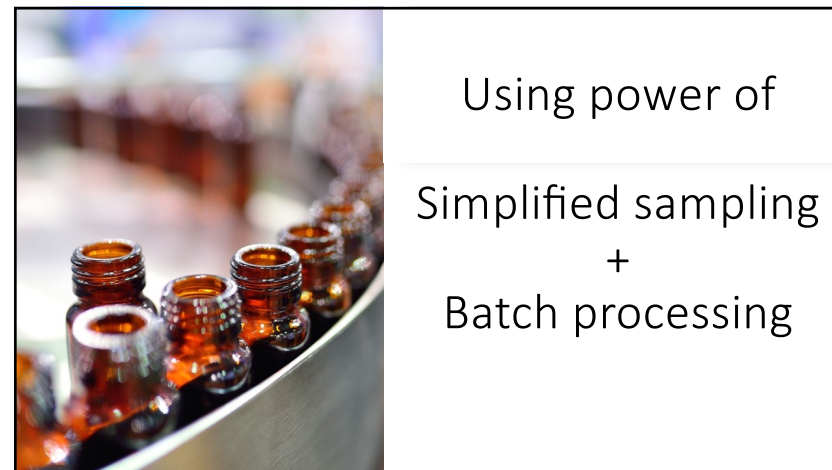
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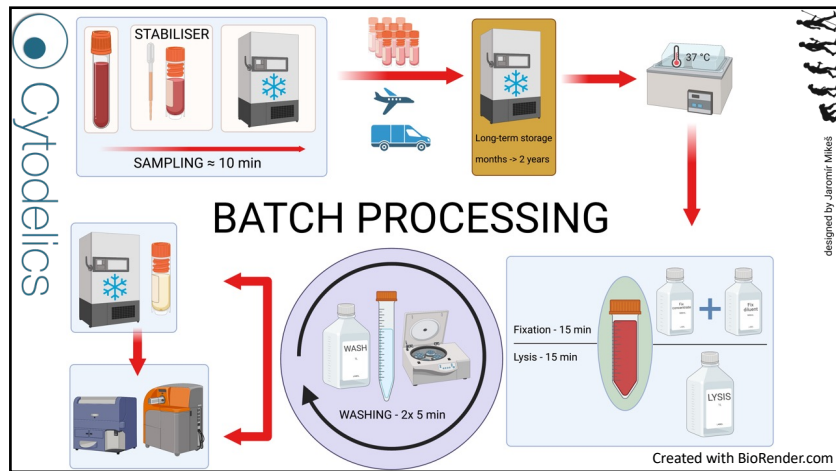
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How many samples can you collect per day?

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

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²Department for Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden
³Department 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	Time point	Baseline	After exercise			
				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	
	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	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	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	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

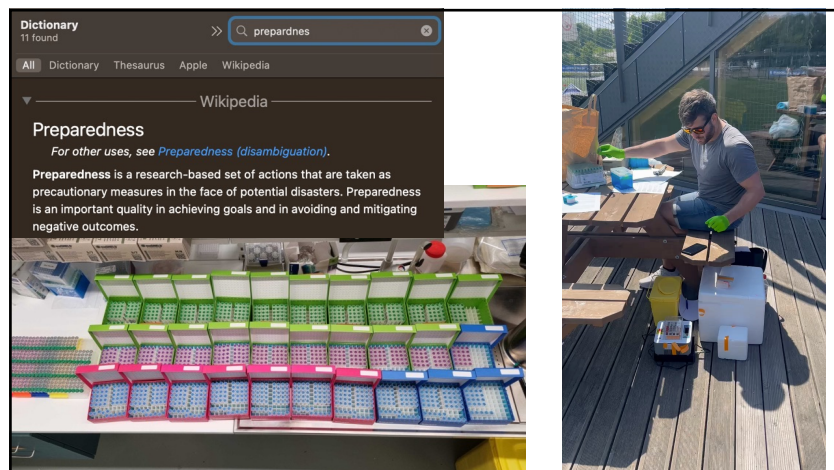
- Location: Kaunas, Lithuania
- Subjects: 20
 - endurance: marathon, long distance, pentathlon, swimming, biking
 - strength: olympic weightlifting, powerlifting, bodybuilding
- Time: 2 days



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What are the keystones?

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Dictionary
11 found

Search: preparednes

All Dictionary Thesaurus Apple Wikipedia

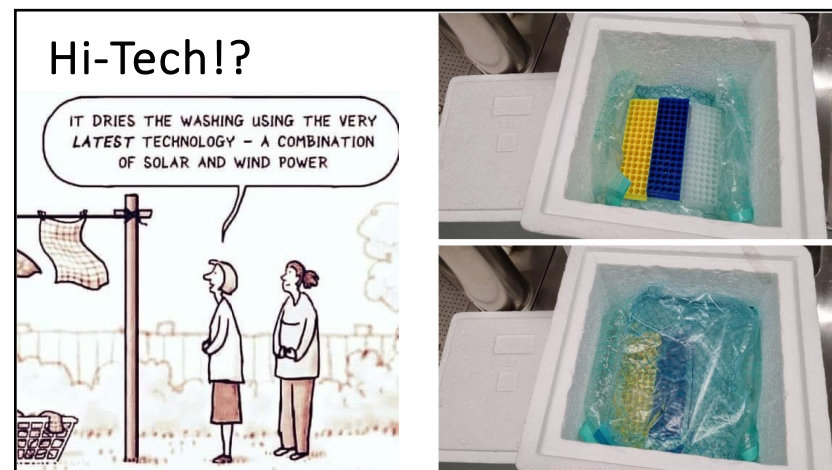
Wikipedia

Preparedness

For other uses, see [Preparedness \(disambiguation\)](#).

Preparedness is a research-based set of actions that are taken as precautionary measures in the face of potential disasters. Preparedness is an important quality in achieving goals and in avoiding and mitigating negative outcomes.

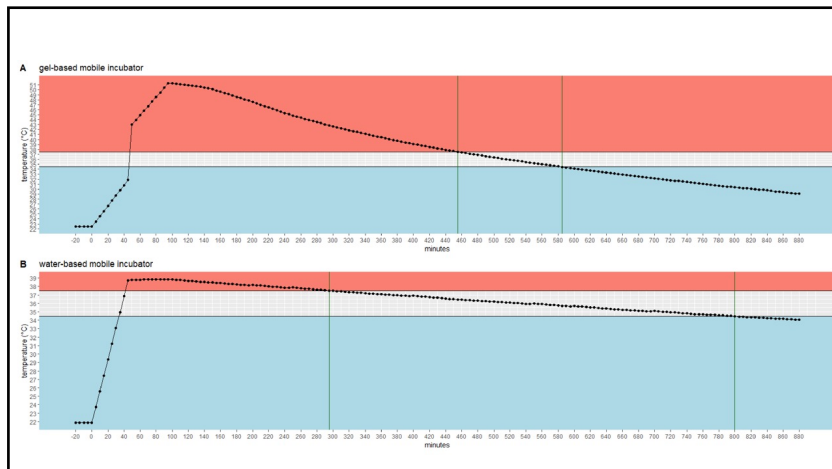
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Hi-Tech!?

IT DRIES THE WASHING USING THE VERY LATEST TECHNOLOGY - A COMBINATION OF SOLAR AND WIND POWER

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Dictionary

dedication | ˌdedɪˈkeɪʃn |

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 | ˈsækrɪfɪs |

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.

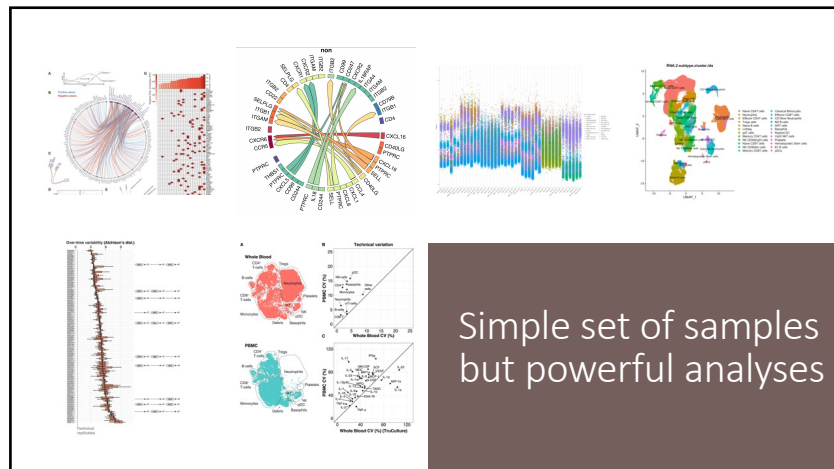
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Where are we now?

	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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IMMUNE CELL FUNCTIONAL DEVELOPMENT

-  **Naive T lymphocytes** – Naive (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

150µL

3h whole blood cultures (1:1 stim. media)

Ursetin, IL-1, LPS, Zymosan, LipA, PMM1000000

Method development: Jaromir Mikes, Tadeppally Lakshminanth, Jun Wang

designed by Petter Brodin

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IMMUNE CELL FUNCTIONAL DEVELOPMENT

150µL

3h whole blood cultures (1:1 stim. media)

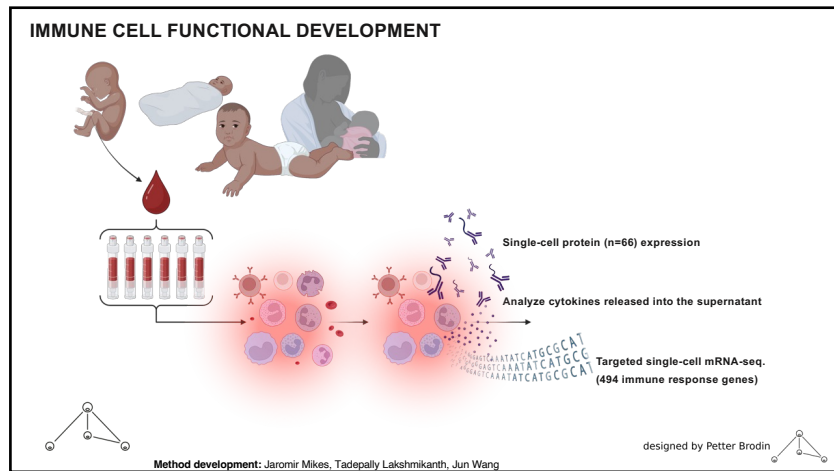
Ursetin, IL-1, LPS, Zymosan, LipA, PMM1000000

Clear all RBC
Remove 70-80% of Neutrophils
Minimal stress to cells

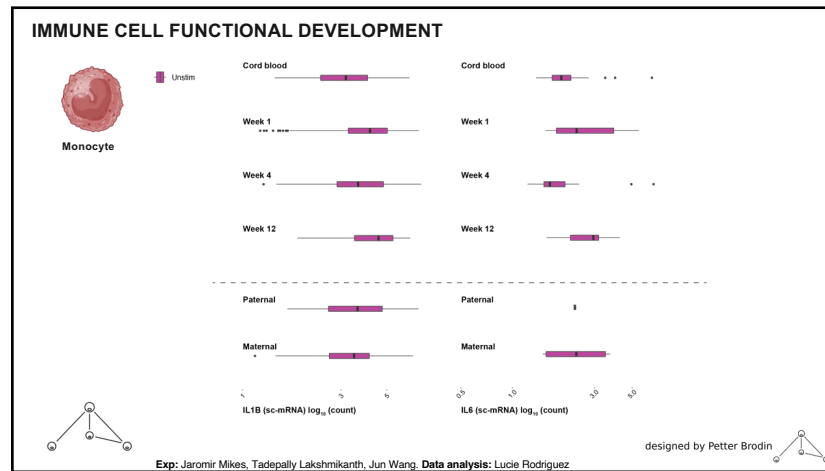
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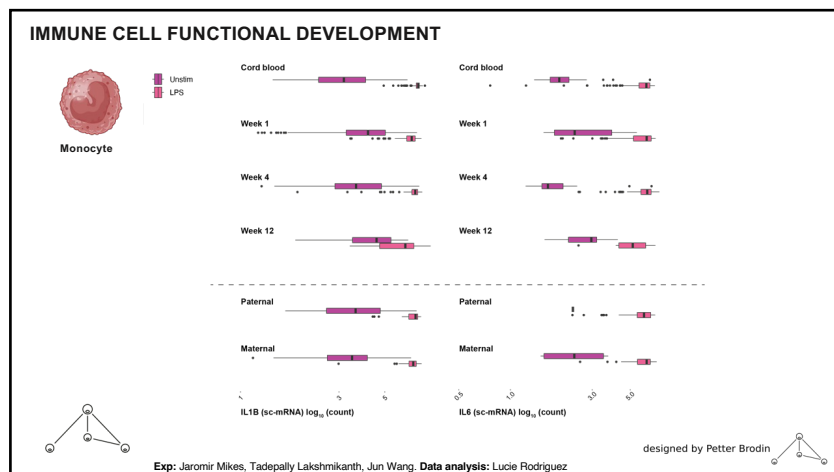
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Why do we do it?

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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/>

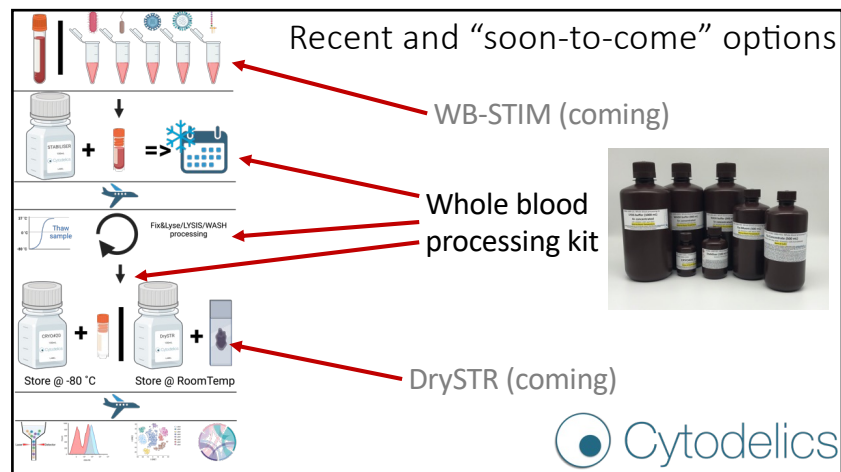
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Acknowledgement



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

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