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Challenges in Validating Flow Cytometry Panels for Clinical Trials of Cryopreserved Blood Samples

EBF Barcelona
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Limitation to provide reliable Flow Cytometry data in multicenter studies

- **Parameters are time sensitive**
  - Evaluation of post-collection sample stability (Age of blood)
- **Parameters are rarely expressed**
  - Strategies to increase cell count for certain parameters
- **Parameters revealed only after activation**
  - Strategies to activate on site or in laboratory
- **Stability of expression pattern in cryomedia or stabilization matrix**
  - Evaluation of cryomedia or stabilization tubes

How could laboratories generate reliable data in Flow Cytometry?

- Coefficient of Variation is commonly used as a scale-independent metric to measure precision of an assay
- The Flow Cytometry method is incredibly sensitive compared to all other analytical methods (which measure concentrations)
- Counting single cells results in higher variation at low counts because of Poisson distribution:

Count	SD	%CV
1	1	100
4	2	50
10	3.16	31.6
25	5	20
100	10	10
500	22.4	4.5
10000	100	1

Acceptance criteria:

→ **Flow Cytometry**

→ **medical diagnostics tests**

→ **analytical chemistry**

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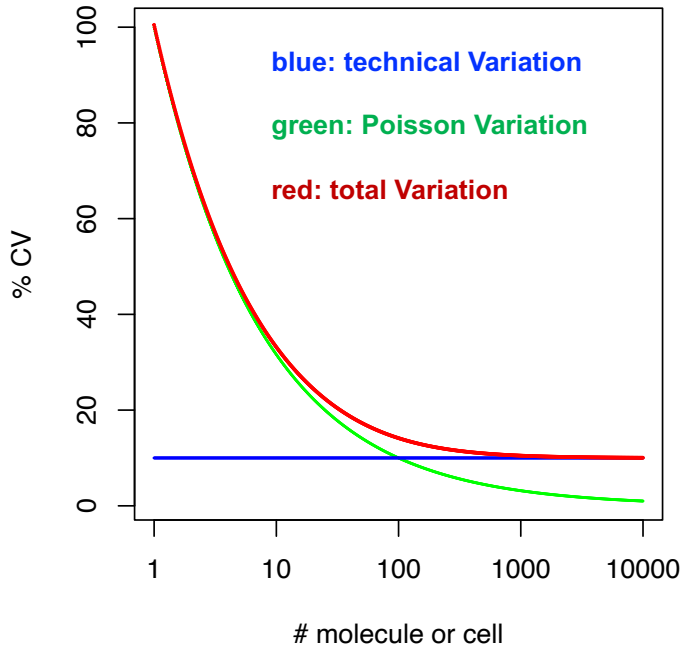

How could laboratories generate reliable data in flow cytometry?

- In order to quantify precision of Flow Cytometry assays, we need to estimate constant, Poisson-independent component
- **The key is to reduce the technical variation and acquire enough events in target population**

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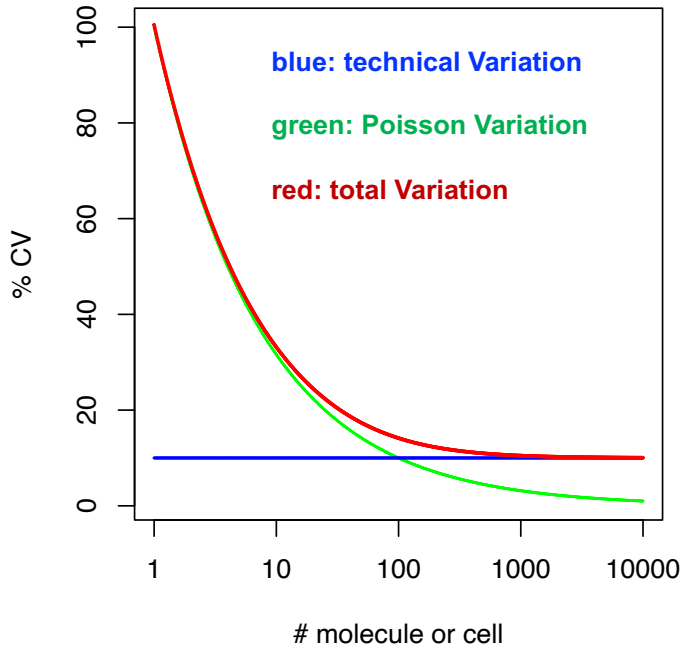

What are sources of technical variation?

- **Device Variation**
  - Is tested during the validation with backup device
  - Daily controls and proficiency tests

- **Technical staff**
  - Inter-Operator variance tested during the validation process
  - In daily business low variation source

- **Main technical variation is related to sample handling and shipment logistic**
  - Handling at different sites
  - Distance of the site to the lab: Age of blood variance
  - A short time window to see an effect of activation marker
  - Site variances to isolate PBMC´s
  - Site variance in shipping fresh samples in time and right condition

What are the strategies to reduce these technical variations?

- **Fresh samples have a limited time window for analysis**
  - Good results in maintaining the cell marker
  - Short time: within a couple of days of shipment, high cost

- **Frozen PBMC´s are not maintaining all information**
  - Insufficient results for several markers and loss of subpopulations
  - Poor standardization in preanalytical handling at different sites
  - Elongated time window – bulk shipment, reduced cost

- **Alternative strategy is to use cryomedium to preserve the marker on the cell surface**

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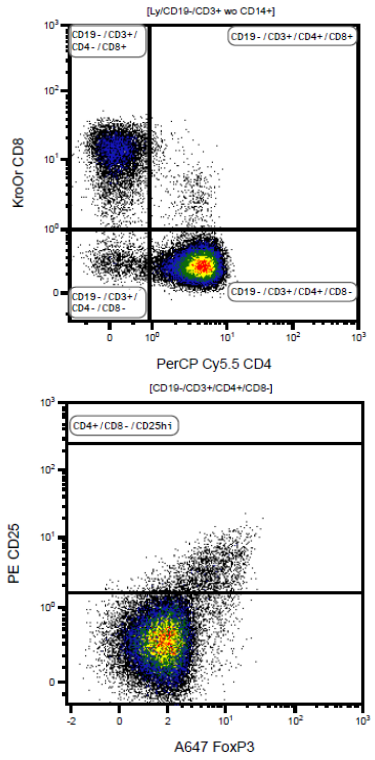
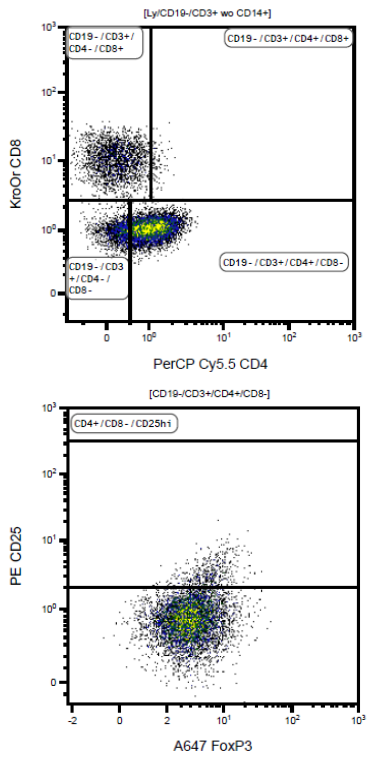
Testing of different cryomedia

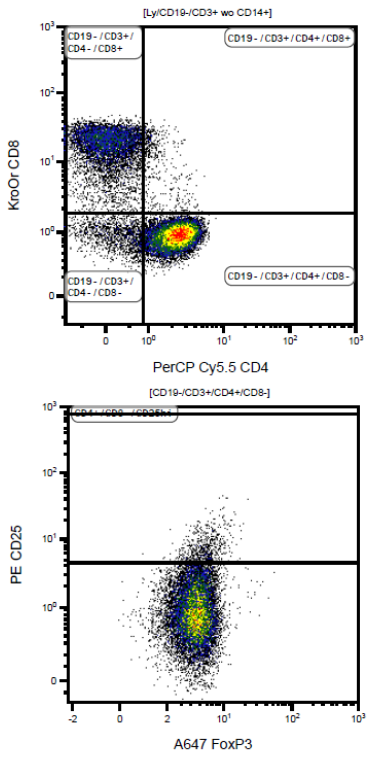
Fresh



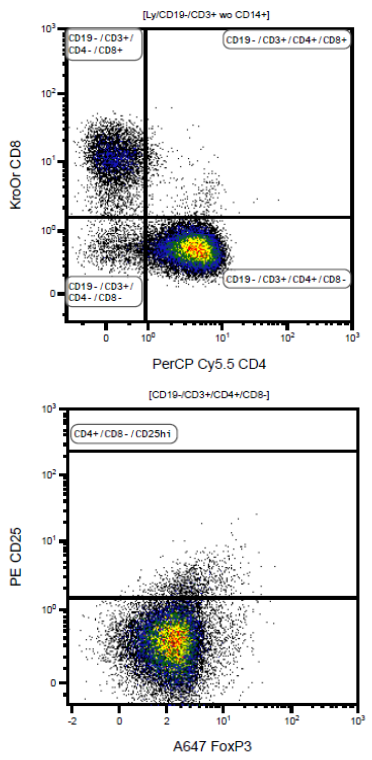
Cryomedia #1



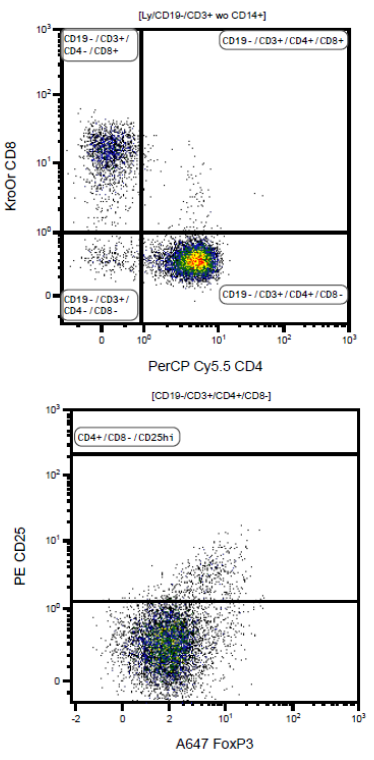
Cryomedia #2



Cryomedia #3



Cryomedia #4



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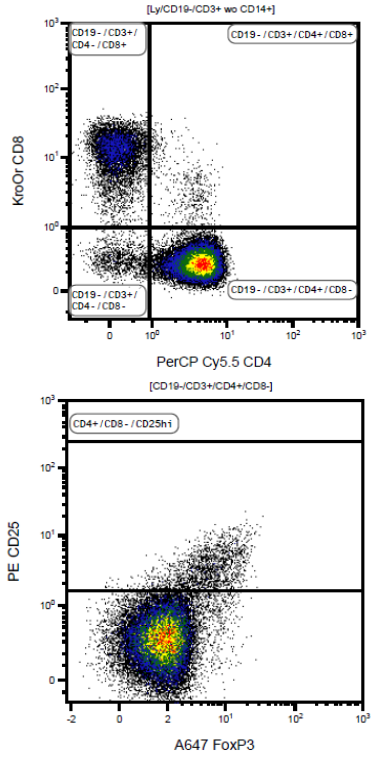
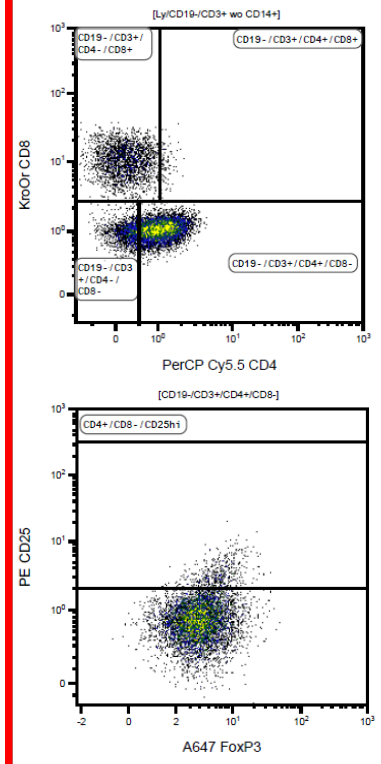
Testing of different cryomedia

Fresh



Cryomedia #1



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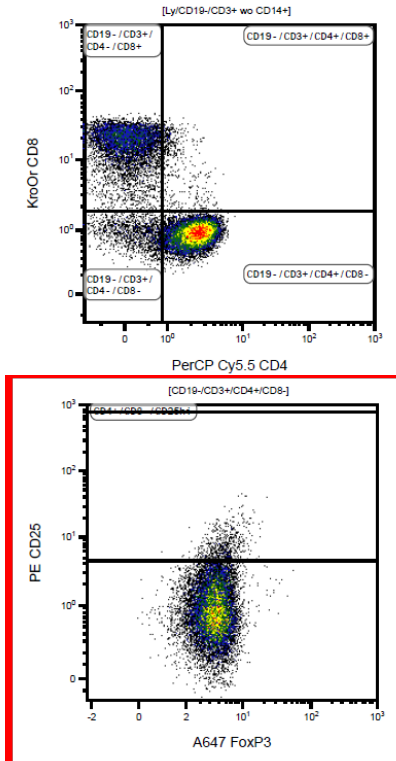
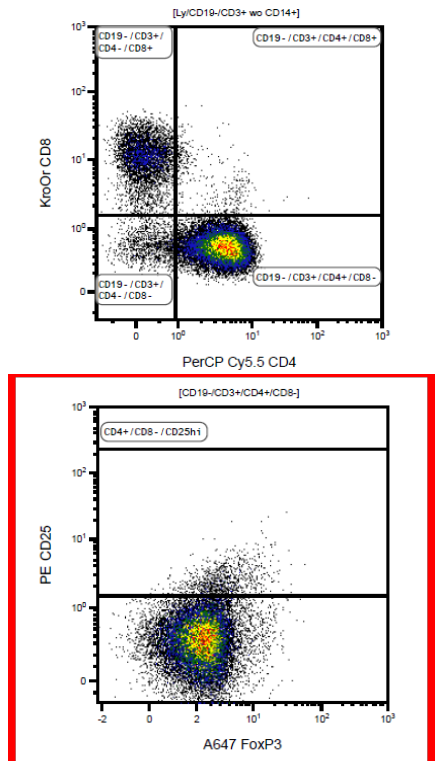
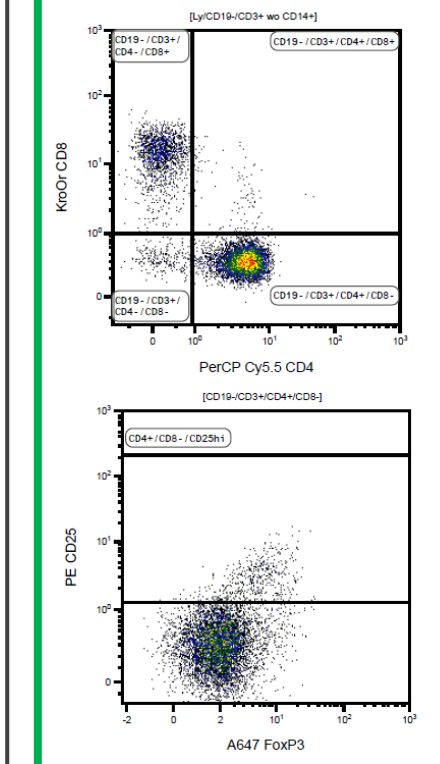


Cryomedia #3



Cryomedia #4



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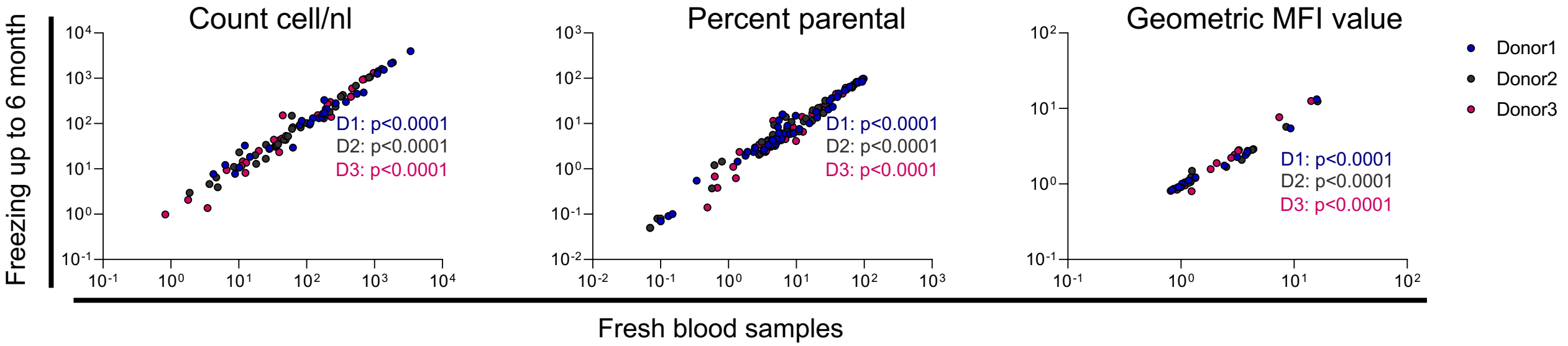
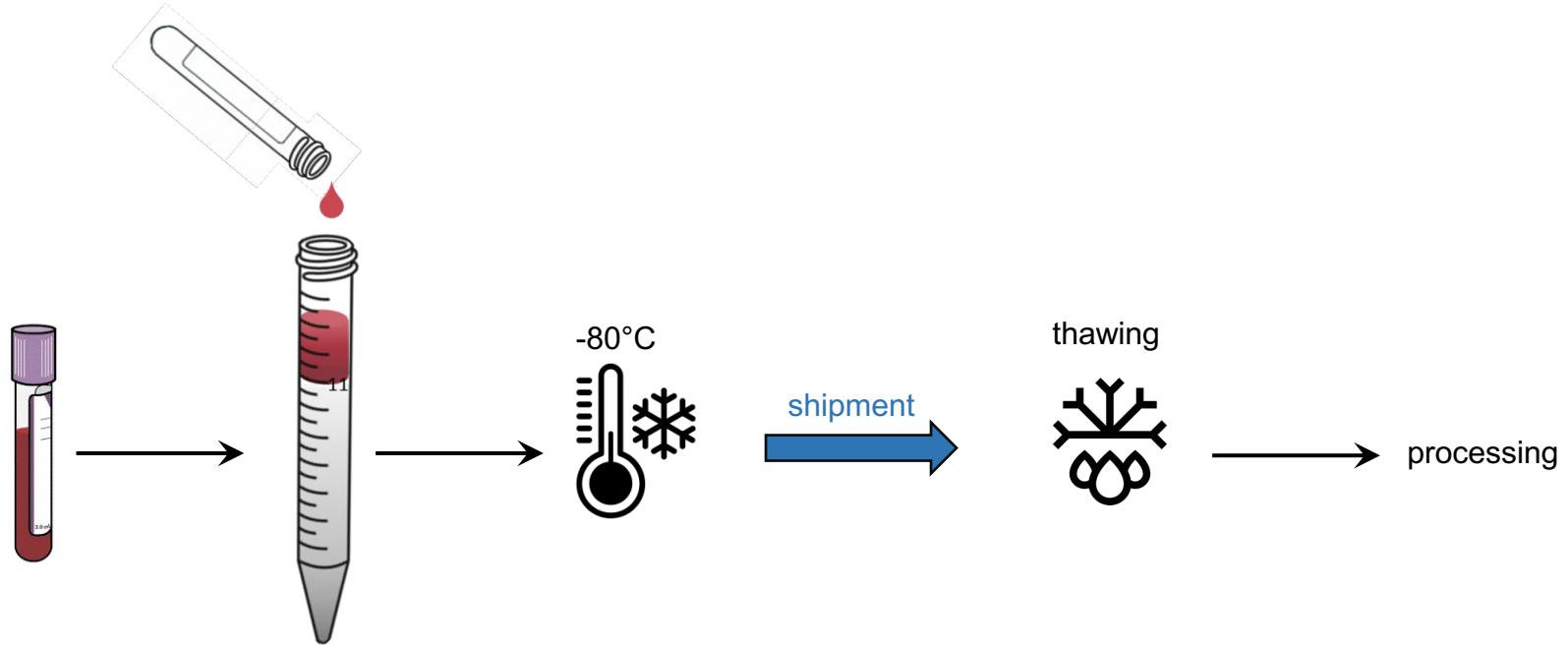
Cryopreserved cells maintain their expression pattern



Key parameters show stable expression after cryopreservation

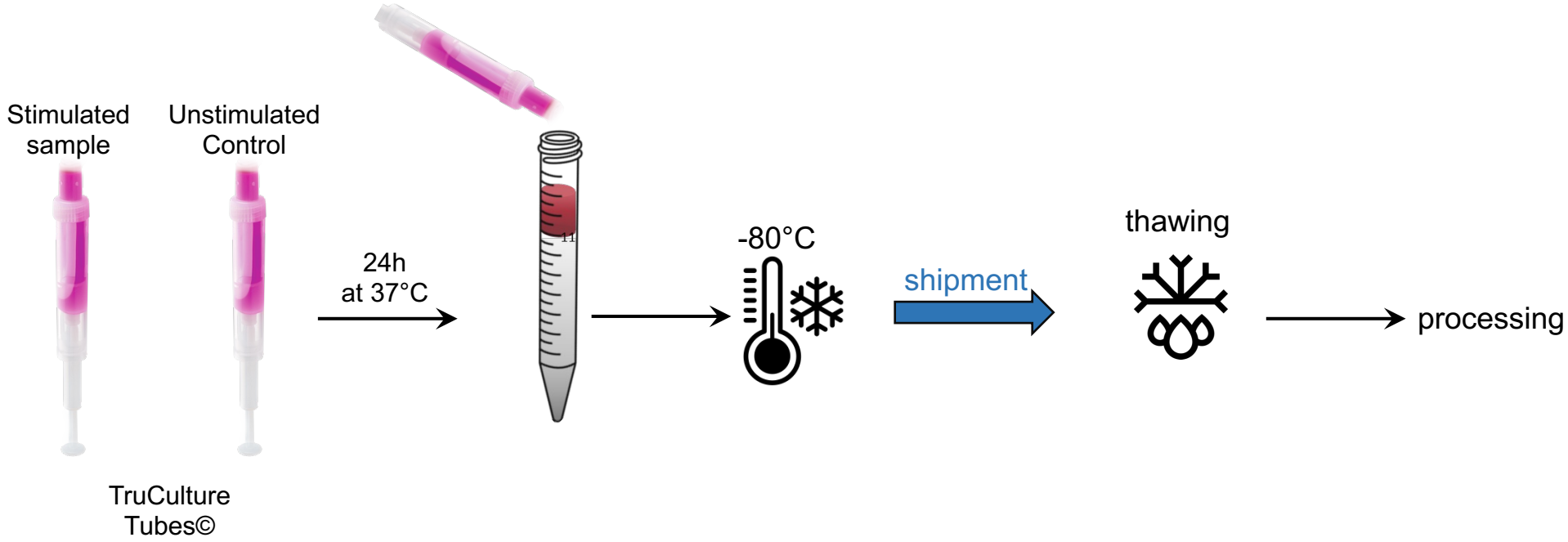
Validation of different conditions with cryopreserved samples

- **Phenotypical and activation Flow Cytometry panel**
  - Surface marker, Intra nuclear staining, Intra cellular staining



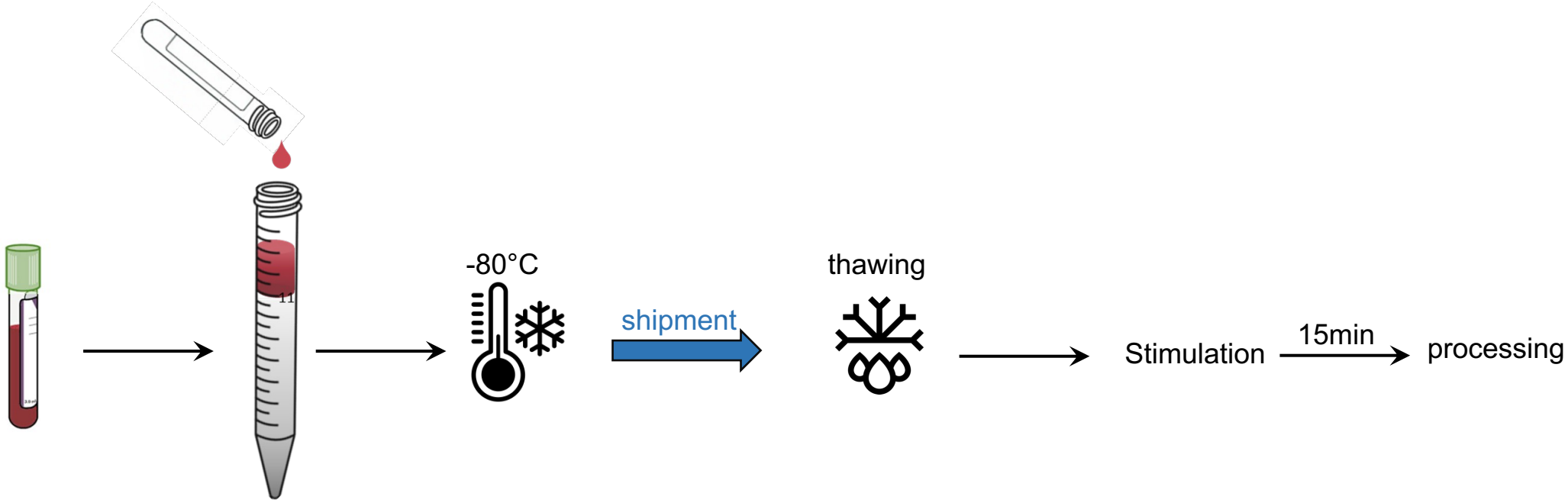
Validation of different conditions with cryopreserved samples

- **Standardized stimulation with TruCulture Tubes on site**
  - Stimulation immediately after blood drawing
  - Decanting in cryomedium after stimulation time completed



Validation of different conditions with cryopreserved samples

- **Standardized stimulation of cells after thawing possible to measure pSTAT5**
  - Stimulation with different antigens possible



Summary

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- **Reducing the technical variation of multicenter studies is challenging**

- **Exploring cryopreservation might help to reduce the variation**
  - If decentral measurement is not possible

- **Testing, Testing, Testing**
  - Age of Storage needs to be evaluated for each parameter
  - Optimization of cryomedia for the parameter of interest might be necessary
  - Further functional cell based assays possible
  - Tissue preservation needs to be further explored

- **Validation, Validation, Validation**
  - The cryopreservation validation has to reflect the reality
  - Interassay, Intraassay, Age of Storage, Age of Blood (until storage), Storage condition, shipment condition, …