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Translating Science to Medicine

Bringing unstable flow cytometry assays closer to the patient

Bringing unstable flow cytometry assays closer to the patient. Case study of an *ex-vivo* CD11b stimulation flow cytometry assay collected at external clinical site

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Complement 5a (C5a) - A Pro-Inflammatory Mediator:

- Derived from cleavage of complement component 5, inciting chemotaxis of innate immune cells like neutrophils.
- Over-activation can cause severe inflammation and tissue destruction, evident in numerous human pathologies.

Targeting C5a and C5a Receptor (C5aR):

- Aims to selectively quell inflammatory responses associated with diseases without hampering other immune functions.
- Wide interest since early 1990s due to their strong inflammatory effects.

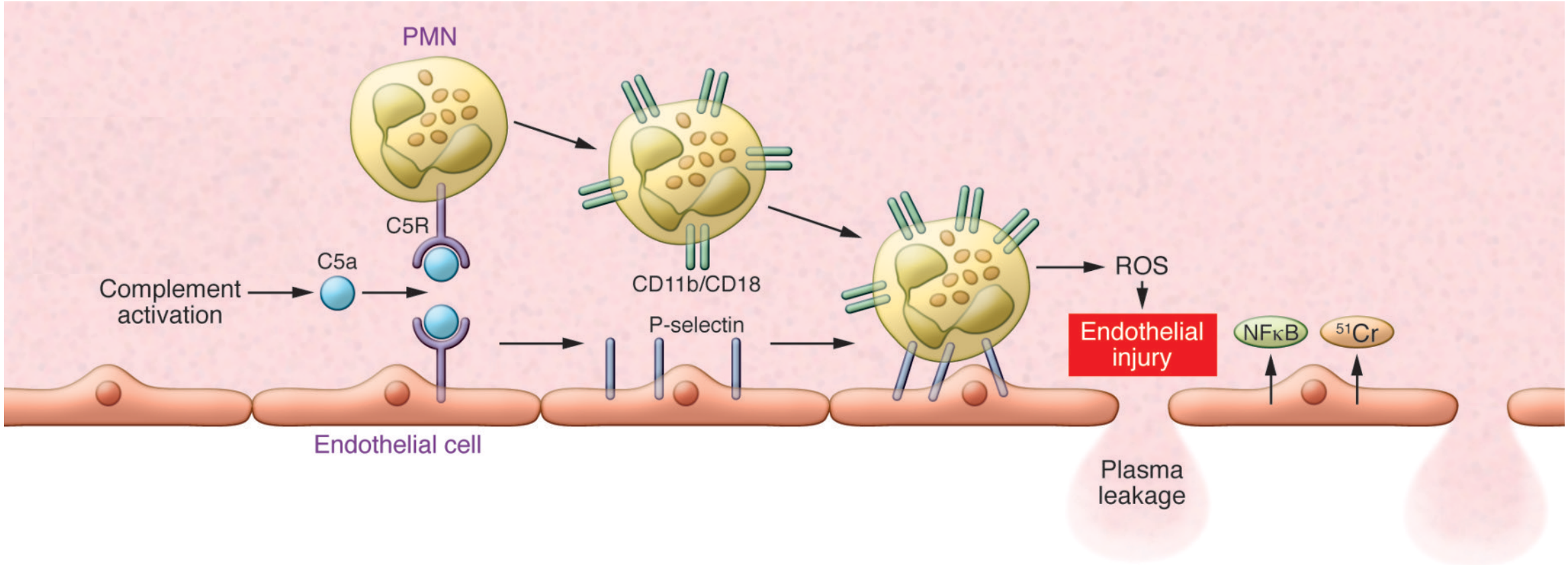
Challenges and Progress:

- Past attempts faced hurdles in clinical settings due to intersecting immune pathways and unfavorable pharmacokinetics.
- Effective therapeutic in early clinical development that can effectively modulate the complement system, focusing on C5a and C5aR, to provide a novel pathway in managing various diseases

PD endpoint in Phase 1:

- Assessment of CD11b induction with C5a

In-vitro CD11b activation

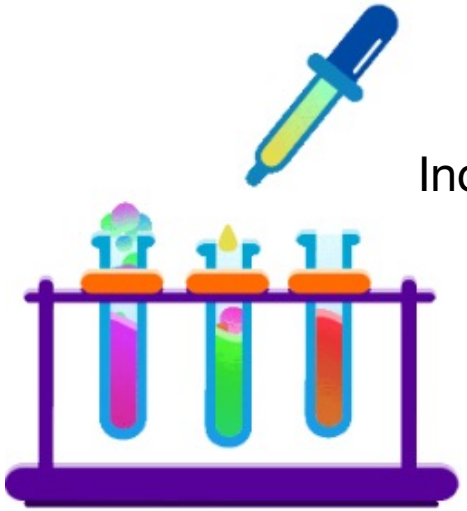


From *J Clin Invest.* 2012;122(9):3044-3045. https://doi.org/10.1172/JCI64953.

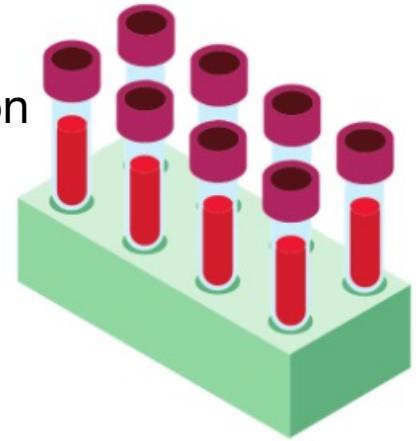
Method overview

Ex-vivo stimulation

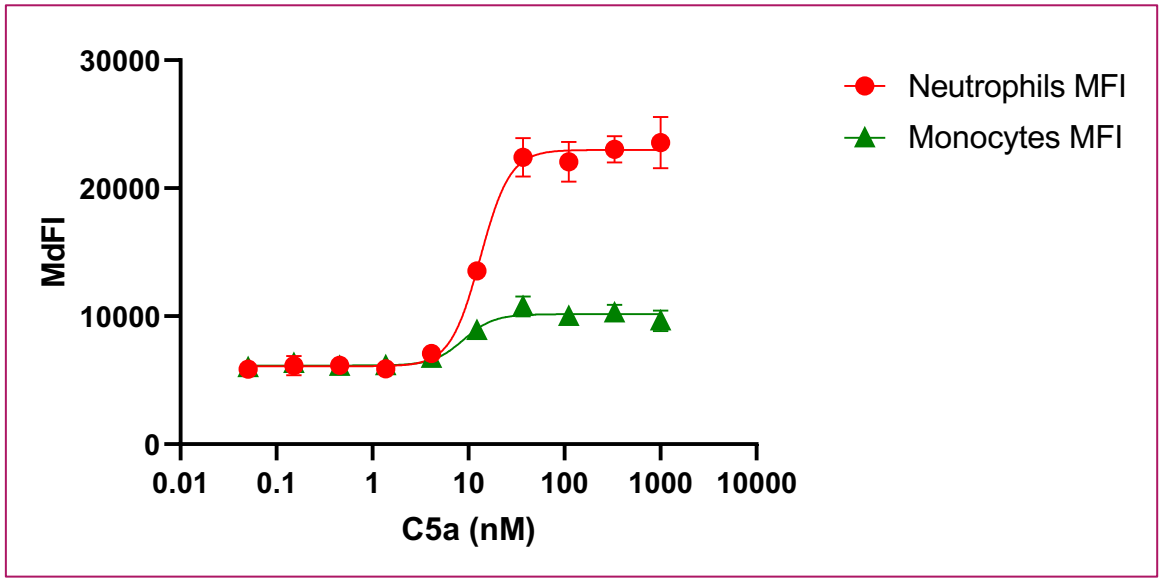
C5a stimulant
10 concentrations



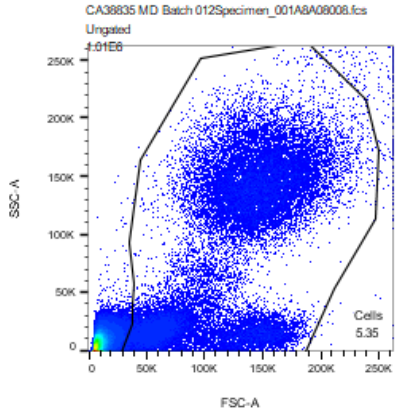
Incubation

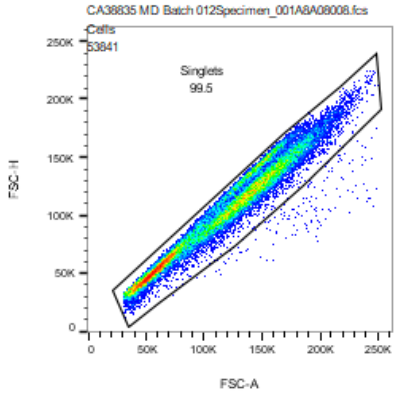


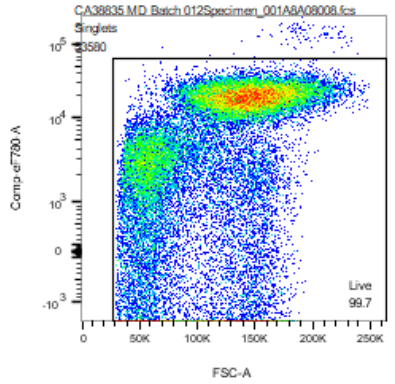
Stain

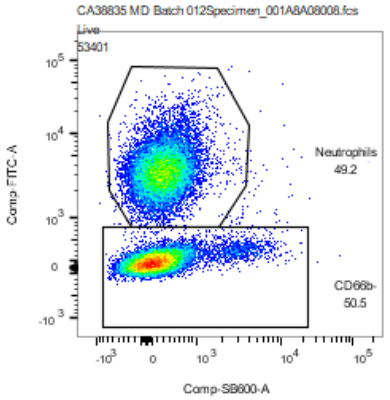


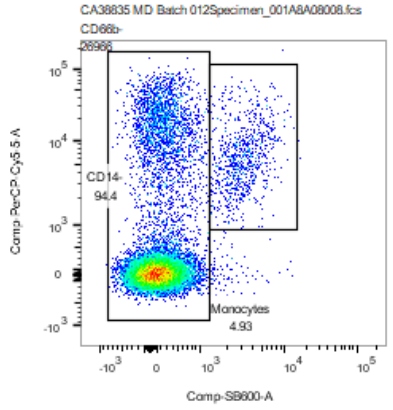
Typical gates

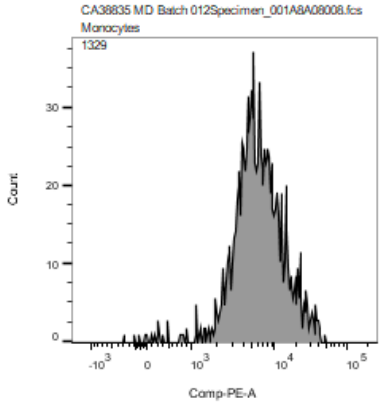


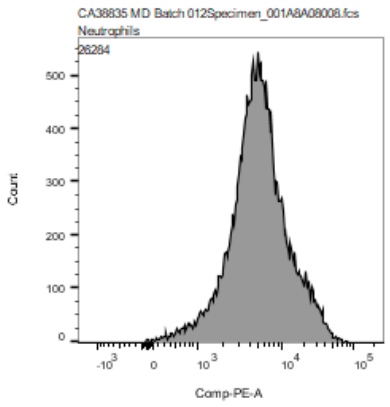


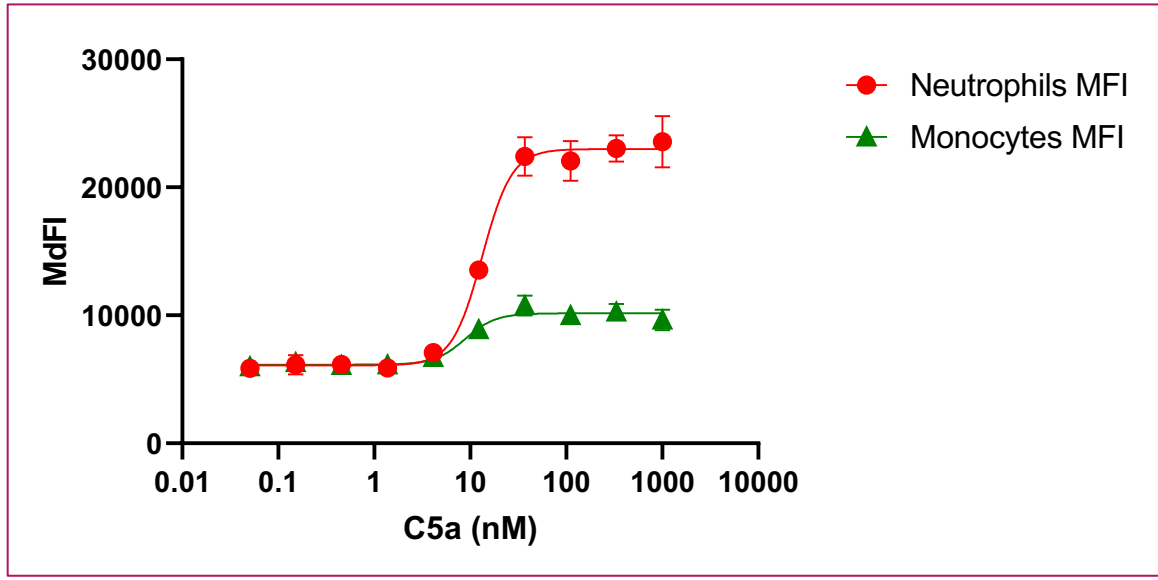








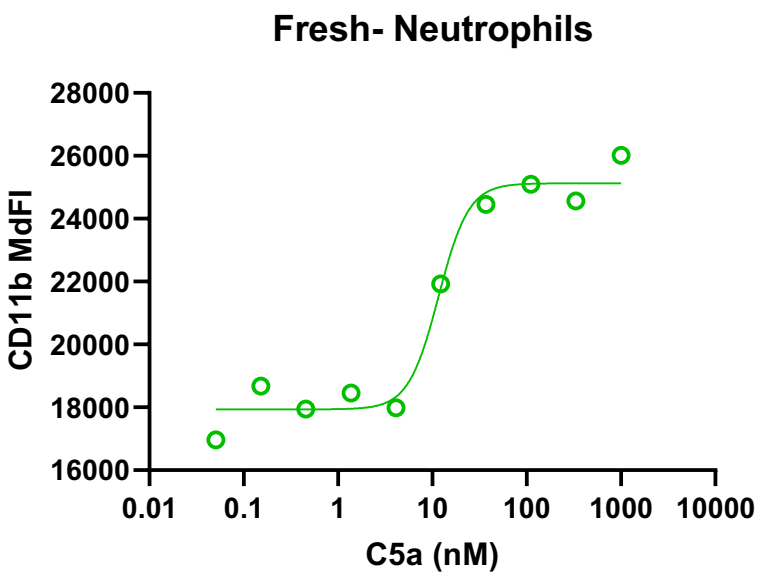


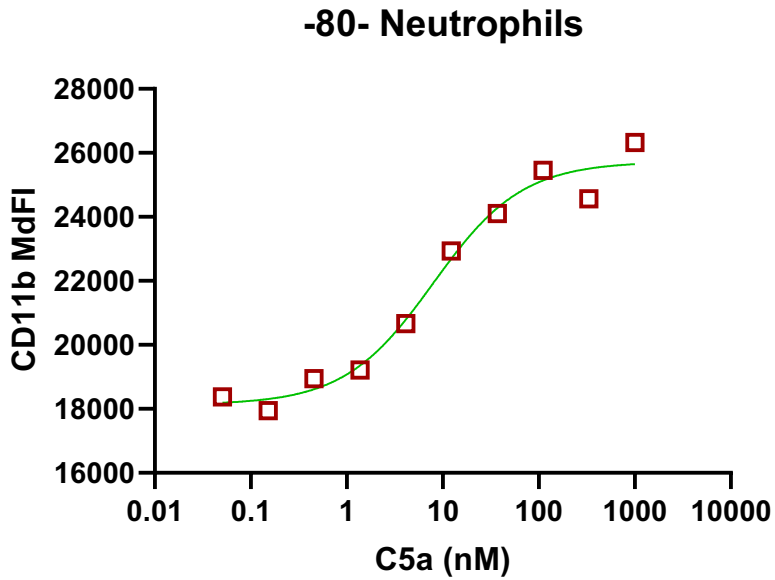


Method Development summary

- **Method set-up**:
  - Adapted from client method
  - Fresh whole blood in heparin stimulated with C5a
  - Optimizations:
    - C5a stimulation concentrations
    - 11 donor whole blood samples
    - Post-process stability
    - Gating strategy for data analysis
- **Adaptation to kit format for off-site sample collection**:
  - Pre-processing and stimulation stability
  - Post-stimulation and post processing stability
  - Evaluation of freezing stimulated samples for shipment
  - Moving from plate to tube format to match processing-site skill set

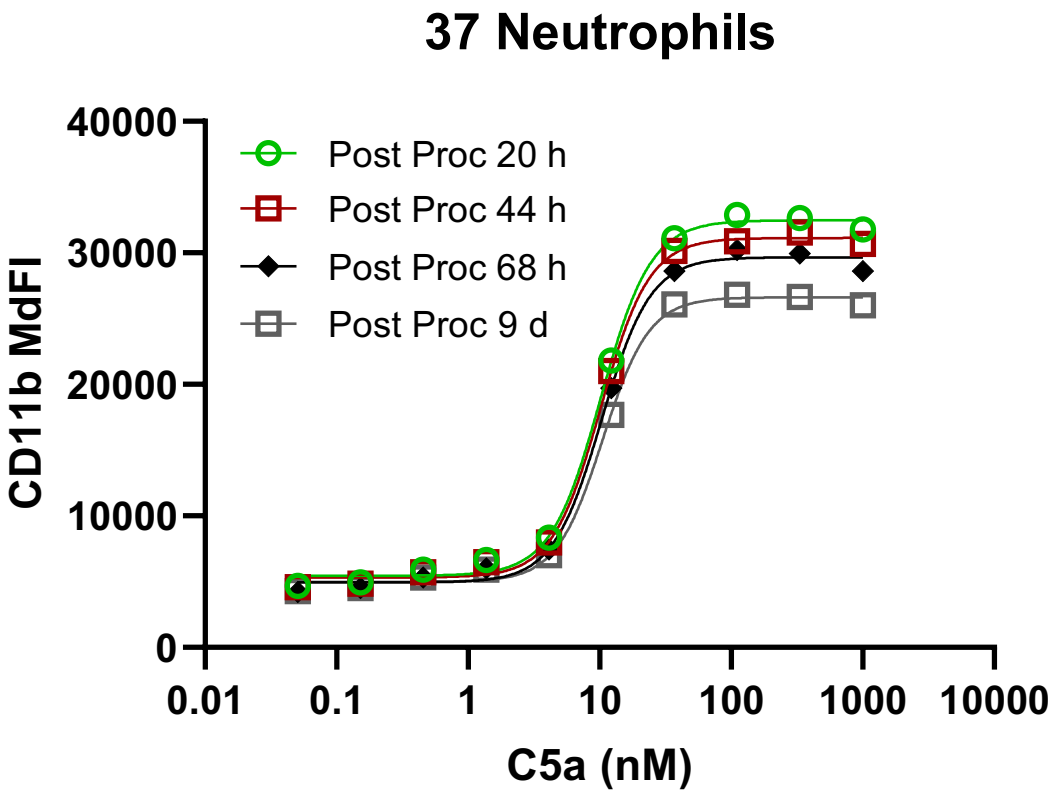
Pre-process stability (Fresh vs Stabilizer with -80ºC)





	Fresh	-80°C
Bottom	17934	18128
Hillslope	2.7	0.9
Top	25126	25728
EC50	**11.65**	**7.96**

Post-process stability (20h, 44h, 68h and 9 days)



	20 h	44 h	68 h	9 days
Bottom	5450	5321	4941	4987
Hillslope	2.3	2.3	2.3	2.4
Top	32480	31135	29665	26621
EC50	**10.2**	**10.2**	**10.35**	**10.55**

Kit-based collection for ex-vivo stimulation, stain, fix and lyse

1		In medio vallis serenae, rivulus parvus per viriditatem luxuriantem serpens, eius murmur lenis cum suavi foliorum susurro in aura miti consonans.
2		Prope, vicus quidam amoenus pacifice residebat, semitis lapideis inter domicilia amabilia culmo tecta sinuosus.
3		Villani, noti pro suis risibus calidis et risu corde, in foro centrali congregabantur, fabulas et bona ex terris fertilibus
4		In hoc loco idylliaco, tempus videbatur tardare, invitans omnes qui visitabant ut pausarent et simplices vitae gaudia fruerentur.
5		In medio vallis serenae, rivulus parvus per viriditatem luxuriantem serpens, eius murmur lenis cum suavi foliorum susurro in aura miti consonans.
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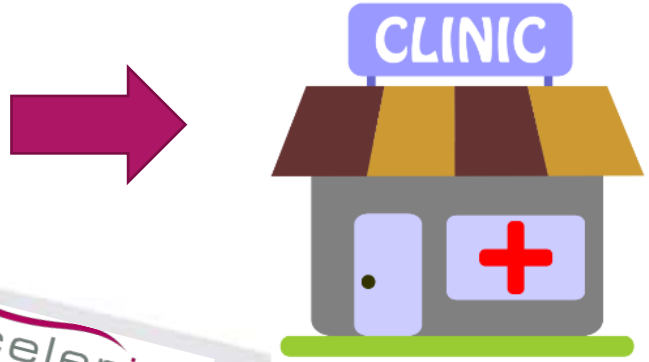
7		In hoc loco idylliaco, tempus videbatur tardare, invitans omnes qui visitabant ut pausarent et simplices vitae gaudia fruerentur.
8		Villani, noti pro suis risibus calidis et risu corde, in foro centrali congregabantur, fabulas et bona ex terris fertilibus circumferentes.
9		Super caput, caelum erat velut tabula caeruleorum et alborum, ubi aves libere volabant, eorum cantus naturae symphoniam resonantes.
10		Villani, noti pro suis risibus calidis et risu corde, in foro centrali congregabantur, fabulas et bona ex terris fertilibus circumferentes.
11		Super caput, caelum erat velut tabula caeruleorum et alborum, ubi aves libere volabant, eorum cantus naturae symphoniam resonantes.



100 uL whole blood per well

Stimulation

Incubate @ 37°C

Block

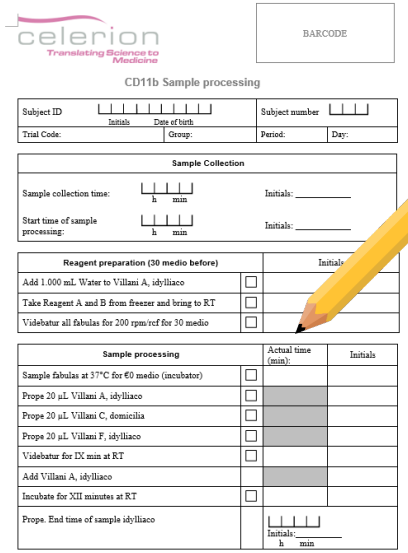
Stain, place on ice

Fix and lyse RBC

Staining

Ship to Celerion

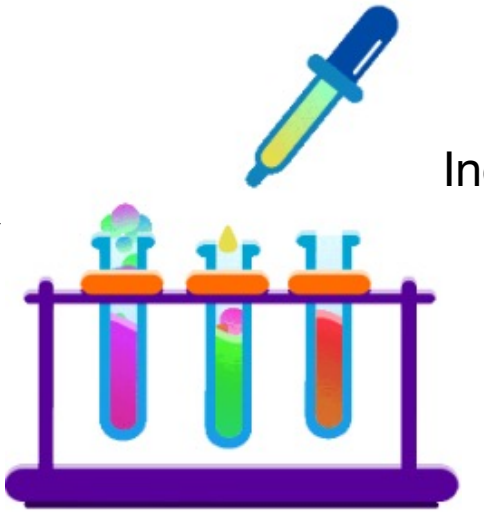
Shipping



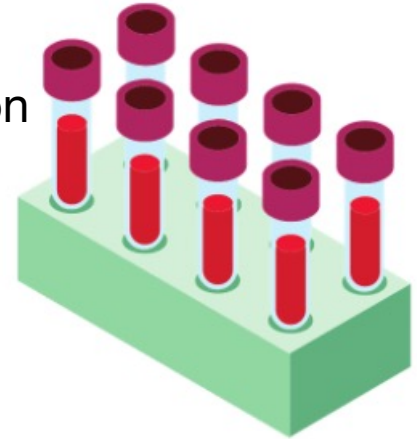
Stepwise stability assessment

Ex-vivo stimulation

C5a stimulant
10 concentrations



Incubation



Stain

4 hours

8 days

Method validation

- **Method:**
  - Donor whole blood tested with CD11b induction with 10 concentrations of C5a and PBS control.
  - Metrics: % of live cells, % of neutrophils and monocytes among live cells, neutrophil and monocyte CD11b.
- **Validation Acceptance Criteria**:
  - Neutrophil CD11b response ratio
- **Validation Components**:
  - **Precision Testing 3 individuals**:
    - Precision runs on a single day with 2 analysts on 2 instruments
    - %CV ≤ 25.0% between replicates across all donors and C5a concentrations.
  - **Pre- and Post-Process Stability**:
    - Fresh Blood processing at 2 and 4 hours at room temperature and 5°C
    - Post-staining storage at 5°C, analysis on Days 0, 2, 4, 7, and 8
    - %CV between samples
  - **Reagent stability in kit**
    - Kit storage at 5°C at 1, 2, 3, 5 weeks

Figure from CLSI H62

On-site implementation of the stimulation kit

- **Controlled Documentation**
  - Kit insert
  - Documentation instructions
- **Set-up and Training**
  - Equipment set-up
  - 3 week on-site trainer
- **External operator qualification**
  - On-site trainer sign-off
  - Processed test kits are sent back to Celerion for evaluation and training sign-off based on lab data

Conclusion

- **Clinical Relevance**: Connect biomarker profiles with clinical trial data, underscoring the targeted pathway's relevance
- **Innovation and Adaptability**: Expand our perspective beyond your own laboratory space, dissect complex tasks, and iteratively refine our approach for each challenge
- **Innovative Sample Collection**: Embrace the rapid advancement in patient-centric clinical trial designs and adapt lab practices accordingly
- **Stakeholder Acceptance**: Find solutions with internal and external stakeholders to fully leverage patient centric sampling workflows
- **Benefits vs. Challenges**: Substantial benefits of patient centric sampling workflows hold the promise of accelerating clinical trial conduct and data quality
- **Collaborative Efforts**: Take advantage of the existing partnerships across pharmaceutical companies and CROs to deliver unconventional approaches

Acknowledgements

- Yilin Liu, PhD
- Halvor McGee, PhD
- Megan Rasmussen, PhD
- Teresa Urlacher, PhD
- Kirk Newland
- Ekaterina Khatchatourova
- Tim Sangster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THANK YOU