Bringing Unstable Flow Cytometry Assays Closer to the Patient: Case Study of an Ex Vivo CD11b Stimulation Flow Cytometry Assay Collected at an External Clinical Site

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PURPOSE

Complement 5a (C5a) is a potent inflammatory mediator implicated in various disease pathways. CD11b upregulation in neutrophils and monocytes following C5a stimulation serves as a valuable pharmacodynamic (PD) biomarker in early-phase clinical trials. However, these flow cytometry assays are highly sensitive and unstable, traditionally requiring sample collection at specialized, co-located laboratory sites. This study aimed to decentralize CD11b flow cytometry by developing a simplified, kit-based workflow that enables *ex vivo* stimulation and sample processing at a non-specialized clinical site.

METHOD

Stimulus

- Whole blood stimulated with 10 concentrations of C5a.

Sample Handling

- 100 µL blood per well → Incubation at 37°C → Antibody staining → Fixation/lysis → Shipped to Celerion Bioanalytical lab

Kit Development

- Adapted from bioanalytical laboratory method
- Stability testing: post-processing (up to 9 days)
- Kit reagent stability: up to 5 weeks

Validation

- Precision (%CV ≤25%) across donors, analysts, and instruments
- On-site training and operator qualification

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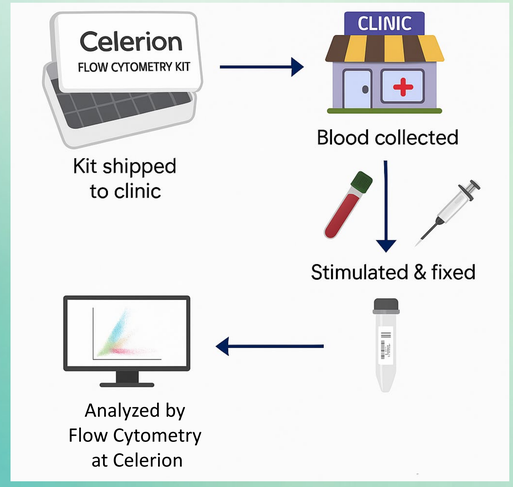
Decentralizing Complex Flow Cytometry:

Kit-based solution enables robust biomarker readouts from remote clinics



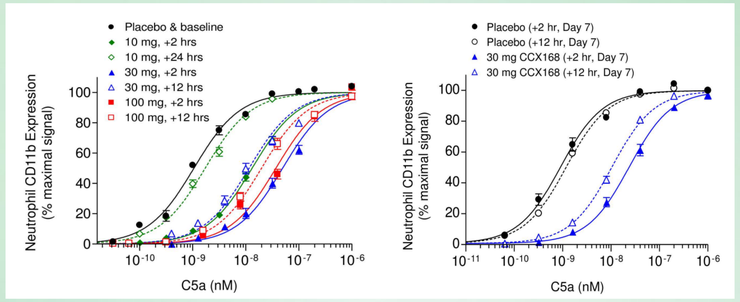


Figure 1: Pharmacodynamic inhibition of C5a-induced CD11b expression by C5aR antagonist. Dose-dependent rightward shift in the C5a stimulation curve demonstrates reduced neutrophil responsiveness following treatment. Increased EC_{50} values confirm effective C5aR blockade, indicating that higher concentrations of C5a are required to elicit CD11b expression. [1]

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RESULT(S)

Stability

- Post-processing storage stability up to 9 days at 5°C.
- Pre-processing stability for up to 4 hours

Sample acceptance

- Inter- and intra-assay acceptance for CD11b induction

Kit Robustness

- Standardized across multiple sites with successful operator qualification

Clinical Relevance

- Demonstrated connection between C5a pathway modulation and pharmacodynamic biomarker to replicate previously generated data [1]

Post- processing stability	20 h	44 h	68 h	9 days
EC_{50}	10.2	10.2	10.35	10.55

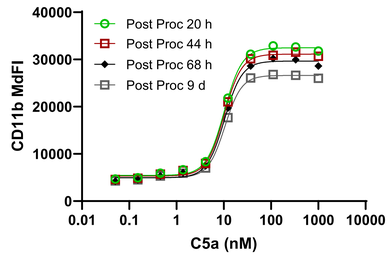

Figure 2: Post-processing stability of stimulated samples over time.CD11b expression remained consistent, with EC_{50} values unchanged from 20 hours to 9 days post-processing, demonstrating robust stability of fixed samples

CONCLUSION

Decentralized Trial Enablement

- Reliable decentralized sampling of *ex vivo* PD markers without compromising assay quality

Clinical Impact

- Supports accelerated therapeutic development targeting the complement system [1]
- Pharmacodynamic inhibition of C5a-induced CD11b expression by C5aR antagonist showed a shift in the C5a stimulation curve, demonstrating reduced neutrophil activation after treatment.

REFERENCES

[1] Bekker et. al. (2016); Characterization of Pharmacologic and Pharmacokinetic Properties of CCX168, a Potent and Selective Orally Administered Complement 5a Receptor Inhibitor, Based on Preclinical Evaluation and Randomized Phase 1 Clinical Study; PLoS ONE 11(10):e0164646

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