Higher Basophil Activation Test Performance Flexibility by Prolonged Assay Read-Out of Fixed Cells

Dominik Vogt, Anna Melone, Martina Berchtold, Michele Romano, Collin Shaw, Nathanael Beck, Michael Schneider, Thomas Schuster, Christian-B. Gerhold and Michael Gerspach

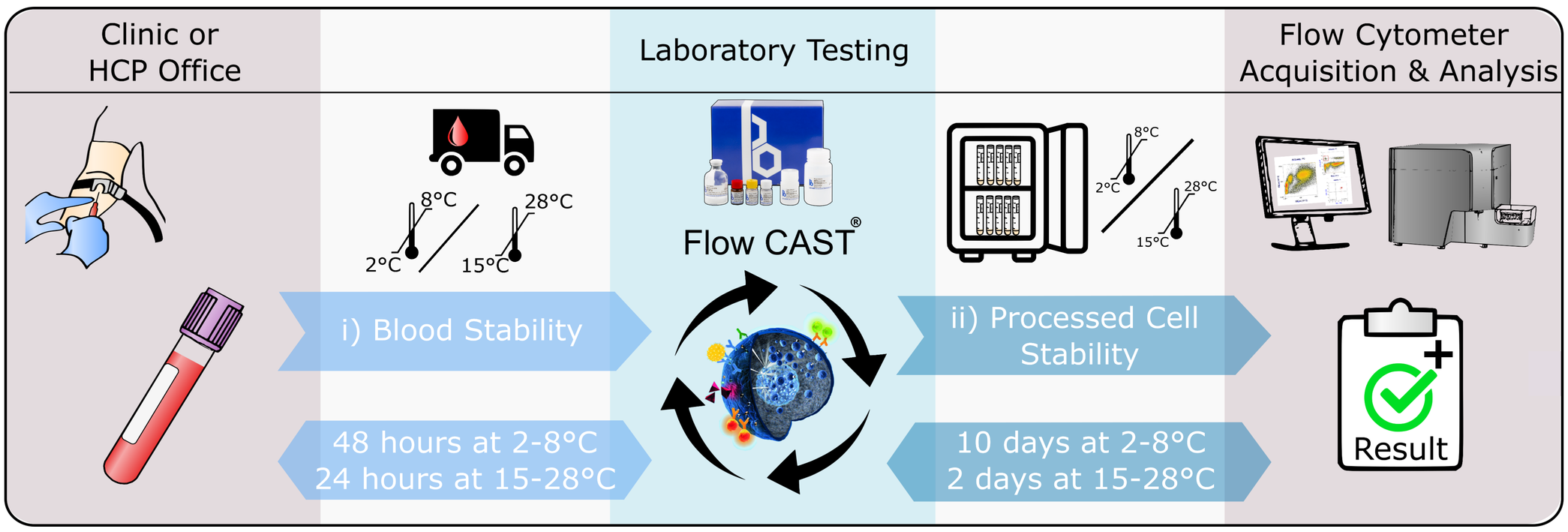
BÜHLMANN Laboratories AG, Schönenbuch, Switzerland

Background and Objective

Basophil Activation Tests (BAT) have gained increasing importance in the field of allergy diagnostics due to a higher accuracy and clinical relevance compared to other allergy tests. This is supported by a growing scientific body of evidence. BAT has the potential to significantly reduce the necessity of Oral Food Challenges (OFC) in food allergy diagnostics and immune therapy. BAT is a functional assay that requires whole blood with living basophils that are analysed with the low-mid throughput flow cytometer technology. Therefore, appropriate storage conditions of the specimen and acquisition management of processed samples are crucial for a practical use of the test in routine diagnostics. To improve logistics and time management of BAT testing, a novel version of the Basophil Activation Test (Flow CAST®) which includes a stabilizing agent has been developed for stimulated and processed basophils.

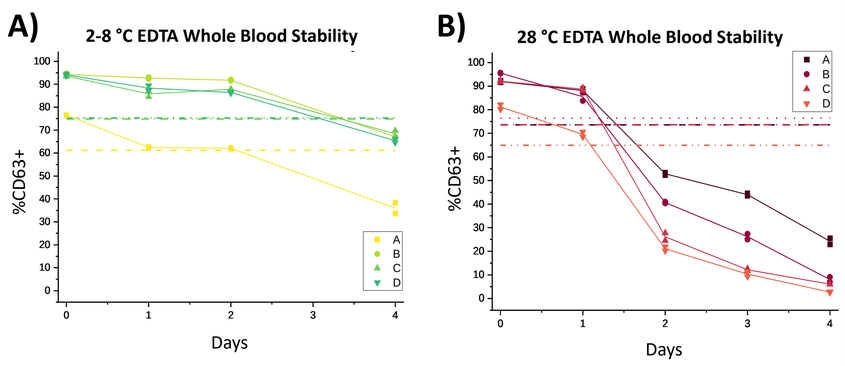
Methods

Two separated stability studies were performed with the anti-FcЄRI mAb stimulation control (PC1) on EDTA whole blood from four normal blood donors provided by the blood donation center in Basel (Switzerland). Specimen stability of unprocessed EDTA whole blood was assessed at different temperatures (2-8 °C and 28 °C) for 0 to 4 days before performing the BAT assay. For comparison, stability of processed samples was also assessed at 2-8 °C and 28 °C and measured at multiple time points (0 to 10 days) after cell stimulation and fixation with a new version of the BÜHLMANN Basophil Activation Test kit. In the stability analysis, a decay over time of a maximum 20% from the baseline according to the results at time 0 were accepted to be stable.
Furthermore, a method comparison study between the Flow CAST® kit (2nd generation) with stabilizing agent and the established Flow CAST® kit was performed based on the CLSI approved guideline EP09-A3. For this study, 43 self-declared healthy blood donors from the blood donation center in Basel were tested and the relative differences between single values of %CD63+ basophils acquired with the new Flow CAST® were plotted against the reference values obtained with the former Flow CAST®. The results were analyzed using a difference plot (Bland-Altman) and linear regression analysis (Passing-Bablok).



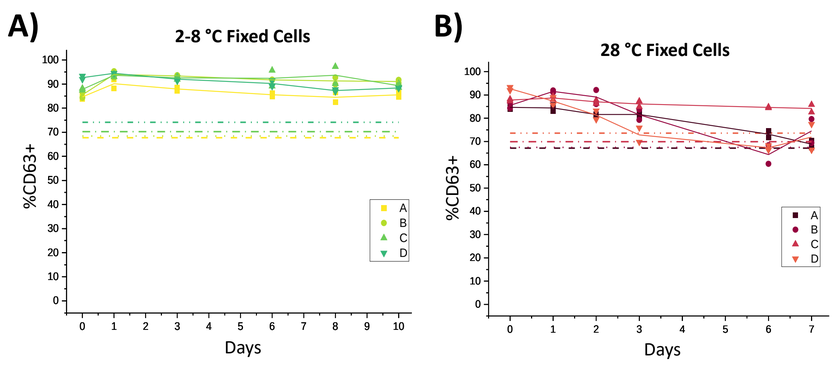
i) Blood Stability

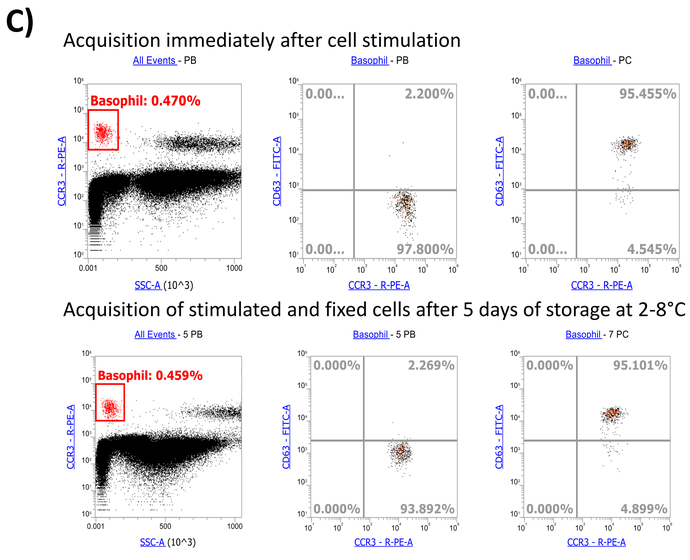
During the short-term storage of EDTA whole blood, the results of day one and day two stored at 2-8°C (A) were within the 80% recovery criteria (dotted line), while for day four all results dropped below 80% from the baseline results of time point 0. (B) Storage of EDTA whole blood at 28 °C leads to a drop of all results below the 80% recovery criteria at day two.



ii) Processed Cell Stability

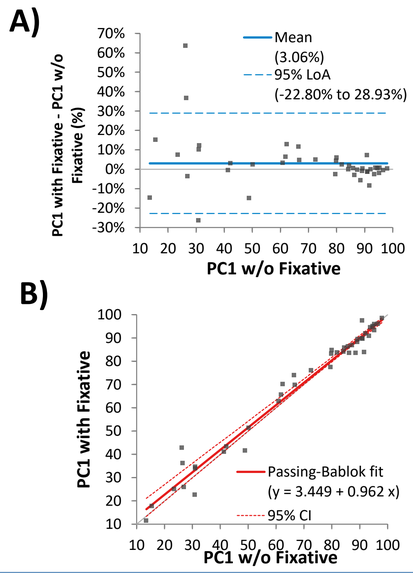
For the short-term storage of stimulated and subsequently fixed cells, all test results remained above the 80% recovery criteria (dotted line) for the study duration of 10 days, if stored at 2-8 °C (A) and for 2 days, if stored at 28°C (B), respectively.
During the flow cytometer acquisition of the processed cells, similar analysis patterns between day 0 and day 5 can be observed (C).





Comparison Study

A 100% agreement between the Flow CAST® with and without stabilizing agent based on CLSI guideline EP09-A3 for 43 samples was achieved.
(A) The relative differences by Bland-Altman analysis showed a mean bias of 3.06% (-1.00 to 7.13%).
(B) Passing-Bablok regression analysis showed a slope of 0.96 (0.91 to 1.01) with an intercept of 3.45 (-0.17 to 7.47) and a correlation coefficient r of 0.986 (r2 = 0.973).



Conclusion & Summary

EDTA whole blood samples stored at 2-8°C give a window of 48 hours until performing a BAT, which allows extended blood sample logistics. The novel stabilizing agent prolonged the stability of activated and fixed basophils up to 10 days at 2-8°C and up to 2 days at room temperature for subsequent flow cytometry acquisition (Table 1). This significantly facilitates time management and hence practicability of BAT testing at laboratories that perform flow cytometry measurements. In comparison to the established method equal analyzing performance can be achieved.

Table 1: Recommended storage conditions for blood and processed cell stability

Stability Study	Temperature	Recommendation of maximal storage time
i) Storage of unprocessed EDTA whole blood	2-8 °C	48 h
	28 °C	24 h
ii) Storage of fixed cells after processing with standard protocol	2-8 °C	5 days
	28 °C	48 h

correspondence: mg@buhlmannlabs.ch

All authors are or were employees of BÜHLMANN Laboratories AG.