Analytical and Stability Validation Studies for Basophil Activation Test according to IVDR

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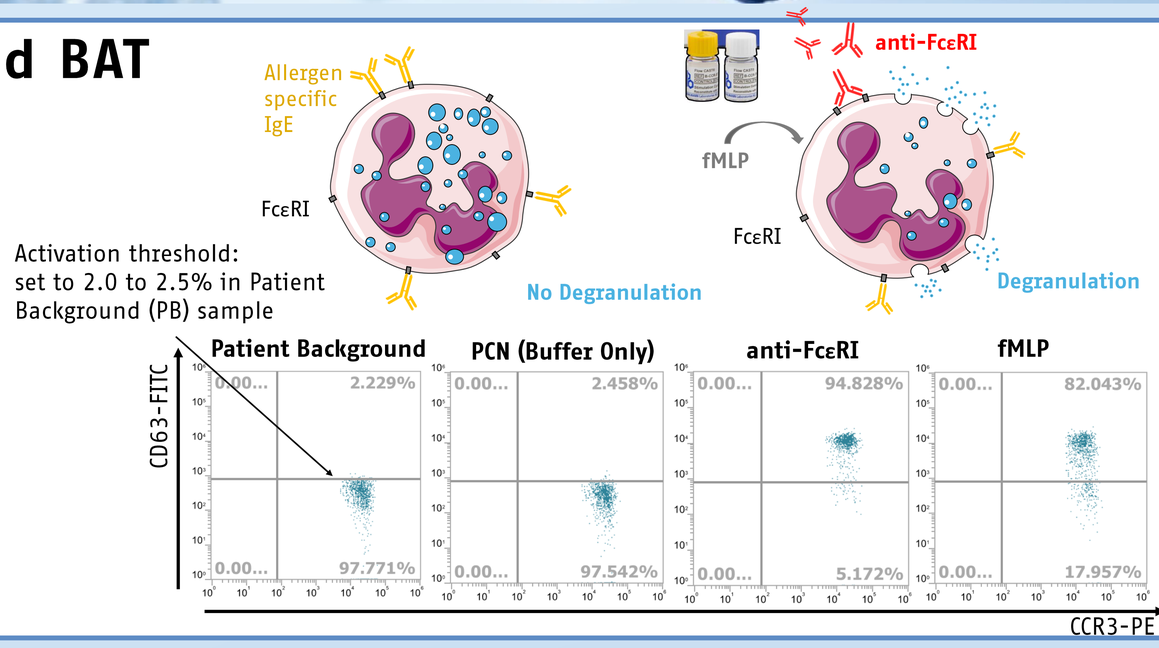
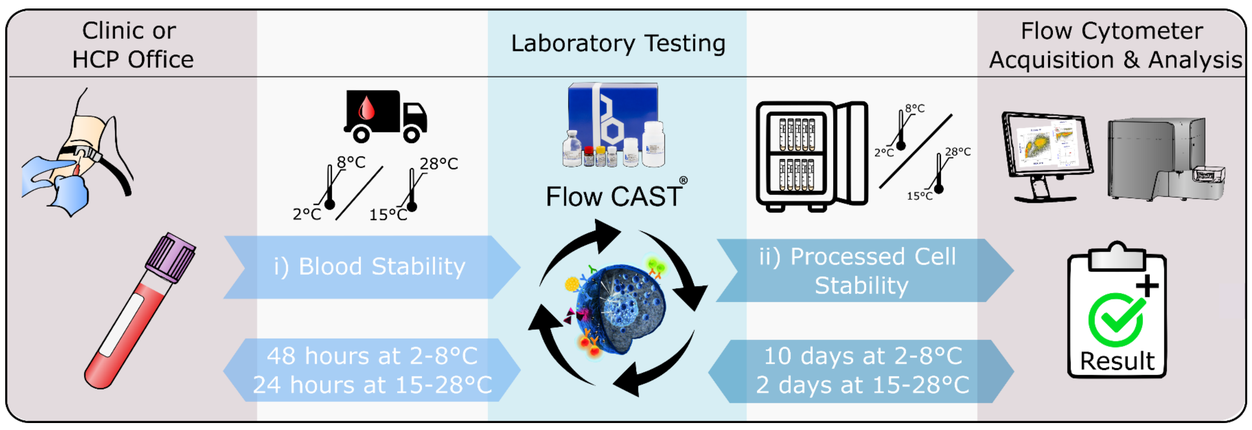
Introduction: The Flow CAST® as a Standardized BAT

Basophil Activation Tests (BAT) have gained increasing importance in the field of allergy diagnostics due to higher accuracy and clinical relevance compared to other allergy tests. In order to provide adequate and useful data for clinical laboratory medicine, thorough assay standardization and validation studies were performed according to the new In-Vitro Diagnostic Regulation (IVDR). This has been done for the BÜHLMANN's Flow CAST® BAT, which quantifies activation as percentage of $CD63^+$ basophils using flow cytometry.

Here, for all studies four healthy blood donor samples were stimulated with stimulation buffer or stimulation control anti-FcεRI mAb or fMLP following CLSI guidelines according IVDR.



Blood and Specimen Stability



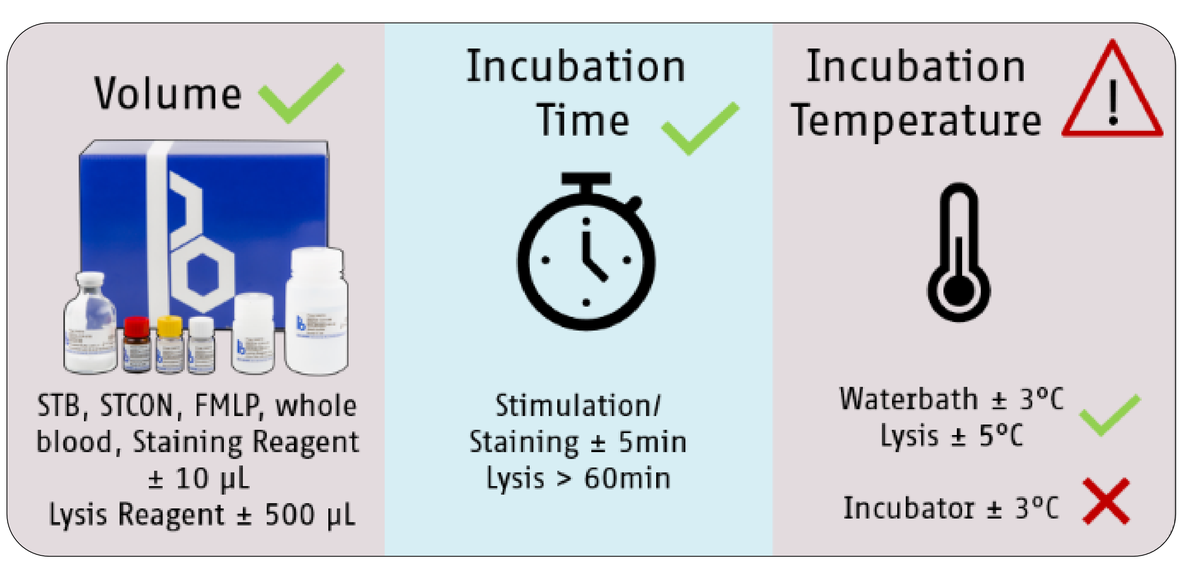
Specimen stability of unprocessed EDTA whole blood and processed (stimulated and subsequently fixed cells) blood were assessed at different temperatures (2-8 °C and 28 °C) for 0 to 10 days. A decay over time of a maximum 20% from the baseline according to the results at time point 0 were accepted to be stable.

EDTA whole blood samples stored at 2-8°C were stable and viable for up to 48 hours before performing a BAT, which allows extended blood sample logistics. The novel stabilizing agent in the Flow CAST® prolonged the stability of activated and fixed basophils up to 10 days at 2-8°C and up to 2 days at room temperature when stored in the dark 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.

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

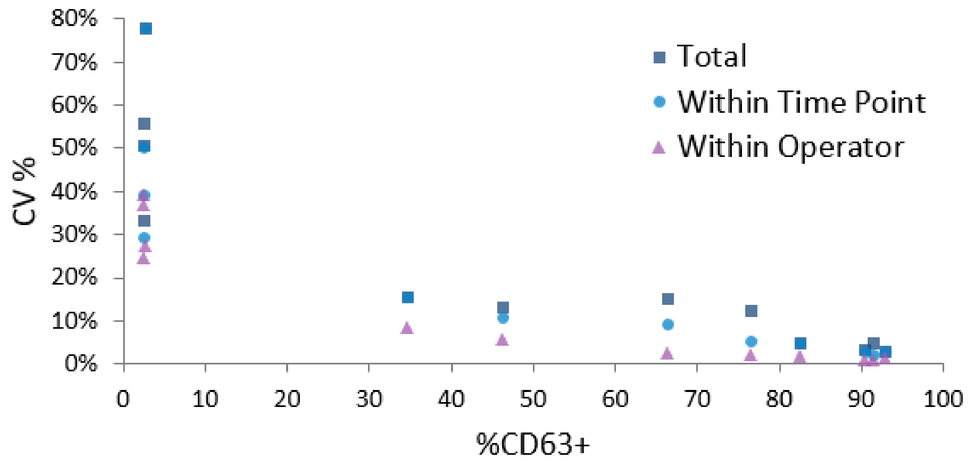
Robustness



The BÜHLMANN Flow CAST® is a robust assay in terms of volume and incubation time changes. Technical precaution is taken regarding the incubation temperature. Small temperature changes can affect test results by using an incubator. Moreover, to guarantee appropriate cell numbers and robust results, lysis time should not exceed 60 min.

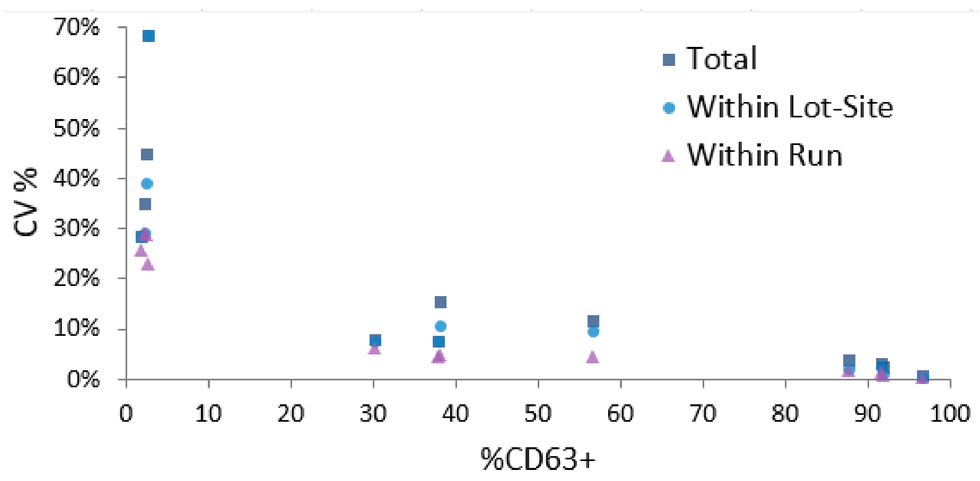
Precision and Reproducibility

Precision



Repeatability (within-run) and within-laboratory precision were established using four donor blood samples using the following assay design: 2 operators x 4 days x 1 run x 4 replicates. A replicate corresponds to an independent stimulation reaction and a full assay procedure. Within-laboratory precision values for controls anti-FcεRI mAb or fMLP were between 3.0 – 15.9% CV. Repeatability values between 1.1-8.8% CV.

Reproducibility



The study design for the reproducibility included 3 instruments/lots x 2 operators x 1 day x 5 replicates. A replicate corresponds to an independent stimulation reaction and a full assay procedure. Testing was performed at two laboratories. Reproducibility values for controls anti-FcεRI mAb or fMLP were between 0.9 – 15.4% CV.

Conclusion

Precision and Reproducibility increases with higher basophil activation level.

The between-run component variance for within-lab precision and between lot/instrument component variance for reproducibility were shown to be the biggest contributing factor to the total test imprecision