Analytical Validation and Stability Studies for Basophil Activation Test to Meet IVDR Certification

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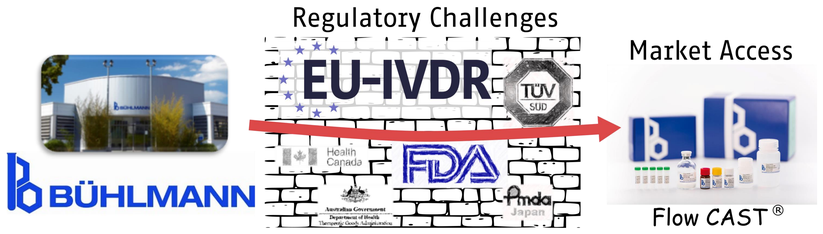
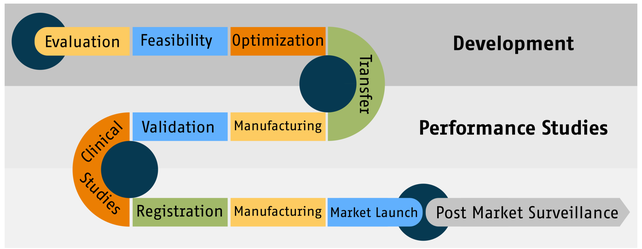
Market Authorization necessary for IVD products

The development and commercialization of In Vitro Diagnostic (IVD) assays represent a complex and intricate journey, marked by various scientific and regulatory challenges. Regulatory compliance plays an increasingly important role, with stringent requirements from organizations like the European, evidenced by the new regulation (EU) 2017/746 IVDR. Meeting these standards necessitates a well-orchestrated effort to manage the manifold development and validation studies of the assay and to provide the appropriate and extensive documentation, which involves the consequential monitoring of post-market performance data during the product life cycle.



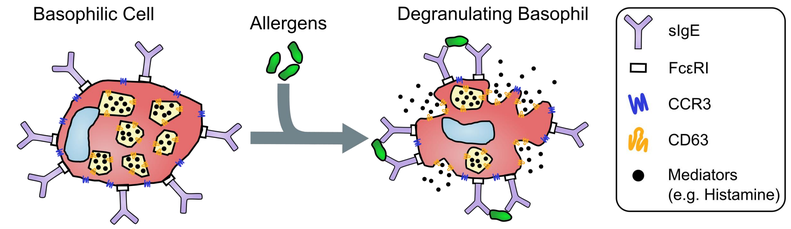
Development Process for Regulatory Compliance



The development process of an IVD assay follows strict rules and requires technical documentation in order to comply with the regulations of the various institutions, such as US Quality Systems Regulations (21 CFR 820), Regulation (EU) 2017/746 (IVDR) and many more. BÜHLMANN is certified according to standards **ISO 13485:2016** Medical Devices - Quality Management Systems (QMS) – (requirements for regulatory purposes) and **ISO 13485:2003** Medical Devices – QMS according the CMDCAS for Canada. The certification is provided by TÜV Süd and TÜV Süd America after accordant audits.

Basophil Activation Test

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. Basophils are a type of white blood cells involved in allergic responses. Monitoring basophil activation can provide insights into a subject's specific allergic sensitivities, helping identify allergens triggering reactions, but there are differences in standardization and limitations in sample collection stability to consider. A recent independent peer reviewed publication (#24 Honer, et al.) **validated the 48 hour stability of EDTA Anticoagulated blood samples maintained @ 4°C, confirming the diagnostic accurate peanut results for at least 48 hours** with the BUHLAMNN Flow CAST assay, addressing a major concern in routine use.

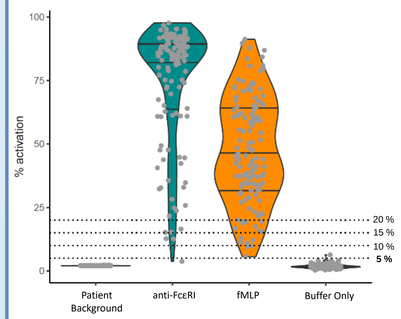


Validation Studies of Basophil Activation Test Flow CAST®

Technical Cut-Off based on Reference Interval

Technical Cut-Off identified at 5% CD63 Activation

Reference interval and technical cut-off were determined according to CLSI C28-A3. The reference interval was established using 120 healthy adult blood donors collected during the period from June to September 2021.



Control	Protocol Option	Mean [%CD63+]	Median [%CD63+]	Reference Interval 2.5 – 97.5 Percentil [%CD63+]
Buffer Only	A	2.4	2.3	0.8 - 4.6
	B	2.3	2.2	0.9 - 4.2
anti FcεR1	A	77.1	85.8	18.0 - 97.7
	B	73.7	83.7	13.2 - 96.4
fMLP	A	49.1	46.5	16.3 - 88.4
	B	41.7	41.1	10.6 - 83.5

Patient Background: samples stimulated only with buffer for setting activation threshold
Anti-FcεRI: positive control, monoclonal antibody against IgE receptor
fMLP: positive control, IgE-independent pathway
Buffer Only: samples stimulated only with buffer to estimate background variance (technical cut-off)
Protocol A: Normal Flow CAST protocol | **Protocol B:** Lyse-no-Wash protocol

Basophils have high FcεRI response and less than 5% are Non-Responder

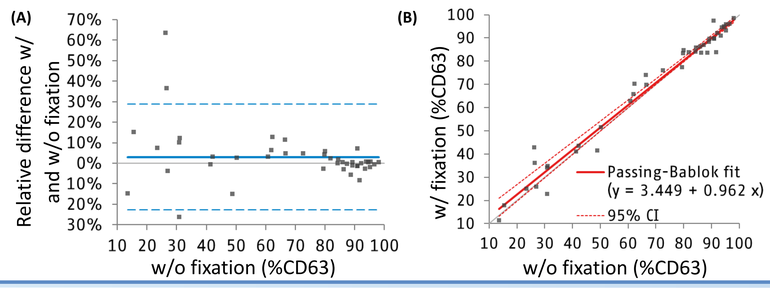
81.5% of donors (106/130) display an activation rate higher than 50% when stimulated with anti-FcεRI. Within the 130 healthy blood donors, only 1 individual showed a basophil activation of less than 5% and 10% (technical- and non-responder cut-offs, respectively) upon stimulation with anti-FcεRI, but this 1 individual 'anti-FcεRI non responder' was confirmed with a fMLP activation of more than 90%.

Comparison Study

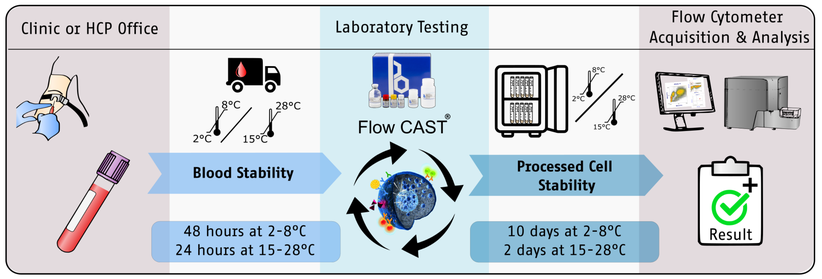
A 100% agreement between the Flow CAST® with and without stabilizing agent using anti-FcεRI stimulation was achieved based on CLSI guideline EP09-A3 for 43 samples.

(A) 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).

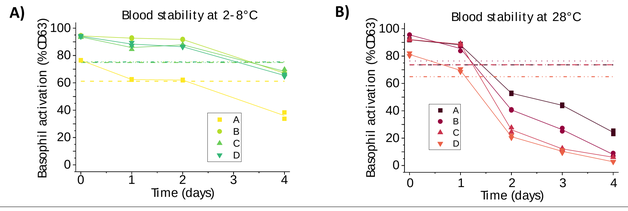


Blood and Processed Cell Stability



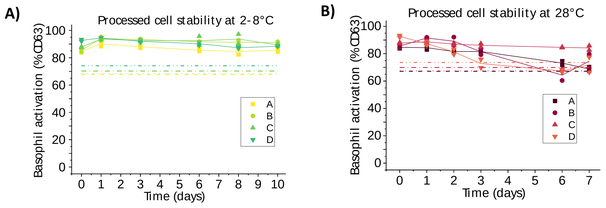
Blood Stability

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% compared to the baseline results on day 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.



Processed Cell Stability

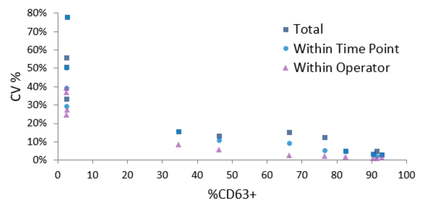
For short-term storage of stimulated and subsequently fixed cells, all test results remained above the 80% recovery criteria (dotted line) for the study duration of 10 days, if stored at 2-8 °C (A) and for 2 days, if stored at 28°C (B), respectively.



Precision and Reproducibility

Precision

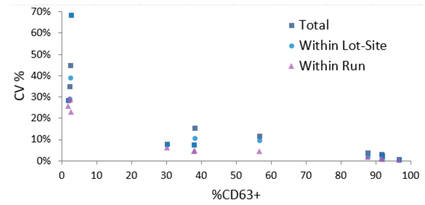
Repeatability (within-run) and within-laboratory precision were established using four donor blood samples using the following assay design: 2 operators x 4 days x 1 run x 4 replicates. A replicate corresponds to an independent stimulation reaction and a full assay procedure. Within-laboratory precision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



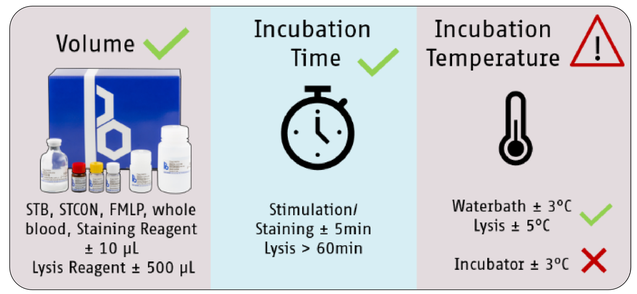
Reproducibility

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.



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.

#= 24-Horner et al., Blood Stored In EDTA Tubes Provides Accurate Peanut Basophil Activation Test Results For 48 hours, DOI: 10.1016/j.anai.2024.06.022

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