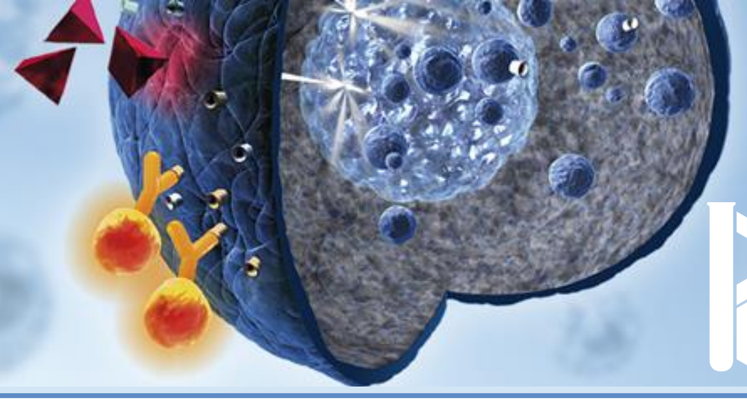
EXPERIMENTAL AND THEORETICAL APPROACHES TO UNDERSTAND THE TECHNICAL CUT-OFF IN BASOPHIL ACTIVATION TEST

Christina U. Bauer[1,2], Anna Melone[1], Dominik Vogt[1], Martina Berchtold[1], Thomas Schuster[1], Christian-Benedikt Gerhold[1], Michael A. Gerspach[1,2]

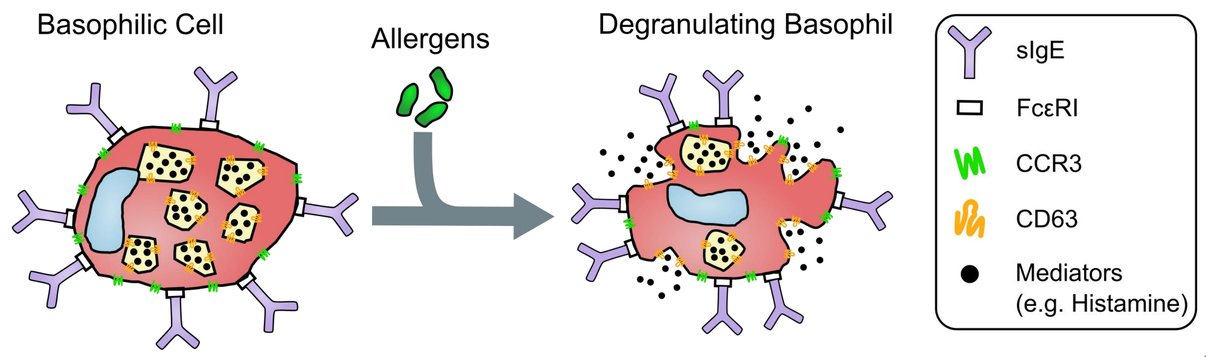
[1] BÜHLMANN Laboratories AG, Schönenbuch, Switzerland
[2] to whom correspondence shall be addressed: cba@buhlmannlabs.ch, mg@buhlmannlabs.ch



Background and Objective

Current diagnostic tests provide unsatisfactory results and often lead to ambiguous conclusion or even misdiagnosis. Due to its high specificity, the basophil activation test (BAT) has gained increasing importance in the field of allergy diagnosis. Unlike other immunoassay techniques such as ELISA, where the readout is a bulk signal, BAT is a single-cell technology where individual basophils are counted and classified into activated or non-activated. Therefore, the question often arises:

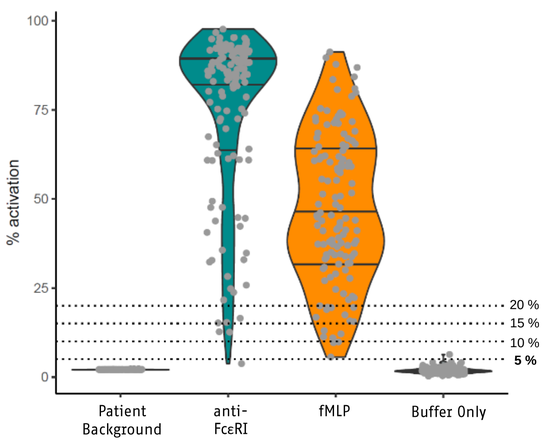
How many basophil cells need to be analyzed to obtain a statistically significant result?



Technical Cut-Off based on Reference Interval & Basophil Response

Technical Cut-Off identified at 5% CD63 Activation

To determine the reference interval and technical cut-off value for the Flow CAST® according to CLSI C28-A3 we used healthy adult blood donors collected during the period from June to September 2021. Blood samples were tested with the standard protocol (A) and the Lyse-No-Wash (B) option. The reference interval was established using 120 donors.



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)

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. The non-responder rate in the Flow CAST® is therefore 0.77% (95% CI: 0.02 – 4.21%). Even if considering higher activation levels of 15% and 20% CD63 for the anti-FceRI would result in a rate less than 5%.

Conclusions

The high standardization level of the Flow CAST® allows to clearly discriminate specific activation above 5% CD63 activation. The majority of healthy individuals show strong activation levels in response to FcεRI crosslinking. The non-responder rate is less than 1%, far below the 5%-15% reported as the main limitation of BAT in IVD.

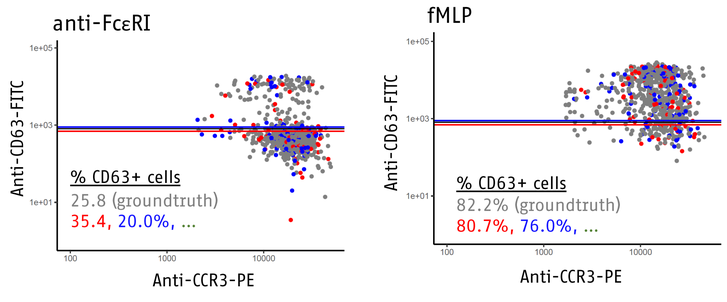
Note: The study was performed between June and September 2021. Studies to investigate potential seasonal variation of non-responder rate are currently ongoing.

Studies on the Reliability of BAT Results dependent on different Basophil Counts

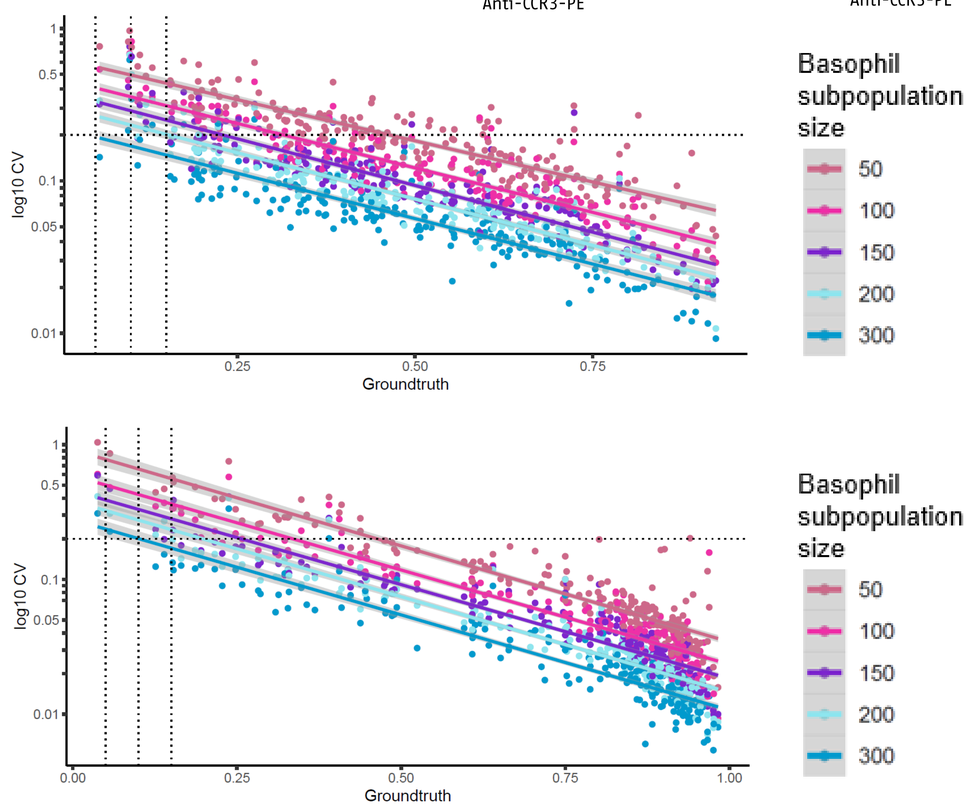
Experimental Results Random Subsampling Approach

207 Flow CAST® datasets have been randomly downsampled to basophils populations of different sizes for 40 iterations and the CV was computed. Subsampling was performed on unstimulated (patient background) and stimulated basophils using two different positive controls, anti-FcεRI Antibody and fMLP.

Example of Subsampling



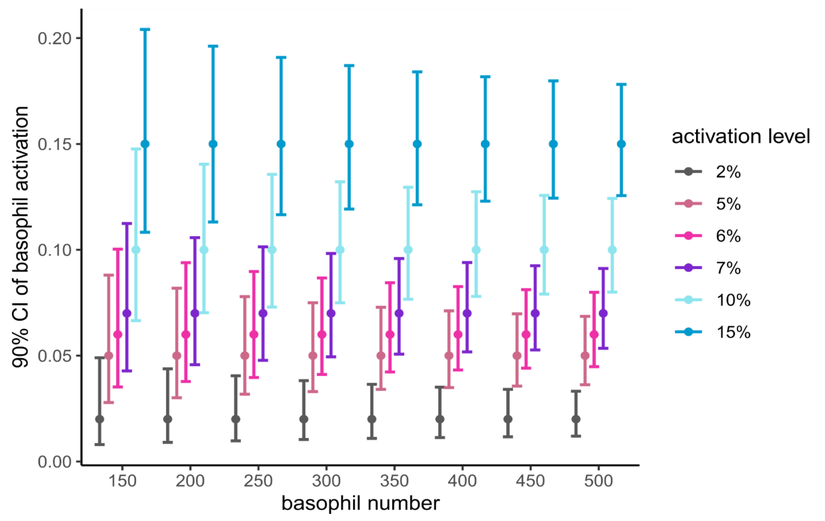
anti-FcεR



fMLP

Theoretical Results X^2-based Theoretical Statistics

The theoretical results are based on a X^2 (Chi2) test statistic and have been obtained with R (version 4.1.2) for a series of combinations of cut-offs and basophil counts. The results confirm a general recommendation for acquisition of at least 500 basophils per sample. If this number cannot be reached, the 90% confidence intervals (CI) of X^2 test could be used as thresholds for confidently negative or positive results (see table). This leaves an increasingly narrow window of activation where results should be interpreted with caution due to lack of statistical confidence.



	Allergen specific cut-off					
	5%		10%		15%	
Basophil count	Negative if <	Positive if >	Negative if <	Positive if >	Negative if <	Positive if >
150 – 199	2.80%	8.80%	6.70%	14.80%	10.80%	20.40%
200 – 249	3.00%	8.20%	7.00%	14.00%	11.30%	19.60%
250 – 299	3.20%	7.80%	7.30%	13.60%	11.70%	19.10%
300 – 349	3.30%	7.50%	7.50%	13.20%	11.90%	18.70%
350 – 399	3.40%	7.30%	7.70%	13.00%	12.10%	18.40%
400 – 449	3.50%	7.10%	7.80%	12.70%	12.30%	18.20%
450 – 499	3.60%	7.00%	7.90%	12.60%	12.40%	18.00%

For each allergen, there is a diagnostically relevant cut-off value, ranging from 5 to 15 % CD63+ cells.

Take-Home Message

- To obtain reliable results in BAT testing, at least 500 basophils need to be analyzed in general.
- However, BAT results at high activation levels (> 50%) require only few basophils (50-100) to be accurate.
- If less than 500 basophilic cells are acquired (for example in case of basopenia), a dynamic threshold depending on the basophil count, allergen specific cut-off and obtained basophil activation level can be considered to prevent unnecessarily high number of non-interpretable assays.
- Flow CAST® data or in general BAT data should only be evaluated if at least 300 basophils are acquired in the unstimulated patient background sample.