Algorithmic methods for quantifying spontaneous activation in basophil activation tests: toward scalable data analysis

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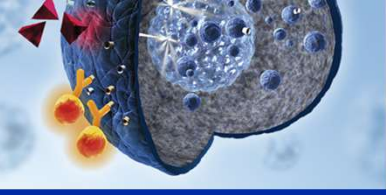
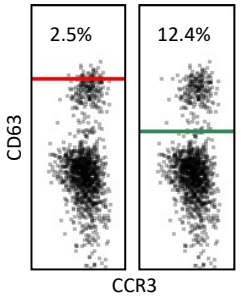


Introduction

- According to BAT analysis consensus, **activation thresholds should be set around the 97.5th – 98th percentile in patient background** (PB) samples (2-2.5% activation). [1]
- In an automated analysis pipeline, this consensus can lead to an elevated activation threshold and **false (reduced) activation in patients exhibiting spontaneous activation** , such as chronic urticaria patients. [2]
- Here we present and compare **two automated methods to recognise spontaneous activation and appropriately set activation thresholds**



Summary and conclusion

- Automated BAT **data analysis pipelines need a built-in method for detecting spontaneous activation**. Although relatively rare, it can lead to false BAT results.
- We present **2 methods (gaussian and percentile) which successfully detected all 4 spontaneously activating patient background samples** out of 598 total.
- Both methods **robustly computed good estimates for sample activation in a reference-free setting for activations of up to 50% for gaussian to 65% for percentile**. Perhaps, paving the way towards fully reference-free determination of activation.
- Due to its simplicity and superior performance, **we recommend usage of the percentile method**.

Determining the activation threshold

This process relies on **comparing the 97.5th percentile (p97)** with an **alternate method of estimating the activation threshold**. Two such methods are presented below.

Gaussian fitting

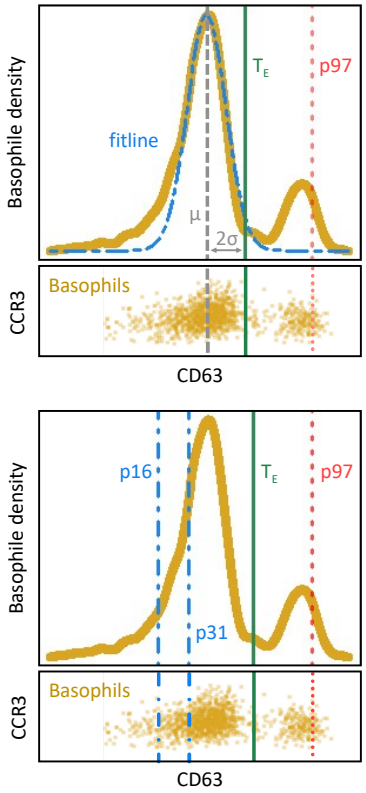
- **In logscale**, the distribution of **CD63 signal of the non activated basophil population is likened to a gaussian distribution** where the **97.5th percentile (p97) mark is situated two standard deviations (σ) from the peak (μ).**
- Through **data fitting** in $\log_{10}$ scale of the non activated basophil (highest) peak, **a reliable estimate for the activation threshold (T_E)** is obtained: **$T_E = 10^{(\mu + 2\sigma)}$**

Percentile range

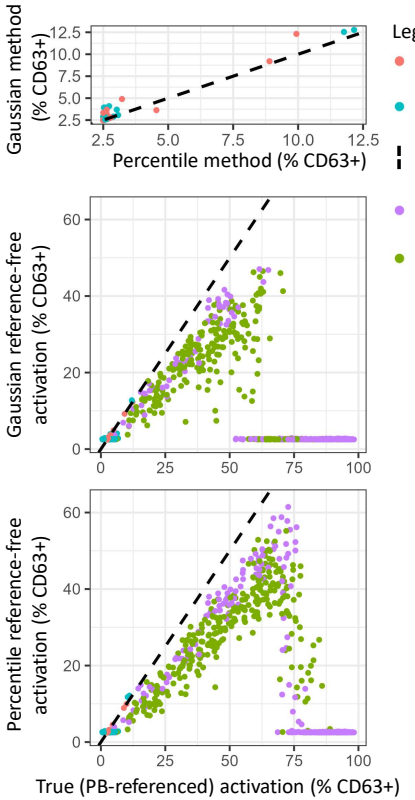
- **Empirically**, the following relation between the 16th and 31st percentiles (p16 and p31) was determined to be a good estimate for the activation threshold: **$T_E = p31+12(p31-p16)$**
- The estimate relies on the **left side of the CD63 signal distribution, which remains unaffected by spontaneous activation**, unlike the 97.5th percentile.

For best analysis outcomes, **pick lowest threshold between the 97.5th percentile and the estimate**:

$T_{Activation} = \min(p97, T_E)$



Reliable detection of spontaneous activation



Both methods **successfully identify the same 4 samples exhibiting > 5% spont. activation out of 598 PB and repeat (PBN) samples** , also providing reliable estimates for the activation threshold.

To mimic **strong spont. activation**, an **additional 598 contrived Flow CAST® BAT samples stimulated with anti-FcεRI (PC1) and fMLP (PC2)** were analysed. Here, both the **ability to detect and correctly quantify activation without a PB reference** was evaluated and compared to the true PB-based activation of the same method.

Comparison	Gaussian fitting	Percentile range
Threshold in PB	Reliable, slight underestimation, not corrected by p97	Reliable, slight overestimation, corrected by p97
Detection of spont. activation	Robust up to 50% activation	Robust up to 65% activation
Reference-free activation	Mostly reliable within range	Very reliable within range
Implementation	Basic processing (fitting, peak-finding)	Simple and straightforward
Transferability to general BAT assays	Directly transferable	May require small adaptations

Poster Nr: TPS - D3.74 / JMA – D1.372

[1] doi: 10.1111/all.15907
[2] doi: 10.1111/all.15090

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