

A consensus protocol for the Basophil Activation Test for multicenter collaboration and External Quality Assurance

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To the editor

The basophil activation test (BAT) has significant potential as a diagnostic tool to better phenotype and manage patients with IgE-mediated allergies, so that only a small proportion of patients need to be challenged. Sample, reagent, laboratory procedure, analysis protocols, and population characteristics can influence BAT performance (1,2). Regulatory approval and clinical implementation require extensive standardization of laboratory protocols, cytometer settings, and results interpretation (3). European national authorities require External Quality Assurance (EQA) of the performance of modern diagnostic laboratories by agencies independent of test suppliers to meet ISO 15189:2012, 15189:2013 and 9001:2015.

Based on an online survey among 59 responding European laboratories performing BAT in 2017 (4,5) (Online Supplement; Results of the online survey), a Task Force was launched in 2018 to create the basis for a BAT-EQA. Round Robins (RR) were organized with seven shipments of 2 donors each to 7-10 European centers

with overnight courier service from Bonn, DE. To minimize variation, prior to shipment, blood basophils were activated with 1 μ l Fc ϵ RI antibody/ml of blood and stabilized with 0.2 mL Transfix (Cytomark, UK) per mL of blood to stabilize activated basophils up to 48 hours for staining (6). Fresh blood was included for stimulation and staining at the participating laboratory sites.

We met after the third shipment to reach consensus on a protocol for BAT (Online Supplement; Proposed SOP for in house BAT). The threshold set on an unstimulated control sample was determined empirically on an independent data set as equal or greater than 2.5% with ROC curves based on data from patients with hypersensitivity to amoxicillin and patients with peanut allergy, (Online supplement, tables S1 and S2). This proposal did not find universal consensus among the authors.

Data analysis started with identification of the relevant region in a scatter plot, followed by identification of basophils with the relevant markers, for instance, using low SSC and CD193 only or CD193 and CD123. Finally, the threshold was set at 2.5% of CD63 expression on resting basophils (Figure 1A). >5% CD63⁺ basophils above that threshold in an activated sample was considered a positive response. This setting was used to obtain the percentage of CD63⁺ cells in centrally preactivated and locally activated blood samples; however, it was not adopted in all labs. Data from participating labs analyzed with their proprietary and the above standardized analysis compared well (online supplement, figure S4).

The first two RR were used to establish coherence between participating laboratories. Data from RR3–RR7 were comparable. The standard deviation of activation measured at all participating centers was 16.8% in preactivated blood (Figure 1B) compared with 49.2% for samples activated and analyzed locally, illustrating the utility of using preactivated blood for EQA. Shipment to Málaga took 48h, and local activation of blood basophils was consistently suboptimal, consistent with a preliminary round robin from 2012, where the clinical outcome was robust up to 24 h. Centrally activated basophils performed as well in Málaga as in other centers.

EQA for BAT is critical to facilitate routine implementation of this assay in the field of in vitro allergy diagnostics. The variability of the responses to our survey highlighted the importance and need for multicenter validation. Full validation and standardization of the BAT protocol and analysis is essential and possible for setting the grounds for controlled multicenter research studies as well as EQA. The BAT-EQA Task Force provides a standard operating protocol (Online supplement; Proposed SOP for in house BAT) and reference materials for the test to standardize and enhance the accuracy of BAT for both clinical and research collaborations and EQA.

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Figure legend:

Figure 1:

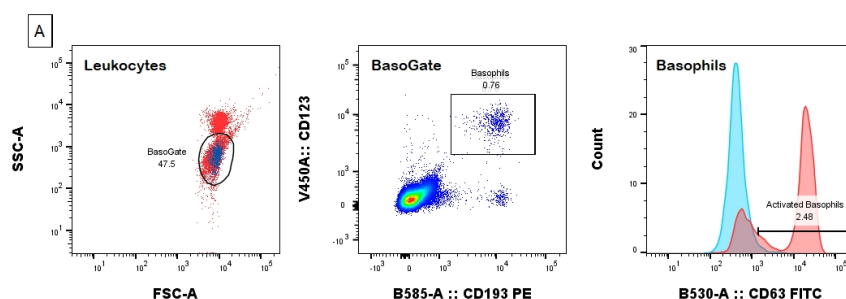
A The consensus analysis process for basophil activation starts with identification of a region containing basophils on a scatter plot, followed by selection of basophils with either two or one basophil specific marker and is completed by setting the threshold at 2.5% of resting basophils (blue), before the fraction of activated basophils (red, 74%) is obtained.

B Data was acquired for preactivated stabilized basophils and for fresh blood. A stippled line indicates the threshold for a positive BAT at 5%. Results with the stabilized cells reflect the efficacy of detecting activation of the same sample and is more focused than that of either heparin or EDTA stabilized blood. The median of standard deviations of each round was 16.8% for the centrally activated, stabilized basophils and 49.2% for the locally activated basophil preparations ($p=0.03$ Wilcoxon Signed Rank test).

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Fig 1b Layout 5 SEM.emf available at <https://authorea.com/users/342682/articles/614984-a-consensus-protocol-for-the-basophil-activation-test-for-multicenter-collaboration-and-external-quality-assurance>