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EAACI POSITION PAPER

EAACI task force report: A consensus protocol for the basophil activation test for 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

M Pascal, SM Edelman and A Nopp are shared first authors.

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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 (TF) was launched in 2018 to create the basis for a BAT-EQA. Round Robins (RR) were organized with seven shipments of two donors each to 7–10 European centers with overnight courier service from Bonn, DE. Each sample was split into two aliquots: (i) To minimize variation, prior to shipment, blood basophils of anonymous donors were activated with 1 µL anti-FcεRI antibody/mL of blood and stabilized with 0.2 mL Transfix (Cytomark, UK) per mL of blood to stabilize activated basophils up to 24 h for staining[6]; (ii) Fresh blood was included for stimulation and staining at the participating laboratory sites.

TF members met after the third shipment to reach consensus on a protocol for BAT (Online Supplement; Proposed SOP for in house BAT). Data analysis started with identification of the relevant region in a scatter plot (Figure 1A), followed by identification of basophils with the relevant markers following different strategies, for instance, using low SSC and CD193 only or CD193/CD123 or CD193/CD203c (Figure 1B).

The threshold was set at 2.5% of CD63 expression on resting basophils (Figure 1C). This threshold set on an unstimulated control sample was determined empirically on independent datasets 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 setting was used to obtain the percentage of $CD63^+$ cells in both centrally preactivated and locally activated blood samples (Figure 1D–F). This proposal, although considered to be more robust by the majority, did not find universal consensus. One laboratory insisted to maintain a threshold "as close to 0% as possible" as their data are based on that approach. Data from participating laboratories

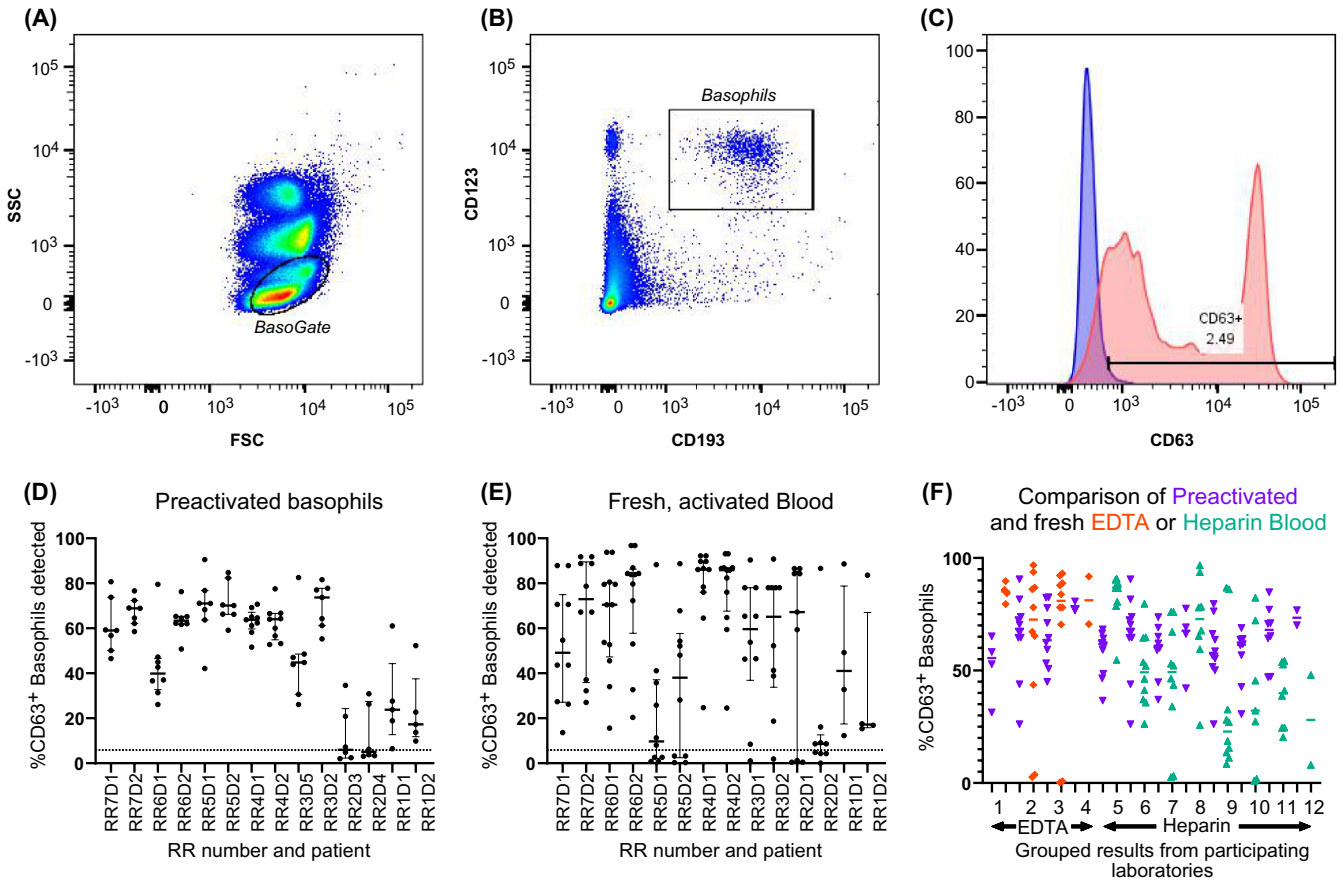

FIGURE 1 The consensus analysis process for basophil activation starts with identification of a region containing basophils on a scatter plot (A), followed by selection of basophils with either two or one basophil-specific marker (B) and is completed by arbitrarily setting the threshold at 2.5% of resting basophils (blue), before the fraction of activated basophils (red, 74%) is obtained (C). Data were acquired for preactivated stabilized (D) basophils and for fresh blood (E). 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. (F) Comparison of performance of individual participants grouped for EDTA and heparin blood.

analyzed with their proprietary and the above standardized analysis compared well (online supplement, figure S4). >5% $CD63^+$ basophils above that threshold in an activated sample was considered a positive response. The first two RR were used to train participating laboratories in the procedure of the analysis. Data from RR3 to RR7 were comparable. The standard deviation of activation measured at all participating centers was 16.8% in preactivated blood (Figure 1D) compared with 49.2% for samples activated and analyzed locally (Figure 1D), illustrating the utility of using preactivated blood for EQA ($p = .03$ Wilcoxon signed rank test). In Figure 1F, individual laboratories performance is presented. After transport, activation of EDTA blood is much better than activation of heparin-stabilized blood ($p = .0091$, Wilcoxon signed rank test). However, as most laboratories used heparin-stabilized blood in daily routine, we developed a method that accommodates both approaches by preactivating blood for EQA.

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 for further work on this matter 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. A consensus protocol has been identified that gives acceptable inter- and intralaboratory variability (according to accepted standards), and that can be implemented across Europe with preactivated blood. This protocol demonstrates that all participating laboratories can contribute giving consistent response when testing basophil activation with appropriate allergens and using the SOP.

AUTHOR CONTRIBUTIONS

The study was conceived by M Pascal, S Edelman, and H J Hoffmann to address escalating demands of regulators for external quality assurance. WJ Geilenkeuser provided logistic support. C Mayorga provided the data for the estimation of the optimal threshold setting. All other authors participated in the RR, provided resources, analyzed their samples, and contributed toward the consensus.

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CONFLICT OF INTEREST STATEMENT

M Pascal, SM Edelman, A Nopp, C Möbs, EF Knol, and C Mayorga have no conflict of interest regarding this work. B Eberlein received methodological and technical support from the company BUEHLMANN Laboratories AG (Schönenbuch, Switzerland) outside the submitted work. Dr Hoffmann reports a grant from the Innovation Fund of Denmark, outside the submitted work. Dr. Patil reports grants from the NIH (5R01AI155630, 1R21AI159732), Food Allergy Science Initiative (FASI), NIAID Immune Tolerance Network (ITN), and Charles H. Hood Foundation Child Health Award. Dr Shamji reports grants awarded to institution from the Immune Tolerance Network, UK Medical Research Council, Allergy Therapeutics, LETI Laboratories, Revolo biotherapeutics and Angany Inc. He has received consulting fees from Bristol Meyers Squibb and lecture fees from Allergy Therapeutics and LETI laboratories, all outside the submitted work. Dr. Santos reports grants from Medical Research Council (MR/M008517/1; MC/PC/18052; MR/T032081/1), Food Allergy Research and Education (FARE), the NIH, Asthma UK (AUK-BC-2015-01), the Immune Tolerance Network/National Institute of Allergy and Infectious Diseases (NIAID, NIH), and the NIHR through the Biomedical Research Centre (BRC) award to Guy's and St Thomas' NHS Foundation Trust, during the conduct of the study; speaker or consultancy fees from Thermo Scientific, Nutricia, Infomed, Novartis, Allergy Therapeutics, IgGenix, Stallergenes, Buhlmann, as well as research support from Buhlmann and Thermo Fisher Scientific through a collaboration agreement with King's College London, outside the submitted work. Dr Geilenkeuser is an employee of Referenizinstitut für Bioanalytik, DE that provided logistic assistance and reagent support for the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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