

# The Measurement of Basophil Activation using a Flow Cytometry Kit for Application in Clinical Trials

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## ABSTRACT

**Background:** Allergens or antigens can cause an allergic reaction in the body due to the IgE class of antibodies. These antibodies develop when the foreign invader triggers their formation. Basophil cells can become activated when the IgE antibody (bound to an allergen) recognizes and binds to the Fc receptor on the basophil surface. Basophils contain many granules inside the cell, which are filled with a variety of active substances that trigger an allergic response upon degranulation. Basophil activation also causes certain markers to be detected on the surface of the cell that can be measured by flow cytometry to determine basophil activity in support of clinical trials.

**Methods:** Experiments were conducted with human whole blood samples using a commercially available basophil activation kit. Whole blood specimens from human donors were collected in EDTA blood collection tubes. Blood was stimulated with FCε-R1 and fMLP and staining reagent for 25 – 30 minutes at 37°C. Cells were lysed, washed and acquired on the flow cytometer.

**Results:** CCR3 was used to identify the basophil population in human whole blood. CD63 and CD203c were then used to identify the unstimulated and stimulated state of the samples. Upon stimulation there was an increase in CD63 and CD203c expression on the surface of the cells.

**Conclusion:** These results showed that Basophil activation can be measured using flow cytometry with the increased expression of CD63 and CD203c on stimulated basophils. This method could be used to help scientists in clinical trials to study the effects of allergens or antigens that cause allergic reactions in the body.

## CONTACT

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## INTRODUCTION

Current methods for allergen testing, such as skin prick tests and serum IgE measurements, are ineffective at giving quick, clear results. These tests often supply contradictory results, making it difficult, and sometimes dangerous, to diagnose an allergy. These assays are also time consuming, sometimes requiring multiple replicates to confirm a diagnosis. However, the Basophil Activation Test (BAT) offers a safer, faster, and more accurate way to test for allergies by utilizing the power of flow cytometry. BAT tests can use two different cell markers to detect degranulation of basophils: CD63 and CD203c. Because of this, BAT tests are more sensitive than IgE serum measurements, while being safer and less painful than in vivo skin prick tests. Their ability to monitor the state of the actual basophil cells instead of the level of antibody used to activate the cells in the blood stream make BATs a potentially powerful and unique research tool. To test basophil activation, we originally planned to use two different stimulation factors, fMLP and FCε-R1. After further research, it was decided that only fMLP was necessary. The purpose of this study was to measure the efficacy of the BAT test as a tool for the research of basophils by flow cytometry in support of clinical trials.

## MATERIALS AND METHODS

Human whole blood samples were obtained and processed according to instructions for ALPCO's InhibiScreen Basophil Activation Test.

### Procedure 1: Testing of Basophil Stimulation

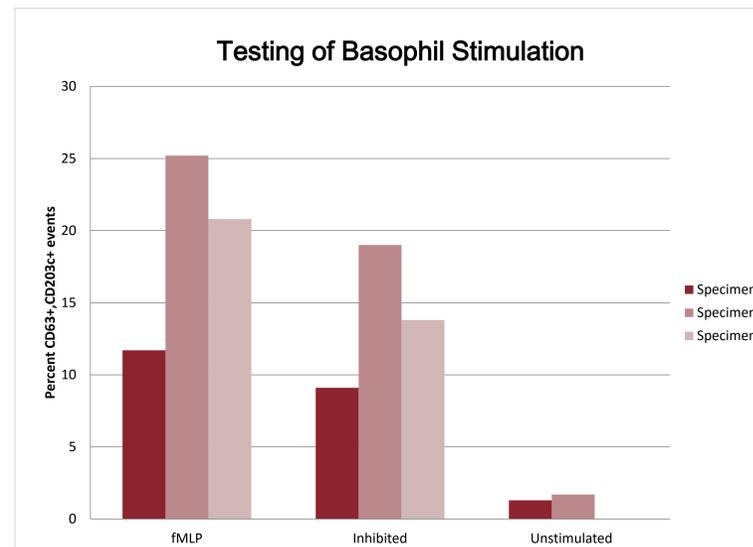
Samples were either inhibited with CAL-101 or left uninhibited, incubated at 37°C for 55-65 min, then stimulated with fMLP. A negative control which was neither inhibited nor stimulated was also processed for each specimen. Samples were stained and incubated at 37°C for 25-30 minutes. After incubation, samples were washed, lysed and analyzed.

### Procedure 2: Dust Mite Allergen Testing

Three samples were processed for each patient: an unstimulated negative control, a positive control stimulated with IgE, and a sample stimulated with dust mite allergen acquired from Buhlmann Labs. Each sample was stained and incubated at 37°C for 25-30 minutes. After incubation, samples were lysed, washed, and analyzed.

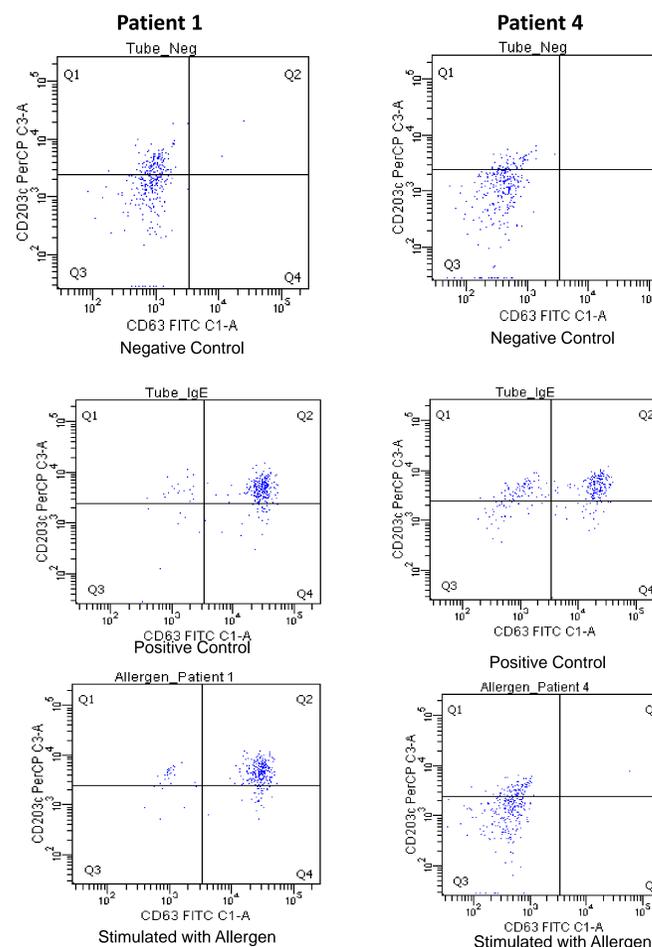
Samples for both procedures were stained with CCR3 PE to determine the basophil population, as well as CD63 FITC and CD203c PerCPCy5.5 to determine basophil activation. Samples were acquired on a BD FACS Canto II equipped with BDFACSDiva 6.1.3 software.

## RESULTS



**Figure 1. Percent of CD63+, CD203c+ events.** Samples were stimulated with fMLP, Inhibited with CAL101, or left unstimulated and uninhibited.

### Dust Mite Allergen Testing



**Figure 2 Comparison of Cytograms for Patients 1 and 4.** Patient 1 shows and allergic reaction to dust mites, while Patient 4 does not.

## RESULTS (cont.)

|            | fMLP | Unstimulated |
|------------|------|--------------|
| Specimen 1 | 33.1 | 1.3          |
| Specimen 2 | 11.7 | 1.3          |
| Specimen 3 | 9.4  | 4            |
| Specimen 4 | 25.2 | 1.7          |
| Specimen 5 | 20.8 | 0            |

**Table 1: Percent Activated Basophils from Stimulation Test** Patient percentage of CD63+, CD203c+ events in the basophil population, indicating activated basophils.

|           | Unstimulated | IgE  | Allergen |
|-----------|--------------|------|----------|
| Patient 1 | .7           | 80   | 77.9     |
| Patient 2 | .4           | 71.7 | 79       |
| Patient 3 | 0            | 42.3 | 49.7     |
| Patient 4 | 0            | 55   | .3       |

**Table 2: Activated Basophils from Dust Mite Allergen Test** Patient percentage of CD63+, CD203c+ events in the basophil population, indicating activated basophils.

## CONCLUSIONS

The purpose of this study was to investigate the efficacy of Basophil Activation Tests (BATs) as a new in vitro platform for allergen detection by flow cytometry in support of clinical trials. We observed that in all of our specimens for both procedures, the BAT detected high amounts of activated basophils in blood stimulated with either fMLP or IgE, while cells which were left unstimulated measured low to no events for basophil activation. When the pathway which fMLP uses to stimulate basophils was inhibited in three of our specimens, the BAT identified a lower level of activated basophils. When stimulated with allergen, a significant difference in basophil activation between patients with allergies and without allergies was observed, while controls were consistent between all patients. This data shows that basophil activation tests can be used to successfully study basophil activation and allergies in a clinical setting by flow cytometry.

## REFERENCES

- Emily C. McGowan, Sarbjit Saini, 2014. Update on the performance and application of basophil activation tests. Curr Allergy Asthma Rep. PMC 2014 Jul 2.
- ALPCO Inhibiscreen Basophil Activation Test, [www.alpco.com](http://www.alpco.com)

## ACKNOWLEDGEMENTS

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