

Basophil Activation Test Using Flow Cytometry (FLOW-CAST test)

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Introduction

Flow cytometry literally refers to the measurement of the properties of cells as they “flow”. It is a flexible testing platform allowing for the rapid assessment of large numbers of cells. It is therefore a very good method for assessing relatively rare cells such as basophils. Basophils generally represent < 1% of the total leucocytes in the peripheral blood.

Principle of the Basophil Activation Test (Flow-CAST)

The basophil activation test (BAT) is based on the *in vitro* reaction of peripheral blood basophils in the presence of a specific allergen. Both IgE and non-IgE mediated reactions can be detected. The test is referred to by a variety of names – FAST (flow cytometry allergen stimulation test), Flow-CAST (used by Bühlmann Laboratories), and BASOTEST (used by Becton Dickenson). The term CAST stands for “cellular antigen stimulation test”. The Flow-CAST test is the testing method used by Lancet Laboratories and therefore the testing method referred to in this newsletter.

In the basophil activation test, the cells are incubated with individual purified allergens under defined conditions to mimic the *in vivo* situation. It measures the response of circulating basophils to that specific allergen.

Controls are performed together with every patient sample. A negative control is included to determine the background level of activation. This result is taken into account in the interpretation of the basophil activation in response to the specific allergen. For the positive control, both fMLP and a highly specific monoclonal antibody that binds with high affinity to the IgE binding receptor (anti-FcεRI mAb) are assessed. These serve as controls for both non-IgE mediated and IgE mediated basophil activation, respectively.

Basophils need to be identified or “gated”. This can be done using a variety of markers. The marker CCR3 is used in the Flow-CAST test.

Basophil activation in response to allergens is assessed by measuring increased CD63 expression on the basophil cell surface. CD63 is an activation marker (see Figure 1). It increases on the basophil cell surface when granules fuse with the cell membrane during the process of degranulation.

The Flow-CAST test is a functional assay which is dependent on the preservation of basophil viability and function.

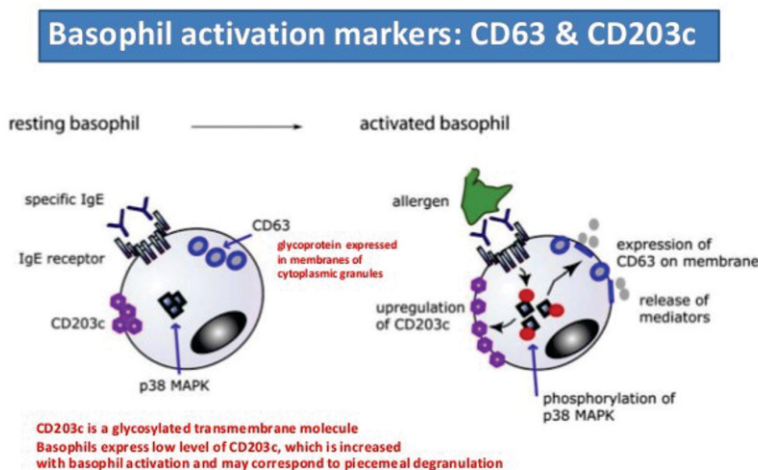


Figure 1. Basophil activation markers: CD63 & CD203c (Adapted from Reference 5)

Important factors in obtaining accurate Flow-CAST results

- A fresh EDTA peripheral blood sample is required. The sample must be stored immediately at 2 – 8 °C. It must NOT be frozen or centrifuged. The EDTA tube must be sufficiently filled. Failure to do this results in a higher EDTA concentration and reduces the sensitivity of the assay. This can potentially result in false negative results.
- Cell stimulation needs to be performed as soon as possible. The Flow-CAST test should be performed within 24 hours of sample collection.
- The patient should avoid systemically administered corticosteroids and cromoglicic acid (DSCG) for a minimum of 24 hours before testing, a longer interval being preferable.
- Flow-CAST testing may be performed when the patient is taking anti-histamine treatment.
- The patient should not currently be experiencing an allergic reaction. This results in a high background level of basophil activation. Basophil activation in response to a specific allergen cannot then be reliably assessed. It is recommended that the test only be performed a minimum of 3 weeks after a significant allergic reaction. It is generally considered that the best time to perform the BAT is 3 weeks to 12 months after an acute event.

Accurate Flow-CAST testing is dependent on sufficient basophils being present in the peripheral blood sample. Low basophil numbers may be observed following recent allergic reactions, tissue migration of the basophils, poor storage or handling of the samples, and following treatment with anti-allergenic medication such as corticosteroids.

Some people are “non-responders” to the positive controls performed during Flow-CAST testing. Approximately 6.1% of individuals are described to be non-responsive to anti-FcεRI antibody and nearly 4.9% to fMLP. The results of FLOW-CAST testing have to be interpreted with caution in the setting of a failed positive control/s.

Differences between BAT/Flow-CAST testing and specific IgE testing (RAST/CAP)

Specific IgE testing (s-IgE) is widely used in cases of suspected allergies. This test involves the serological detection of allergen specific IgE antibodies. A positive result indicates that an individual has been sensitised to an allergen. It does not directly show that the individual will react with basophil activation, mediator release and allergic inflammation. It also cannot detect non-IgE mediated mechanisms.

The Flow-CAST test is not a replacement for specific IgE testing. Specific IgE testing usually remains the first-line laboratory test when assessing inhalant and food allergies. Flow-CAST testing is a sensitive technique in these situations, but it is generally more expensive than specific IgE testing and may not provide additional information. Flow-CAST testing may, however, be of use in complex cases, and when other tests are inconclusive.

In the setting of suspected drug or food additive sensitivity, specific IgE has been shown to have a poor sensitivity. Drug specific IgE may disappear within weeks of the last exposure. The reactions may also be non-IgE mediated. Flow-CAST testing has good specificity and moderate sensitivity in this setting. It may reduce the need for provocative challenges.

Indications for BAT/Flow-CAST testing

Basophil activation testing is a useful test in the evaluation of:

- Food additive (colourants and preservative) sensitivity.
- Drug allergy and non-steroidal anti-inflammatory drug (NSAID) sensitivity.
- Cases of suspected non-IgE mediated sensitivity.
- Suspected Latex allergy.
- Complex or inconclusive cases of suspected inhalant, food or insect (usually bee or wasp) venom allergy. The BAT may reduce the need for oral food challenges.

A list of commercially available purified allergens is attached. The different categories of allergens have different reference ranges. The quality of a BAT is dependent on the quality and standardisation of the allergen of interest. Frequently requested allergens are kept in stock in the flow cytometry laboratory.

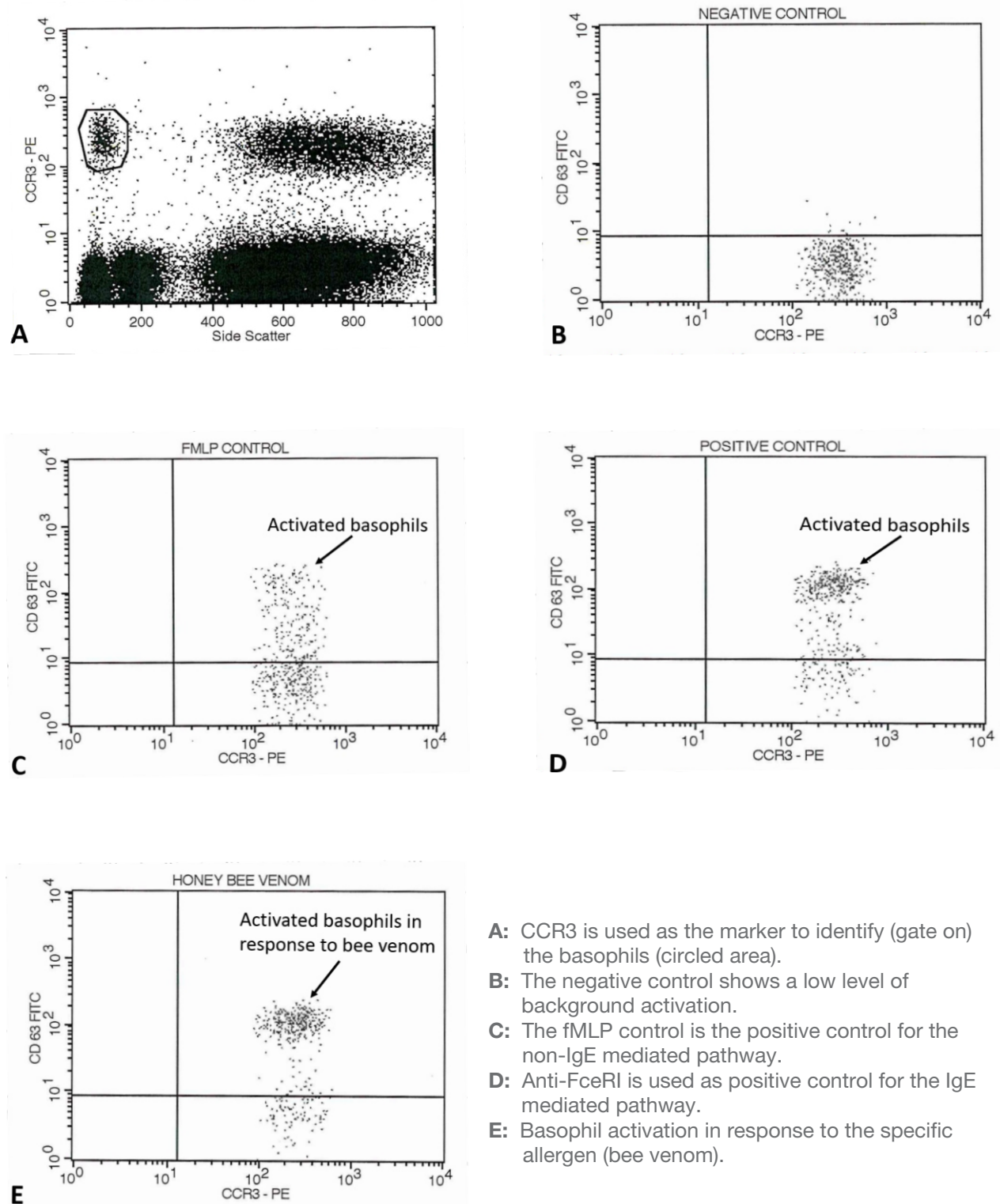
Interpretation of Flow-CAST Results

It is essential that the results of Flow-CAST testing be interpreted in conjunction with a thorough clinical history and the results of all other tests performed (including laboratory tests, skin prick tests, and provocation tests). Functional assays have significant limitations. Provocation tests and skin prick tests typically remain the gold standard.

As the Flow-CAST test has a higher specificity and lower sensitivity, a positive result is usually particularly meaningful.

A negative Flow-CAST test result and failure to demonstrate basophil activation to a particular allergen **DOES NOT COMPLETELY EXCLUDE** the potential for a severe clinical reaction to the allergen. This is particularly important in the case of “weaker” allergens on this testing platform, namely drugs and food additives.

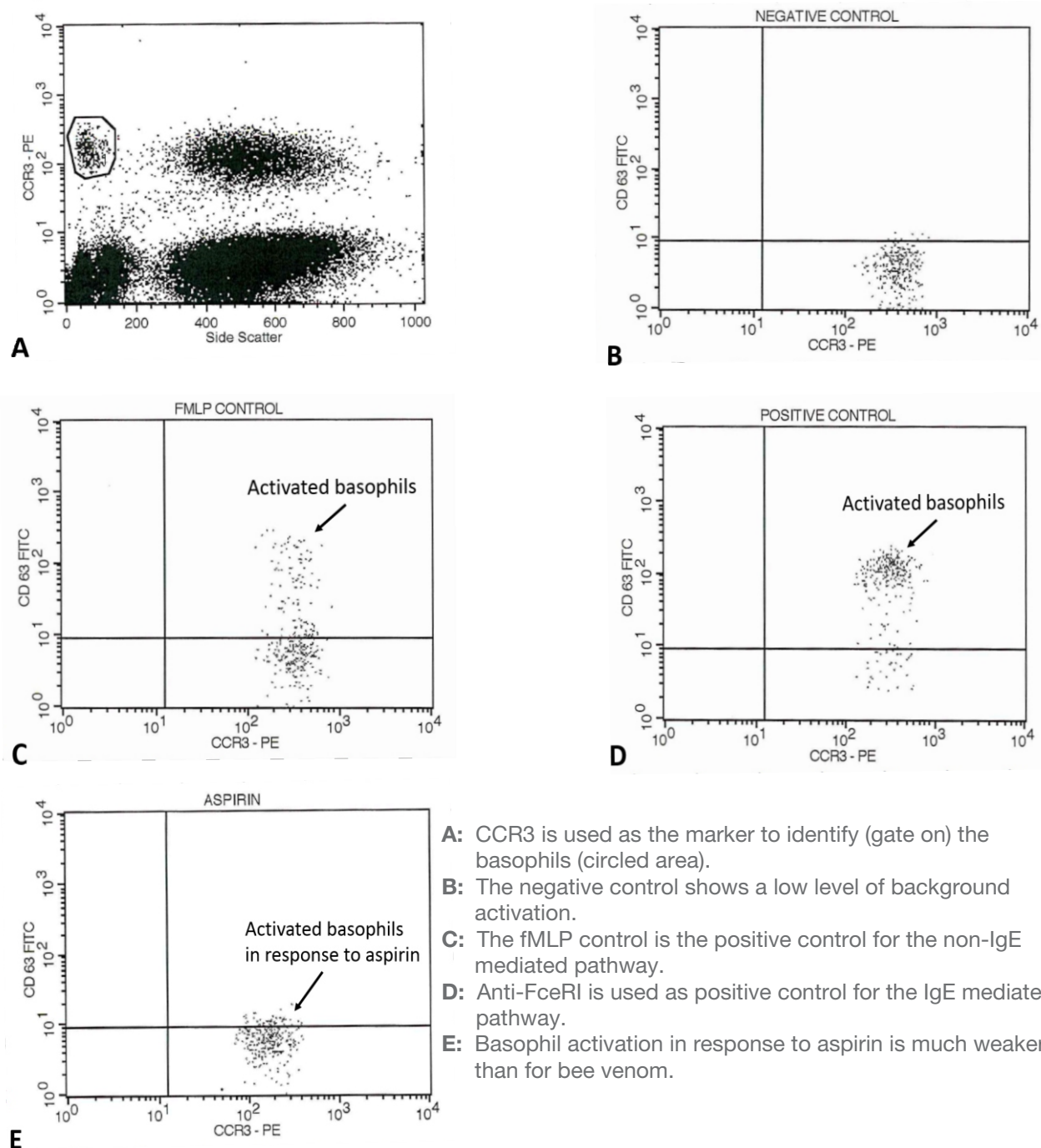
Figure 2. Example of a Positive Flow-CAST test result to Bee Venom



- A:** CCR3 is used as the marker to identify (gate on) the basophils (circled area).
- B:** The negative control shows a low level of background activation.
- C:** The fMLP control is the positive control for the non-IgE mediated pathway.
- D:** Anti-FcεRI is used as positive control for the IgE mediated pathway.
- E:** Basophil activation in response to the specific allergen (bee venom).

Figure 3. Example of a Positive Flow-CAST test result to Aspirin

Aspirin is a much “weaker” allergen than bee venom, and different reference ranges apply.



References

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