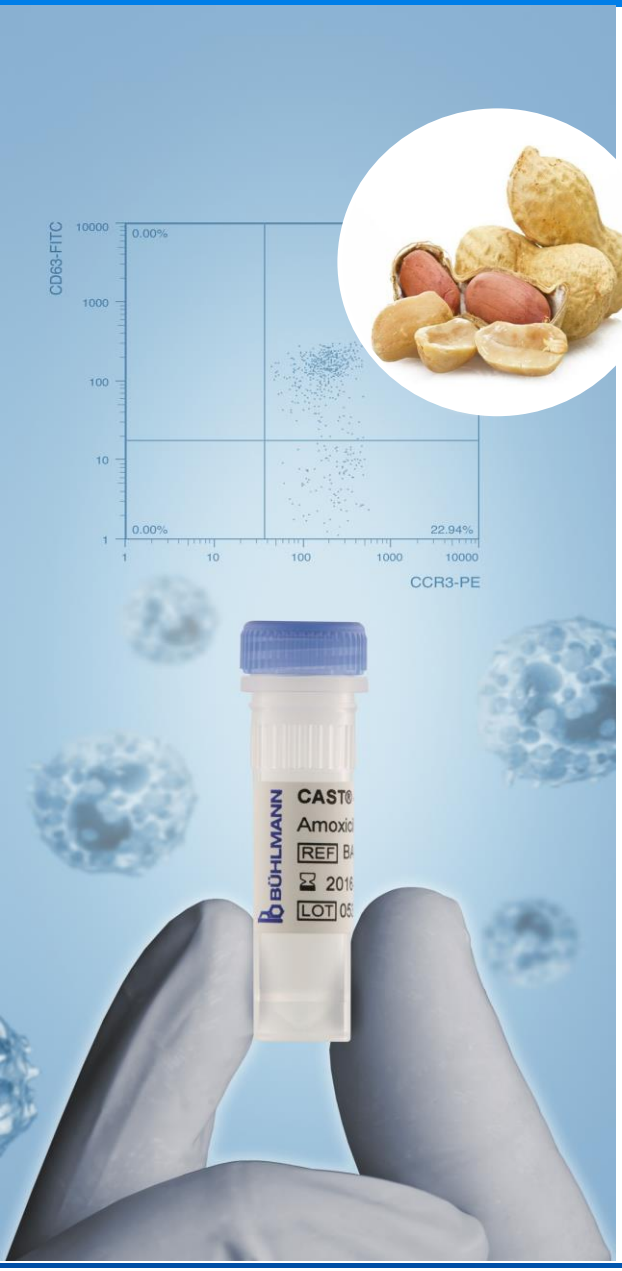


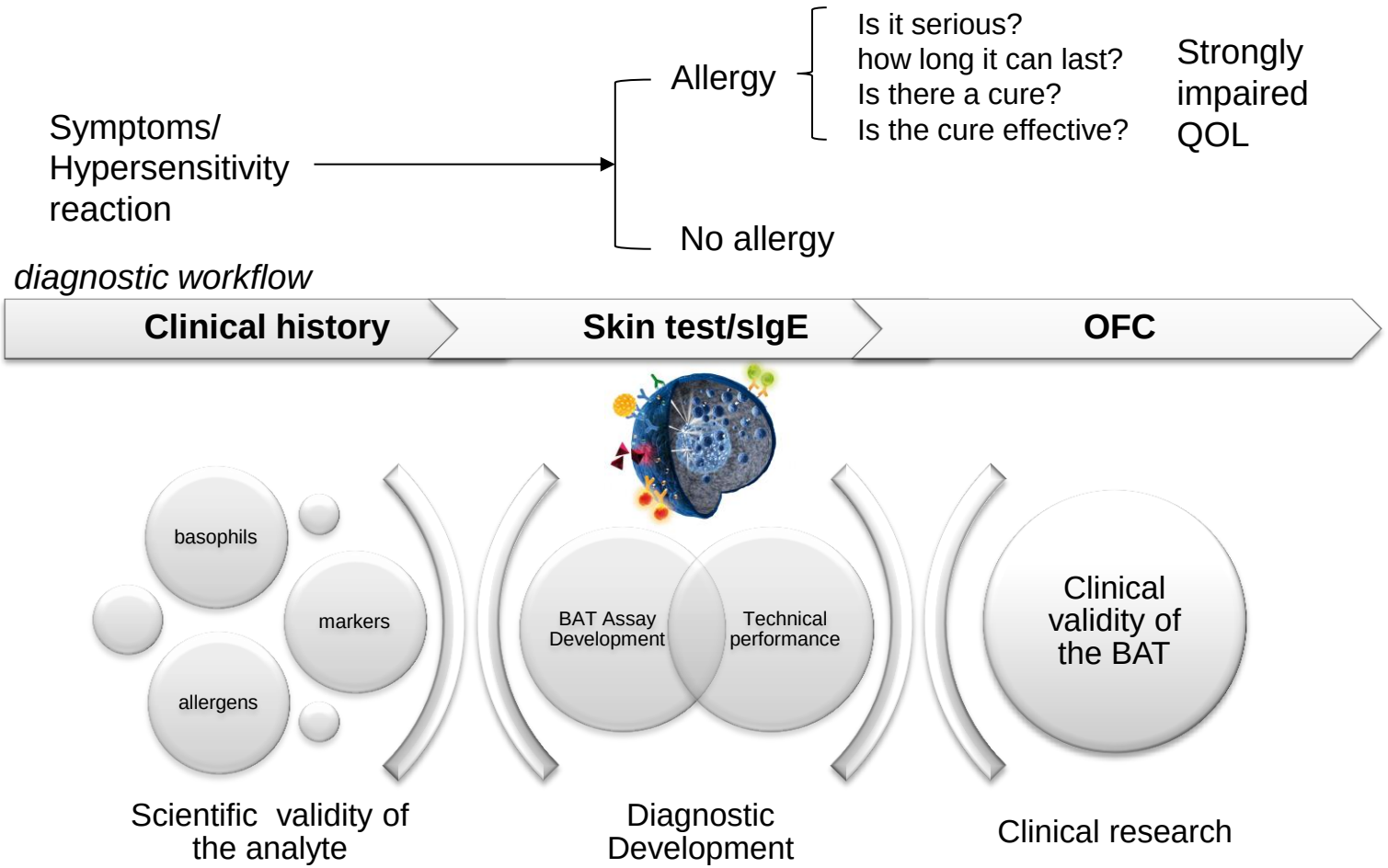
Blood Basophil Stability in BAT Test

Michele Romano, PhD
Product Manager

EuroBAT Meeting 2018

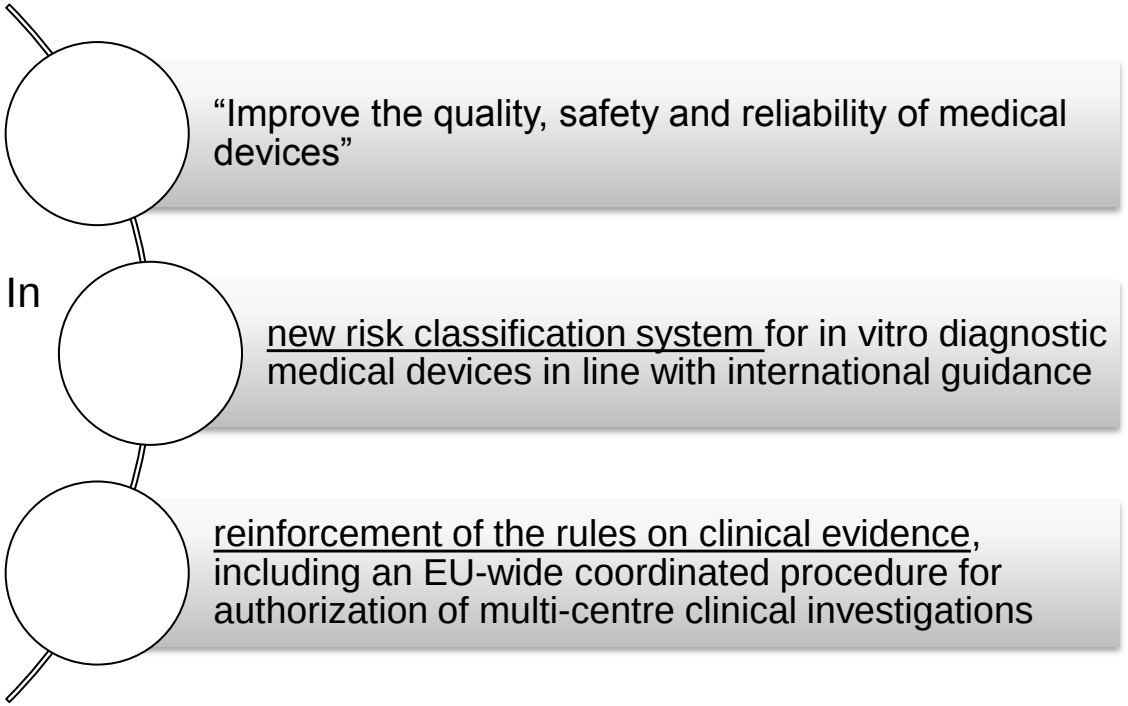
18/10/2018

Synergy is the Key for Spreading BAT in Clinical Practice



Basophil Activation Test: a Diagnostic Perspective

IVDR
2022 is the horizon of new In Vitro Diagnostic Medical Devices Regulations



Sources:

- https://ec.europa.eu/growth/sectors/medical-devices/regulatory-framework_en
- http://ec.europa.eu/growth/content/new-eu-rules-medical-devices-enhance-patient-safety-and-modernise-public-health-0_en

Basophil Activation Test: a Diagnostic Perspective

Flow CAST® is standardization of BAT assay

- Technical performance
- Pre-analytics
- Clinical performance

Blood Stability:

- Kind of blood
- Time from blood taking
- Temperature of storage
- Is blood logistic feasible?

some confusion and misleading info

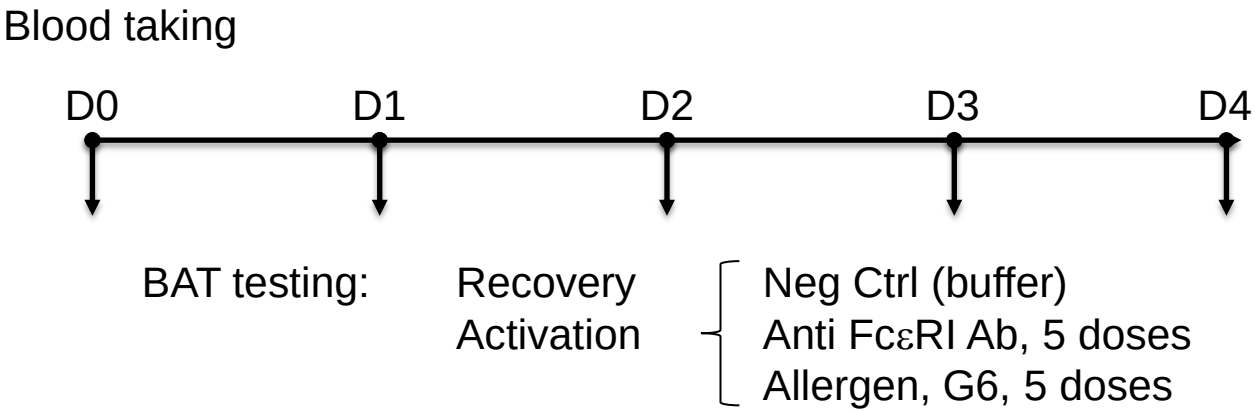
Basophil Stability in Whole Blood

- EDTA
- Heparin
- ACD

- Stability over 4 days of storage

- Different temperature of storage 2-8°C, 20°C, 28°C

- BAT was analyzed by Flow CAST® reagents



Flow CAST® Procedure and Reagents

1. Stimulation & Staining

- Stimulation Buffer
  - with Heparin, IL-3 and Ca^{2+}
  - with Heparin and Ca^{2+}
- Stimulation Control
  - anti-FcεRI
  - fMLP
- Staining Reagent
  - anti-CCR3-PE
  - anti-CD63-FITC
  - Anti-CD203c-PE-DY647
  - Anti-CD63/CD203c-PE-DY647

2. Lysis

- Lysing Reagent

3. Analysis

- Wash Buffer

Flow CAST® Protocol

1. Stimulation & Staining

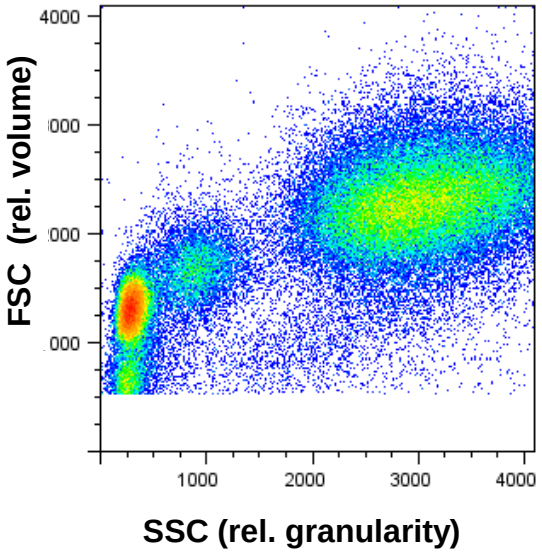
- Pipetting scheme:
  - 50 µl Stimulus (neg Control, anti FcεRI, Allergen)
  - 100 µl Stimulation Buffer
  - **50 µl EDTA/Heparin/ACD Blood**
  - 20 µl Staining Reagent
- 15 minutes incubation at 37°C

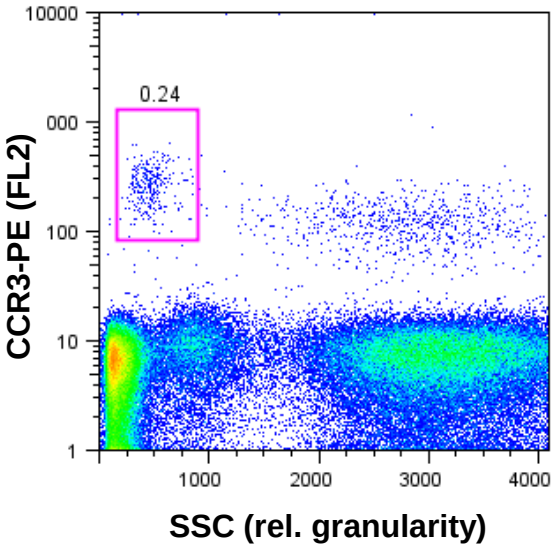
2. Lysis

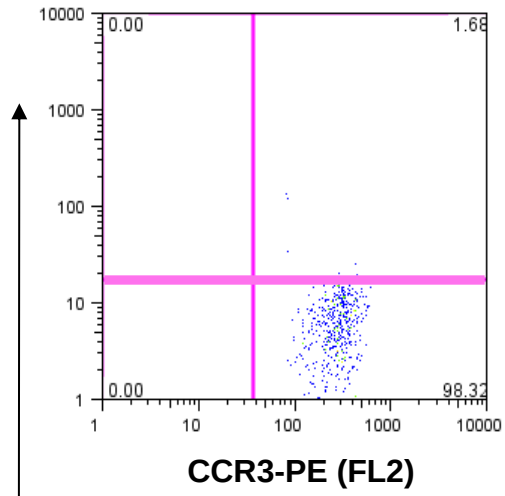
- 2 ml of Lysing Solution
- 5-10 minutes incubation at RT
- 5 minutes centrifugation at 500g
- Reconstitution of cell pellet with Wash Buffer (volume according to the instrument)

3. Flow Cytometry Analysis

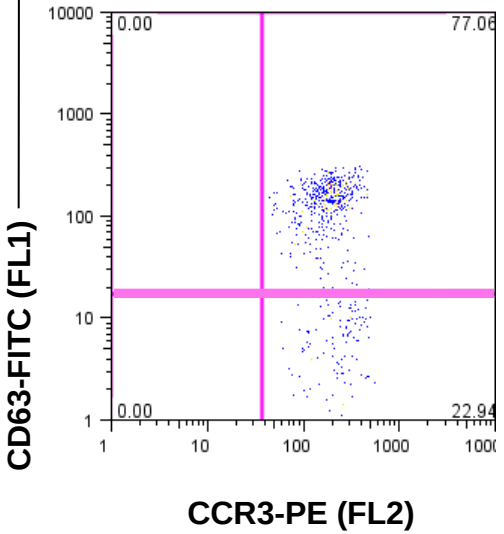
Flow CAST® Analysis





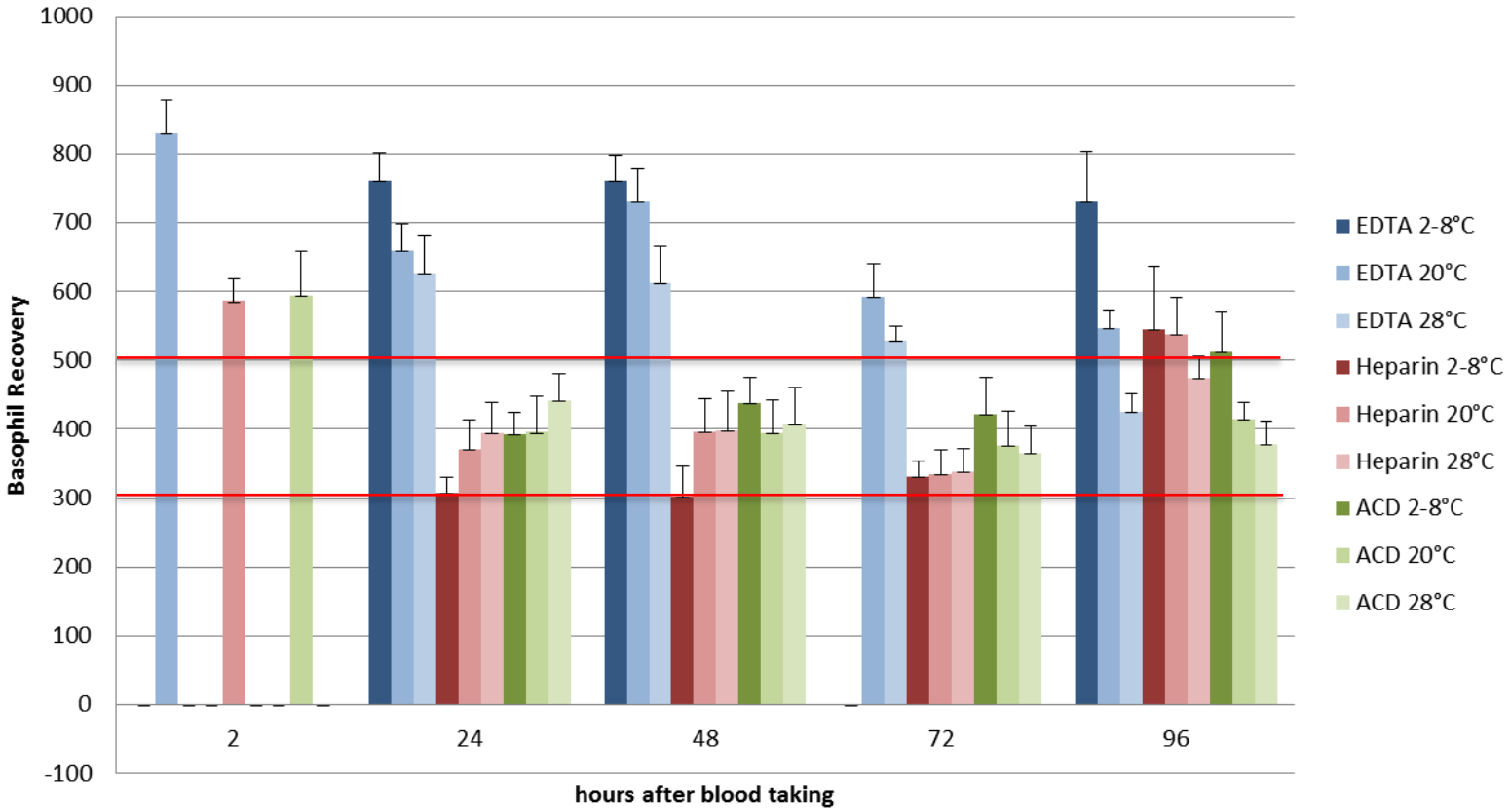


Patient Background (PB) ~ 2%

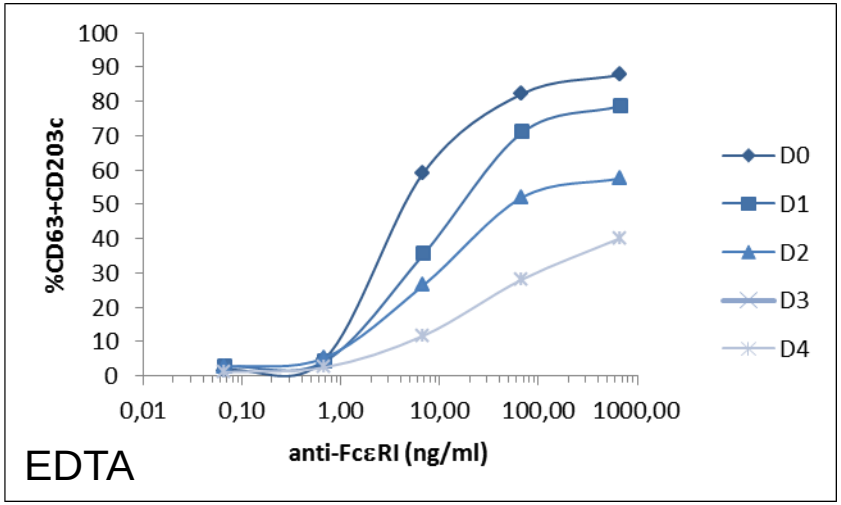


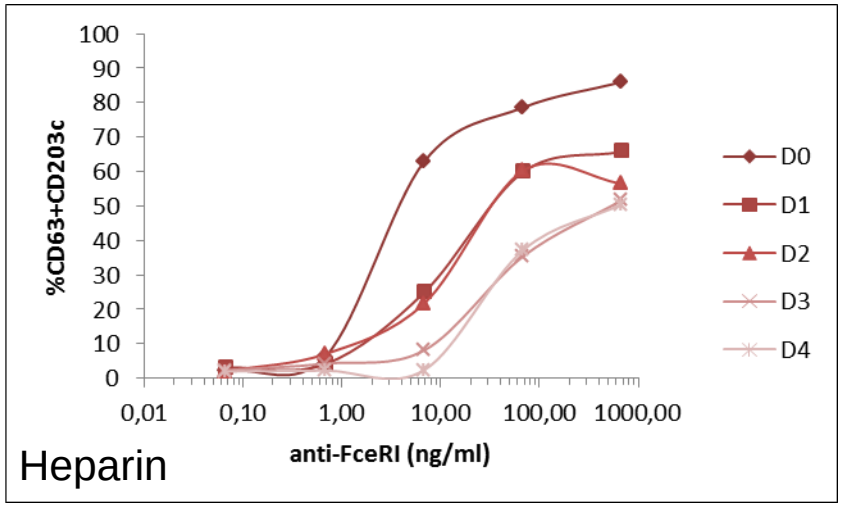
Patient Control (PC) (α-FcεRI)

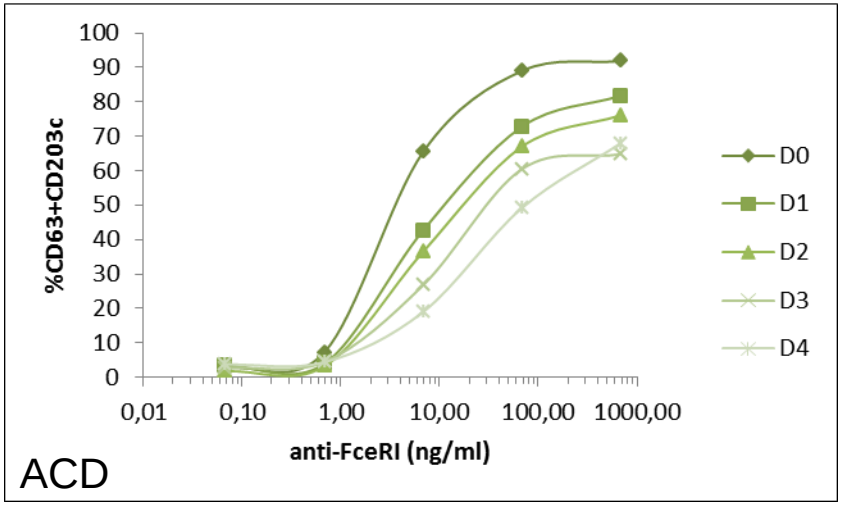
EDTA blood has the Best Recovery of Basophils



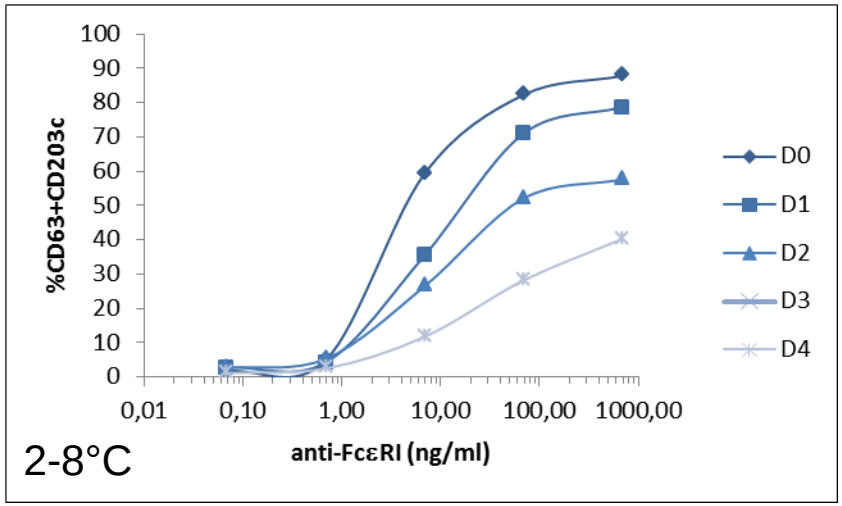
Basophil Activation is Retained Over Days

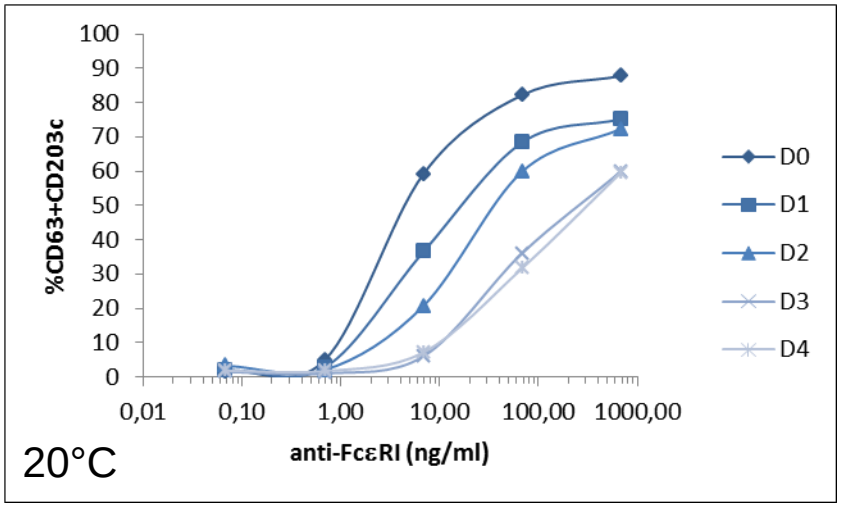


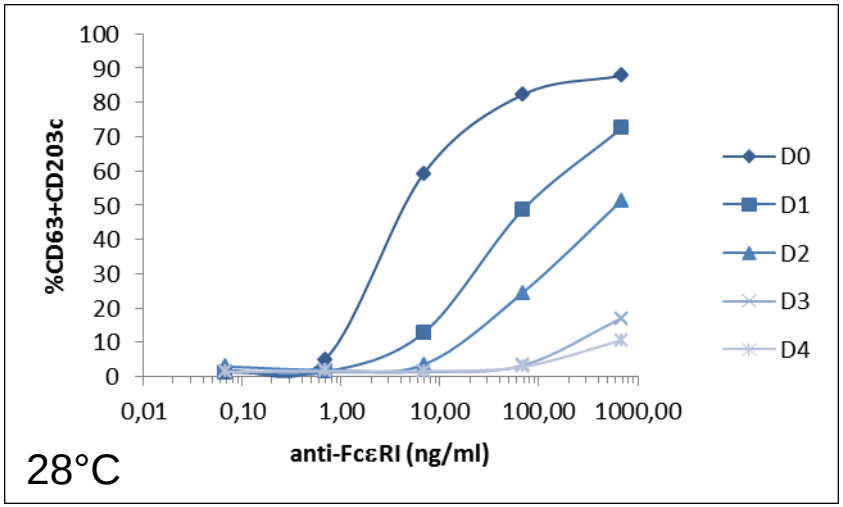




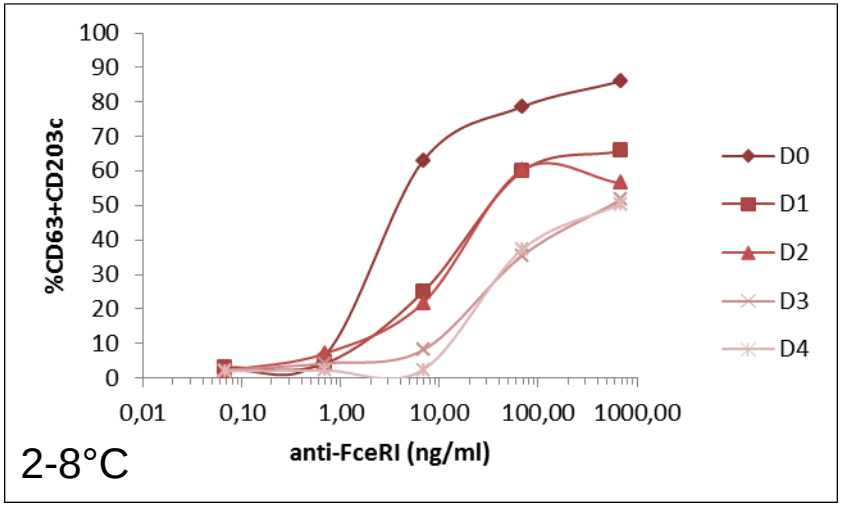
Basophil Stability is Influenced by Storage Temperature EDTA

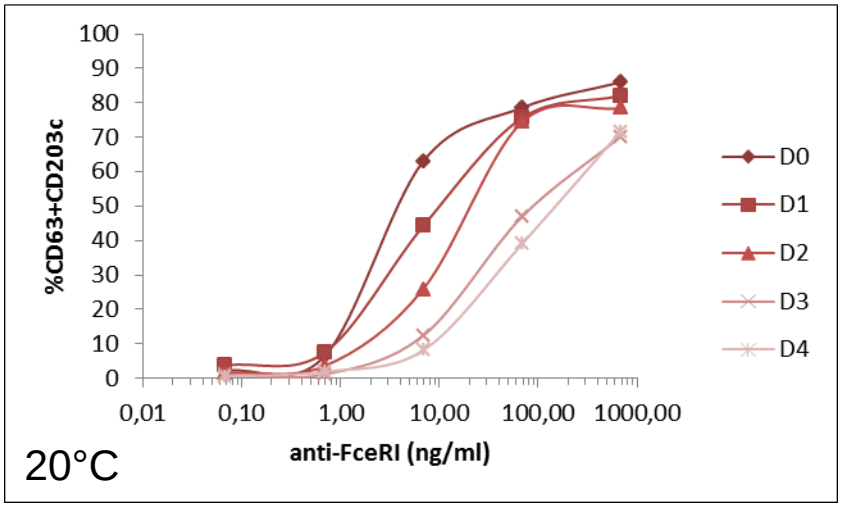


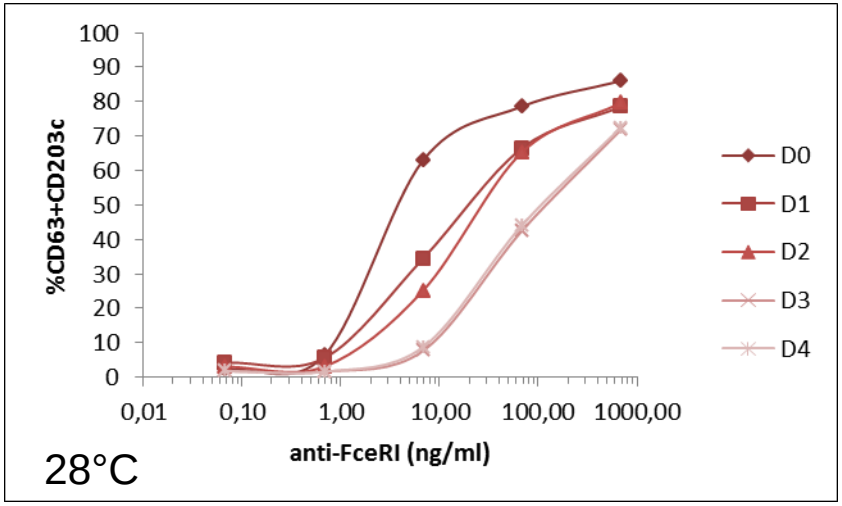




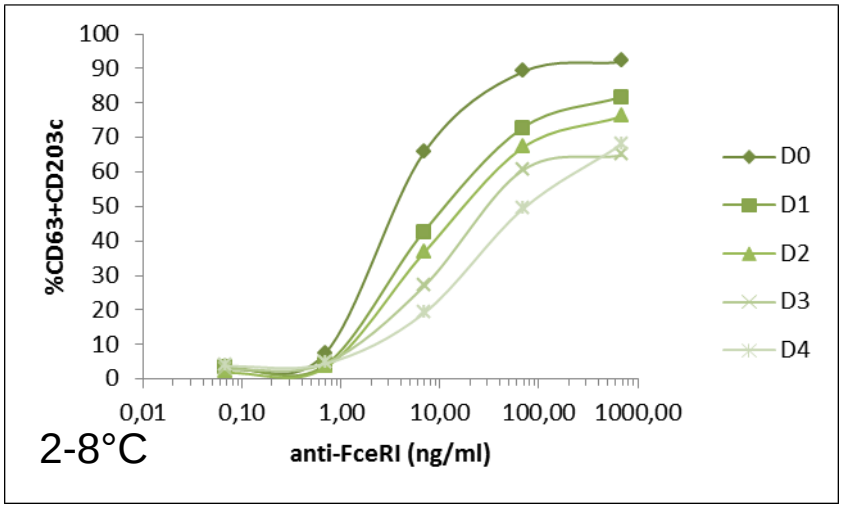
Basophil Stability is Influenced by Storage Temperature Heparin

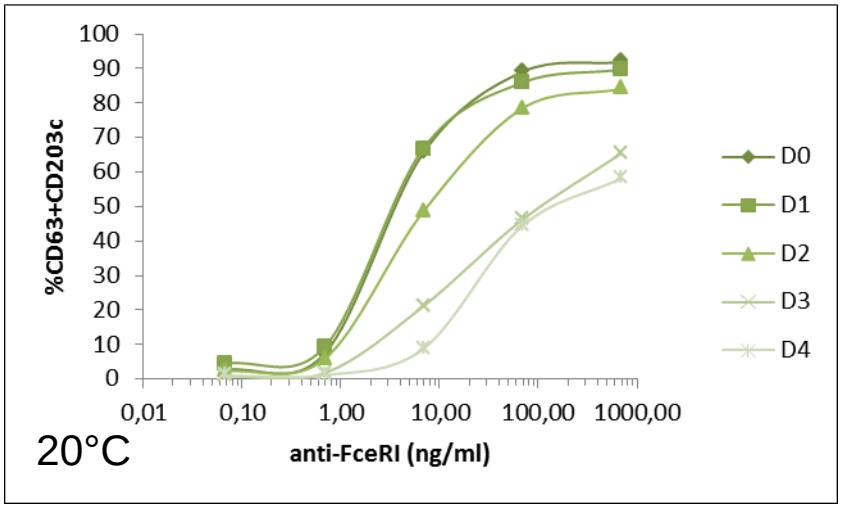


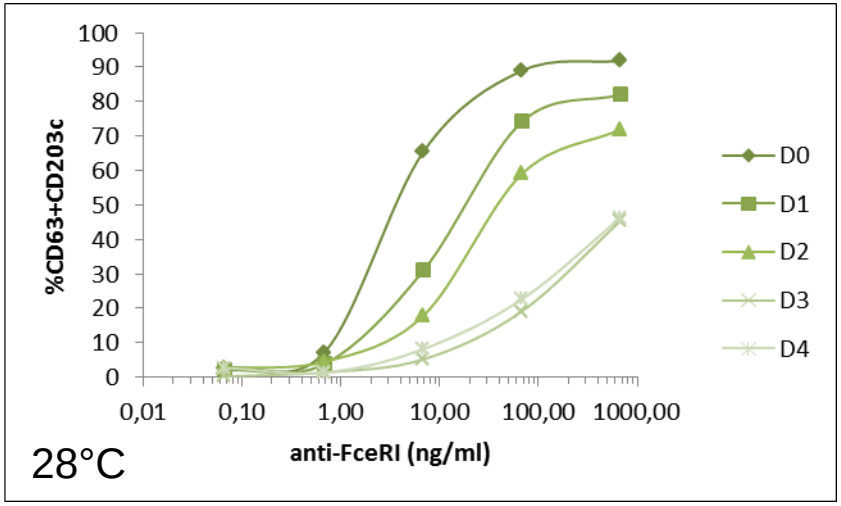




Basophil Stability is Influenced by Storage Temperature ACD





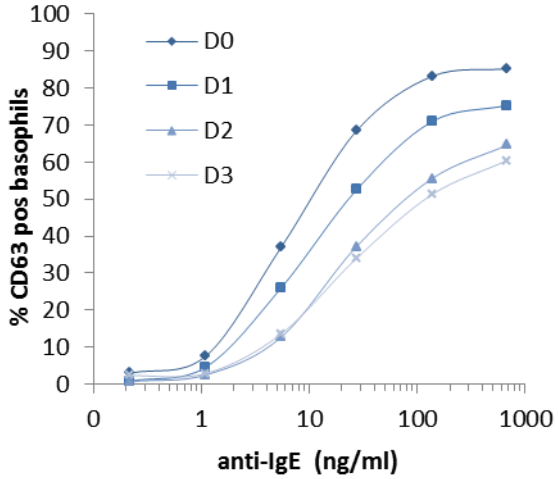


BAT Test is Feasible Over Days After Blood Taking

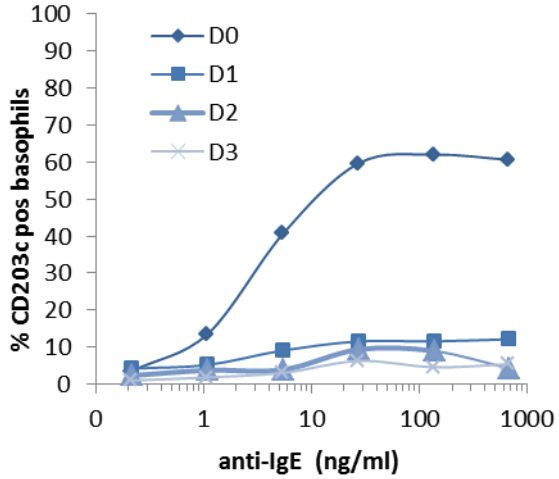
- Basophils are stable in terms of recovery and activation over days
- 24/48 hours of storage allow reliable BAT test results
- Different kinds of blood result in differences in basophil recovery and basophil activation
- EDTA shows the best recovery with the Flow CAST® test system
- The temperature of storage is another sources of can influence variability in basophil activation

Blood Stability – Anti IgE

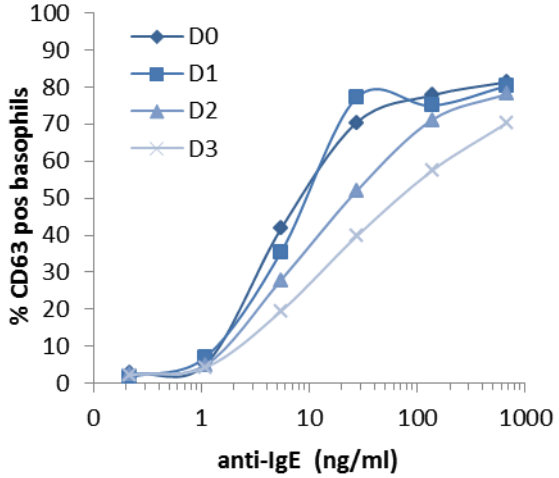
CD63

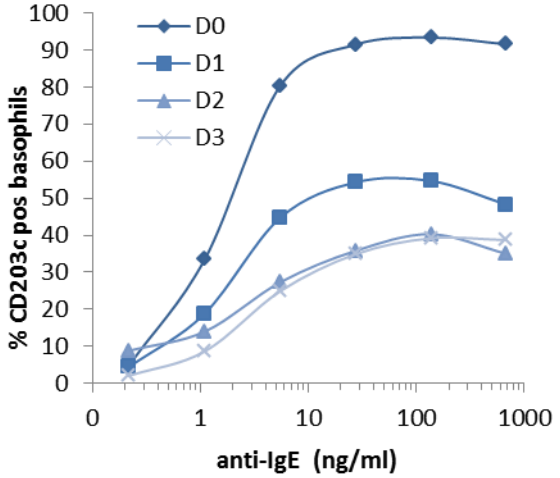


CD203c



With IL3

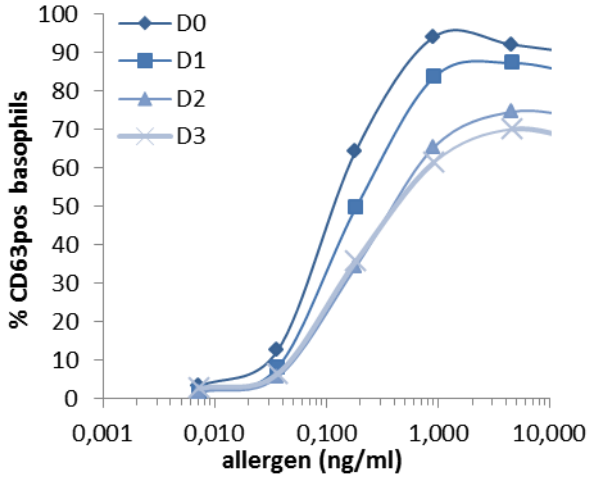




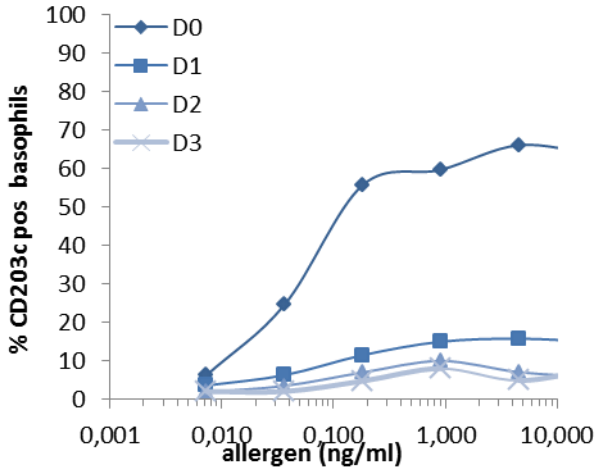
W/o IL3

Blood Stability – Allergen

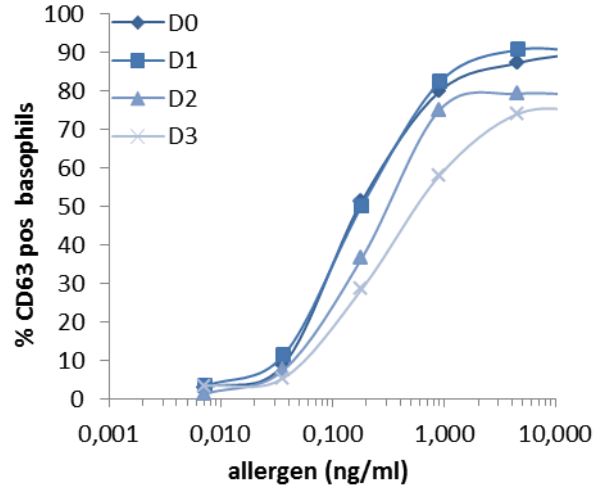
CD63

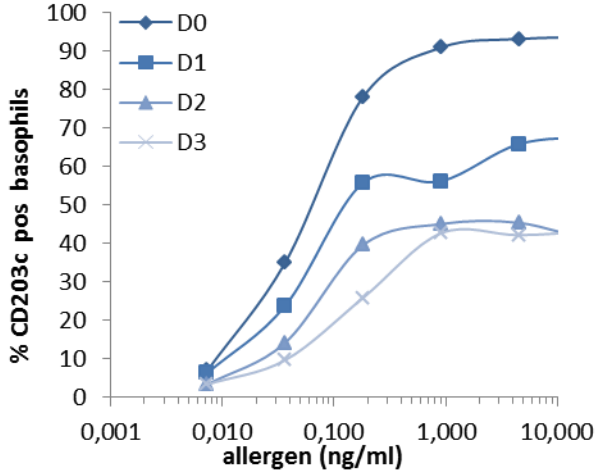


CD203c



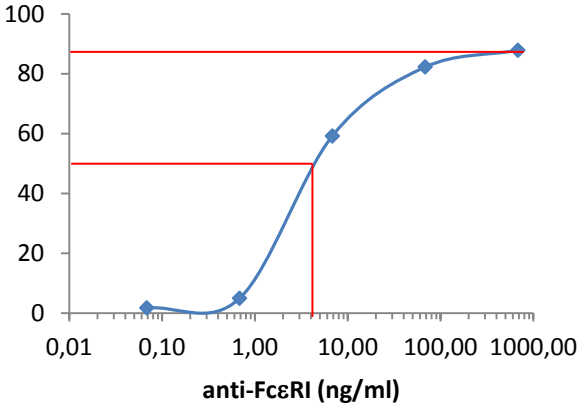
With IL3

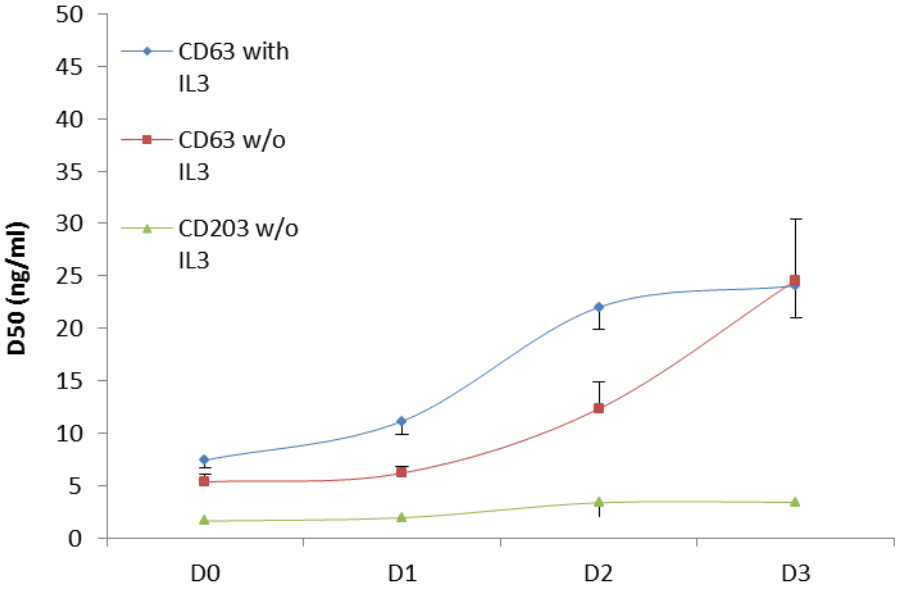


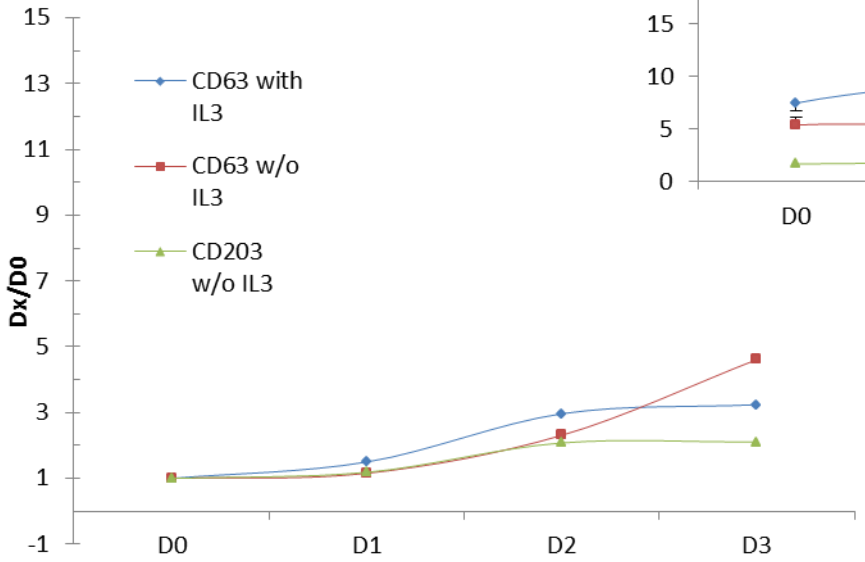


W/o IL3

Variability in Basophil Sensitivity







Flow CAST® BAT Testing Allow Blood Logistic

- Statistically significant recovery of basophil at least at 24/48hrs
- CD63 is the preferred activation marker for blood storage, CD203c lose activation window in 24 hours
- EDTA shows the best recovery with the Flow CAST® test system
- 2-8°C is the appropriate temperature of storage for blood
- 24/48 hours of blood storage is feasible for allergen specific basophil reactivity measurement
- 24/48 hours of blood storage has an impact on variability of allergen specific basophil sensitivity. This variability, together with other source of variability must be taken in account in follow up study based on basophil sensitivity.

CONTACT

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