Indirect Basophil Activation Test (iBAT): A User-Friendly Protocol for Plasma and Allergen Testing

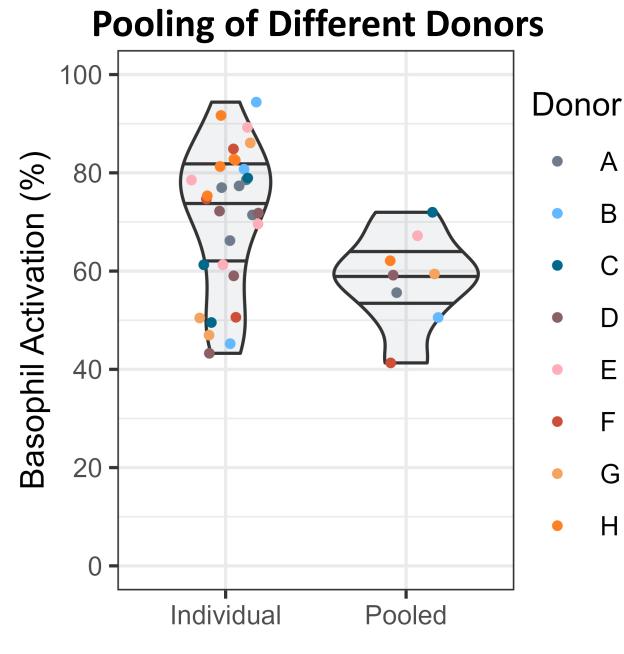
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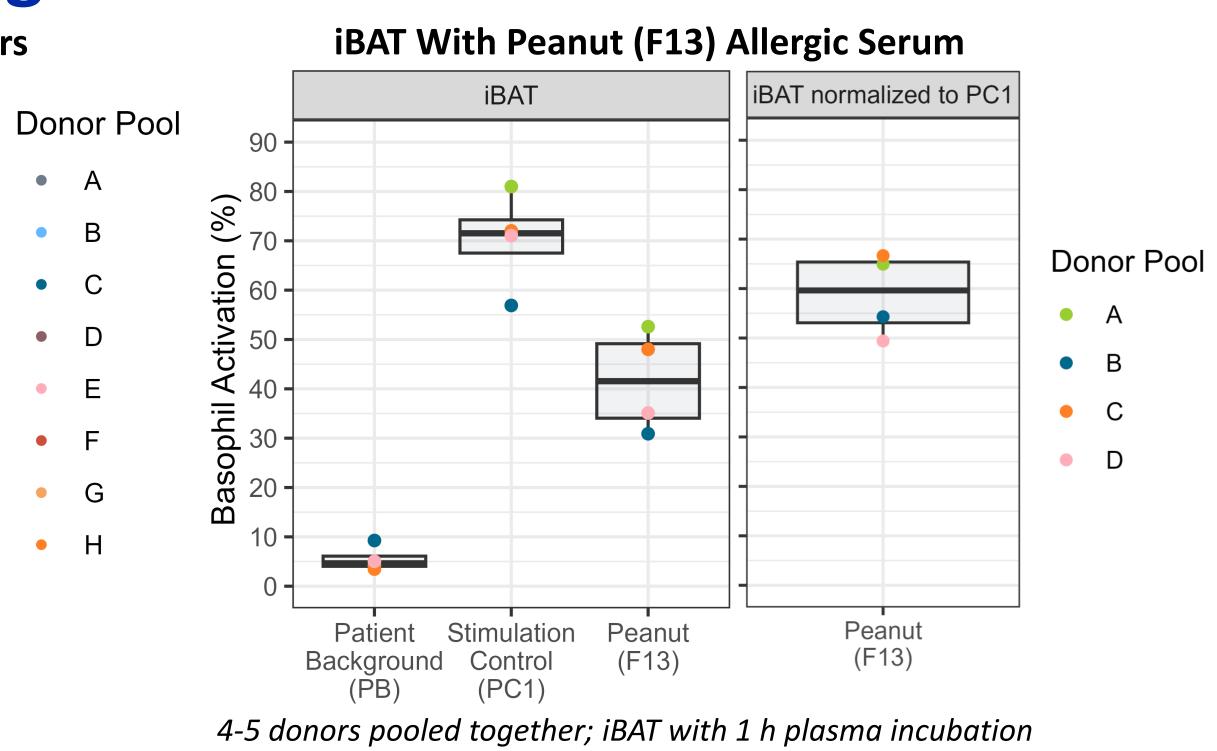
Introduction

The indirect basophil activation test (iBAT) is an in vitro assay that uses basophils from non-allergic blood donors that are sensitized through the incubation with serum or plasma from allergic individuals to their culprit allergen. The subsequent allergic reaction is induced by the specific allergen *in vitro* and measured by flow cytometry.

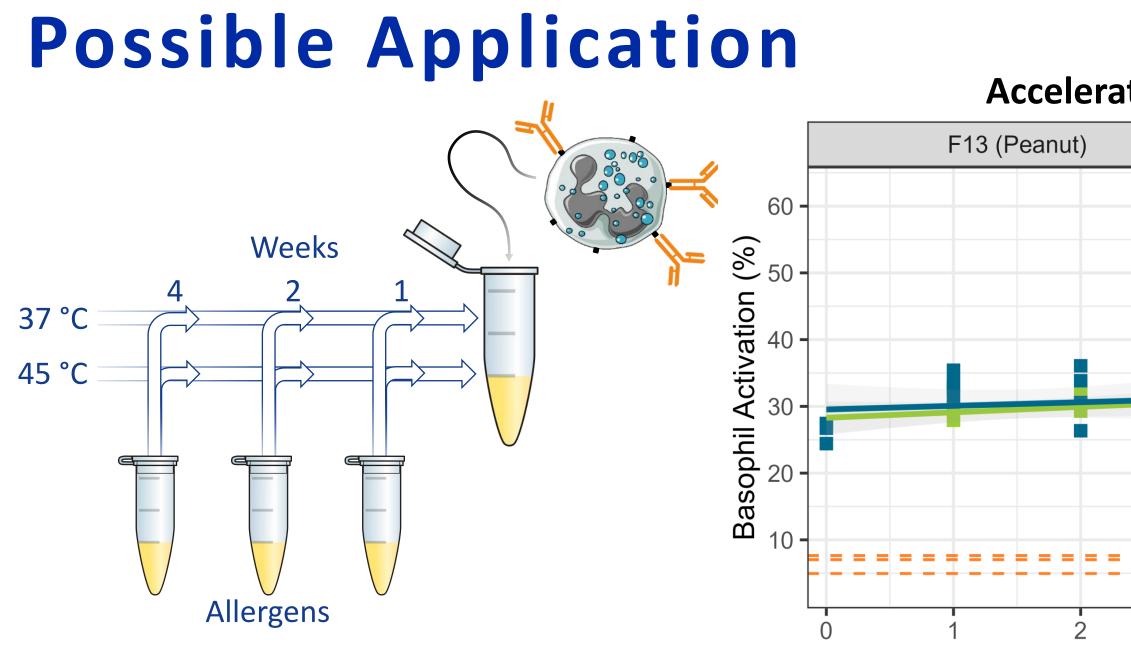
Advantage: unlike BAT, it does not require fresh blood from allergic patients.

Donor Pooling



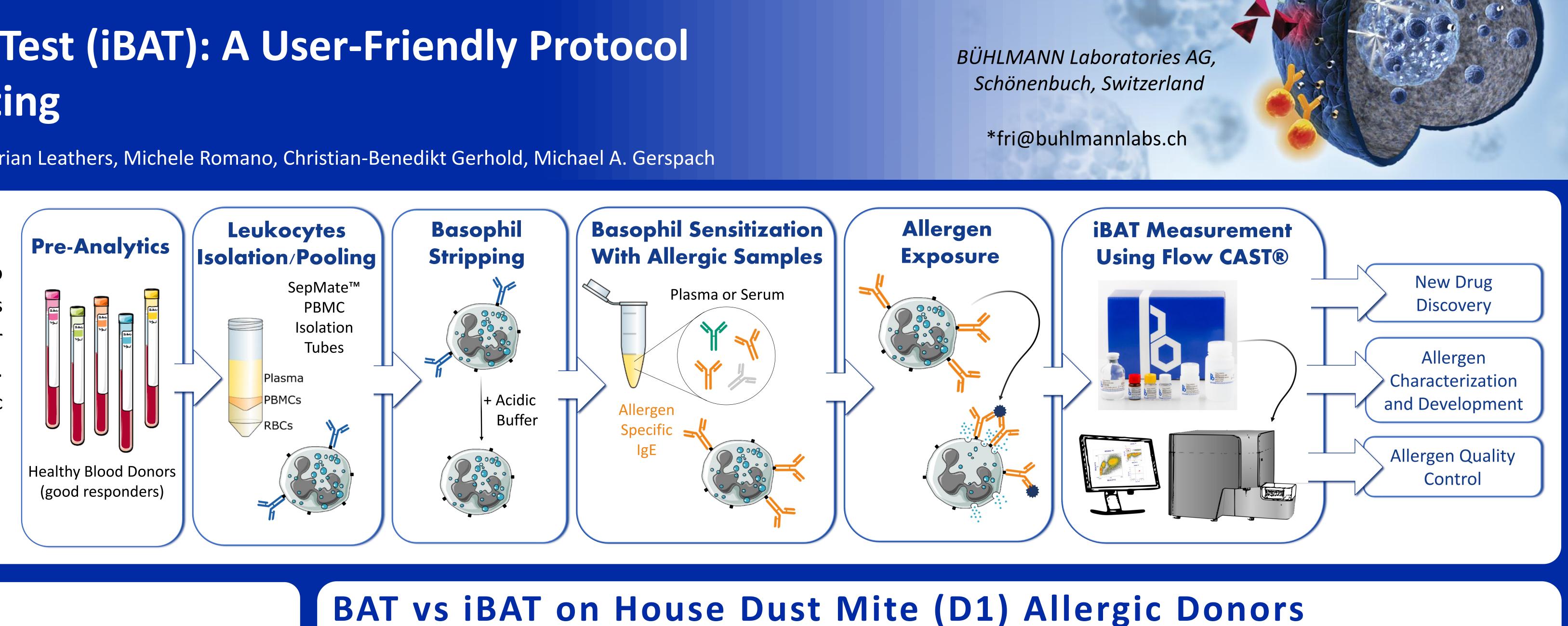


Pooling PBMCs (Peripheral Blood Mononuclear Cells) from different donors helps achieve a more predictable and reproducible indirect basophil activation.

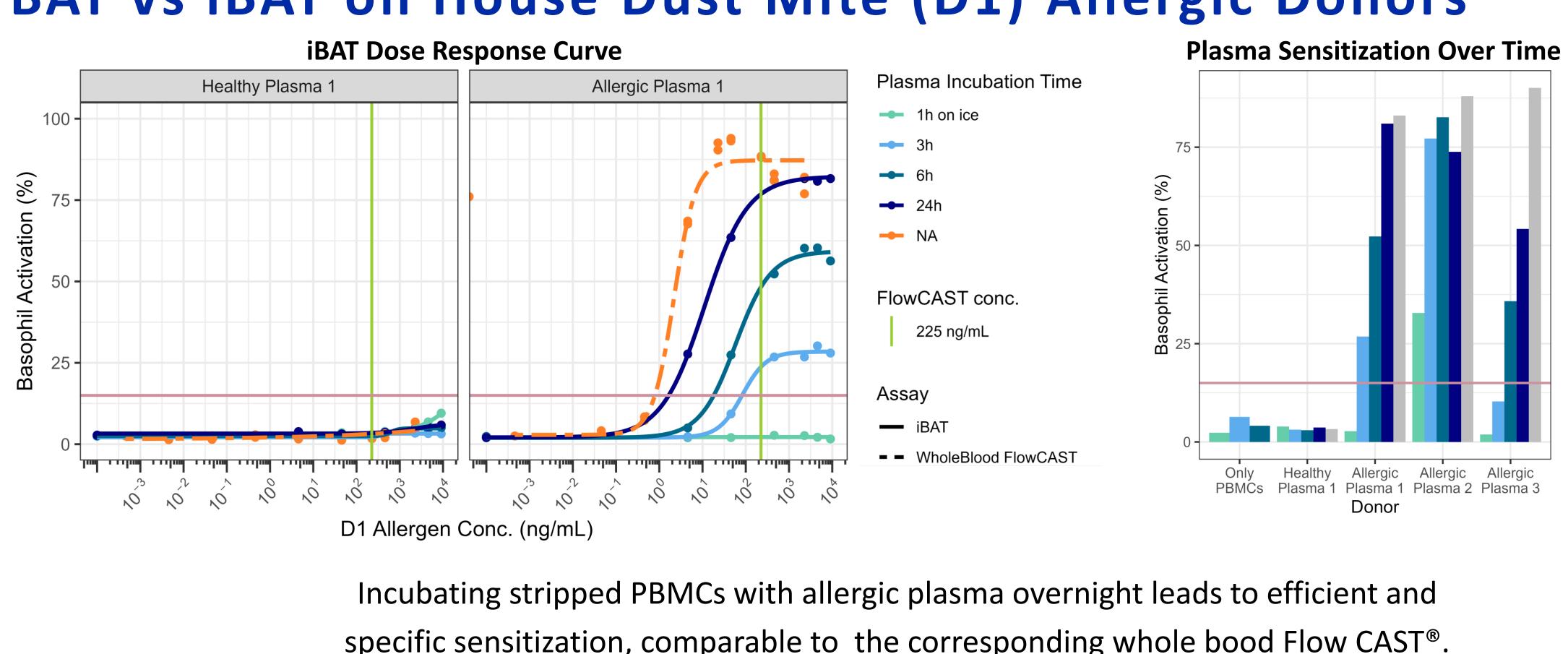


iBAT is successfully used for accelerated stability studies for several allergens.





Accelerated Stability Studies I1 (Honey Bee Venom) Allergen Tested ■ F13 • 11 Storage Temperature (°C) - 37 **--** 45 – – PB Storage Weeks



Advantages: *Key points:*

Allergens tested: Applications:

EAACI 2025, Glasgow | Poster prefix D2.12



specific sensitization, comparable to the corresponding whole bood Flow CAST[®].

Summary

A reliable and user-friendly iBAT protocol was developed using **BÜHLMANN Flow CAST®**.

- iBAT is accessible and easy to implement in any laboratory. Pooling donors decreases the heterogeneity, thereby allowing for roboust and reproducible results. iBAT can be performed in 1-2 days, depending on the purpose and characteristics of the plasma. Isolated pooled PBMCs can be stored for up to 72 hours at 8 °C in BÜHLMANN stimulation buffer, offering increased experimental flexibility. Peanut (BAG-F13), Hazelnut (BAG-F17), House Dust Mite (BAG-D1), Cat Epithelium (BAG-E1), Wasp Venom (BAG2-I3), Honey Bee (BAG-I1).
- iBAT can be useful in allergen and plasma characterization, quality control and drug development. Furthermore, preliminary data suggest its potential in allergy diagnosis.

Cut-off **—** 15% Assay/Plasma Incubation Time iBAT - 1h on ice iBAT - 3h iBAT - 6h iBAT- 24h

D1 Conc. = 450 ng/mL

Whole Blood BAT

We are happy to share our protocol

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