



RAPID EVALUATION OF COMPOUND ALLERGENICITY BY MEASURING HISTAMINE RELEASE ON STIMULATED WHOLE BLOOD

APPLICATION NOTE

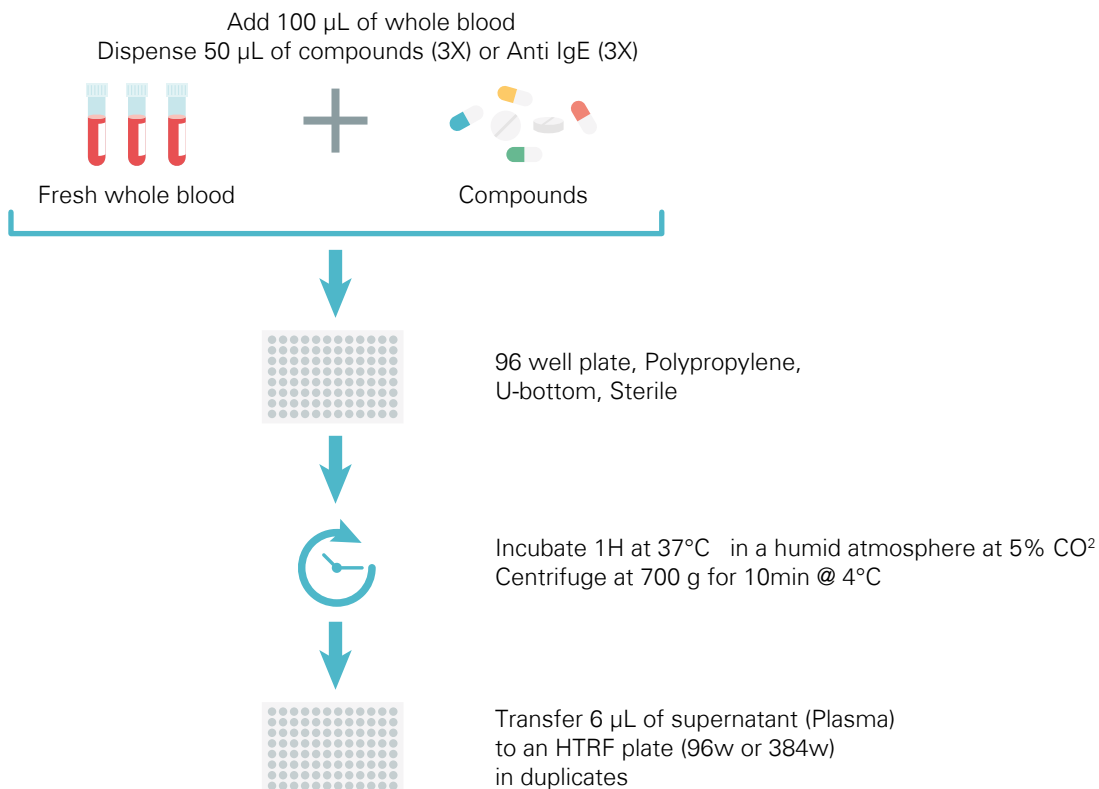
ABSTRACT The measurement of histamine release in blood from different donors in response to compound stimulation represents a clinically proven in vitro test for the assessment of any toxic side effects. The use of whole blood eliminates cumbersome purification of Peripheral Blood Mononuclear Cells (PBMC) and therefore significantly simplifies the assay procedure.

An allergic reaction is a heightened immune system response that occurs upon exposure to certain substances called allergens. The allergen binds to specific IgE antibodies at the surface of mast cells and triggers the release of histamine, which can lead to serious respiratory illness or more severe reactions such as anaphylaxis. Therefore it has become crucial to evaluate the potential adverse effects of drug compounds caused by histamine release (IgE-dependent or IgE-independent stimuli) during the early stages of their development.

This study demonstrates the capability of the HTRF Histamine-Dynamic kit to measure histamine release in whole blood samples treated with different allergen mixes. Investigating histamine release in fresh blood enables a rapid assessment of immunogenicity reactions and thus aids in efficiently predicting the safety of drug candidates.

MATERIALS & METHODS

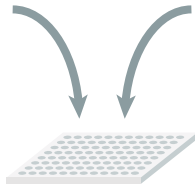
FLOWCHART



HISTAMINE ADD & READ ASSAY

6 μL of plasma

- 4 μL of acylation buffer
- 2 μL of acylation reagent



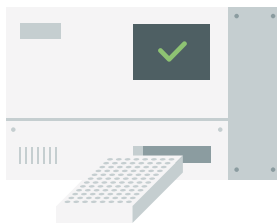
Acylation step:
15 min @ RT



4 μL of each conjugates



Read on an HTRF compatible reader



Fresh blood from different donors was collected in heparin-tubes and dispensed into 96 well plates. The whole blood samples were stimulated either with 50 μL of Anti-IgE at 15 $\mu\text{g}/\text{mL}$ (3X) or 50 μL of allergen mixes at the concentration recommended by the supplier for 1H at 37°C in a humid atmosphere at 5% CO₂. The plate was then centrifuged at 700g for 10 min at 4°C and 6 μL of supernatant (i.e. plasma) were transferred to the assay plate for histamine detection as per the kit protocol.

BIOLOGICAL MODELS & REAGENTS

Whole blood samples were obtained from the French National Blood Service.

HTRF Histamine-Dynamic kit (#62HTMDPET)

HTRF 96 well low volume plate (#66PL96001)

Positive control: Monoclonal antibody anti-IgE (#MABETS07)

Plasma sample diluent (#62DLPDDD) for standard curve preparation

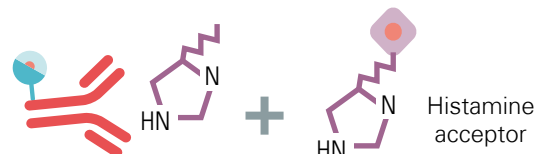
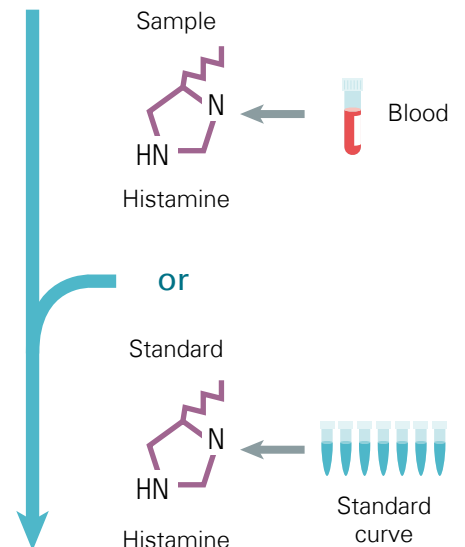
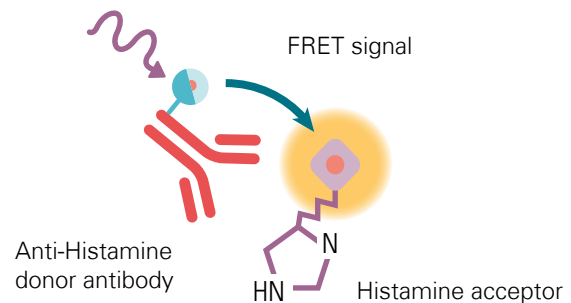
Allergens:

- 6 Grass mix allergen (Bühlmann # BAG-GX1)
- House dust mite allergen (Bühlmann # BAG-D1)
- Cat epithelium (Bühlmann #BAG-E1)
- Inhalant mix allergen (Bühlmann #BAG-IX1)
- Common Ragweed allergen (Bühlmann #BAG-W1)

HTRF HISTAMINE-DYNAMIC KIT

The HTRF Histamine-Dynamic assay is a no-wash immunoassay where native histamine produced by cells is detected in a competitive assay. The format includes a specific antibody labeled with Europium Cryptate (donor) and histamine labeled with d2 (red acceptor).

- Histamine from the plasma competes with the binding between the two conjugates and thereby prevents FRET. The protocol involves the acylation of the histamine from standards and plasmas in order to maximize the assay sensitivity. The standards must be prepared in diluent or in the same medium as the samples.



DATA HANDLING

- The HTRF Ratio was calculated for each well of standard or sample independently, using the following formula:

$$\text{HTRF Ratio} = \frac{(\text{signal } 665 \text{ nm})}{(\text{signal } 620 \text{ nm})} \times 10^4$$

- Generation of the standard curve

The standard curve was plotted using the four-parameter logistic model, from ratio means and standard deviations.

RESULTS

INTER-DONOR VARIABILITY

Sensitization to foreign proteins from the environment affects up to 40% of the population worldwide, with different levels of magnitude. Therefore, the inter-donor variability regarding the allergic response measured in blood samples has to be taken into account when assessing a compounds' effect.

All the results presented in the following sections were obtained on whole blood samples from 4 healthy donors.

HISTAMINE RELEASE UPON STIMULATION WITH DIFFERENT MEDIATORS

Four independent blood samples (Donor A to D) were used to measure the spontaneous release of histamine, as well as upon stimulation with 5 allergen mixes including 6 grass mix, house dust mite, cat epithelium, inhalant mix and common ragweed allergen.

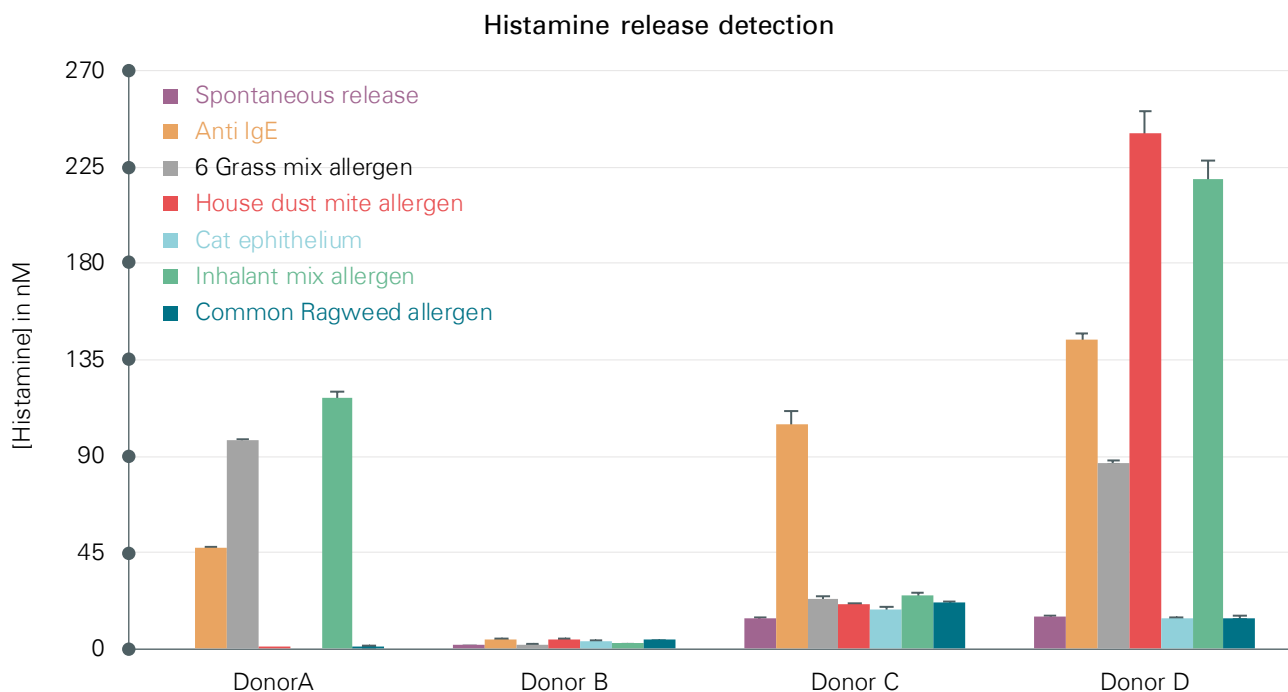
A positive control was generated by the anti-IgE treatment that triggers the human blood basophil response via FcεRI cross-linking, leading to histamine release.

The figure below displays the response of the 4 randomly chosen donors.

The data obtained show different response profiles:

- Donor A positively reacted to the anti IgE control and efficiently responded to the inhalant mix. The patient can be categorized as a medium allergic responder.
- Donor B did not respond to the different stimuli including the positive control and is considered as a "nonreleaser" profile. This donor type is not suitable for drug safety studies.
- Donor C positively answered the anti IgE control but did not display any sensitivity to the different allergen mixes tested. This donor can be classified as a "releaser" donor with a low allergic sensitivity for the conditions tested.
- Donor D was the strongest responder, showing a high histamine release triggered by the anti IgE positive control, the house dust mite and the inhalant mix allergens, and a medium sensitivity to the 6 grass mix.

Allergic reactions depend on many factors that are variable between individuals, including the presence of specific IgE, the concentration of blocking antibodies, and the ability of basophils or mast cells to release mediators. Therefore, it is recommended that these studies be performed on 5 to 10 independent donors to obtain reliable conclusions when testing compounds.



CONCLUSION

The allergic statistics from the American Academy of Allergy Asthma & Immunology (AAAAI) demonstrate that 10% of the world's population and 20% of all hospitalized patients are sensitive to drug allergens. Moreover, these adverse drug effects can be responsible for up to 20% of fatalities due to anaphylaxis. Monitoring the allergenicity profile of drug compounds is therefore crucial to assessing toxic side effects.

Compound-mediated histamine release from human blood samples represents a simple and accurate approach to study in vitro allergic reactions. The new HTRF Histamine-Dynamic kit displays unique properties such as a no-wash protocol, extended detection range, low sample consumption and high robustness, enabling the generation of relevant physiological data on fresh blood.

The HTRF histamine Dynamic assay thus provides a very simple and straightforward way to mimic the allergic reaction occurring in vivo and to reliably predict compound toxicity in early drug discovery.

FOR MORE INFORMATION

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