LATERAL FLOW KIT DESIGN FOR THE DETECTION OF HUMAN ANTI-PAN-ALLERGEN E IMMUNOGLOBULINS IN SERUM: Towards More Accesible Allergy Diagnosis

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Abstract | Molecular mechanisms determining the allergy process and its public health repercussion have been a matter of study since the allergy term designation. The so-called allergens, which provoke this condition, can trigger a cross-reaction (CR) phenomenon whereby people respond against them even if they have not been previously sensitized. Within these, pan-allergens are considered some of the responsible agents of most CR processes in Europe. When it comes to diagnosing these reactions, the Lateral Flow Immunoassay (LFIA) stands out among all the other methods. In addition to the great precision and quickness of its results, this test is currently a promising bet to enable the allergy diagnosis to the population, avoiding the requirement of health service intervention. In this project a novel and attractive LFIA kit to detect pan-allergens and CR is designed, by analyzing and enhancing if possible each of its different components.

1 Allergic disease

• **Allergy:** This can be defined as an adverse immune-mediated overreaction to otherwise innocuous environmental substances, that is, allergens.

• **Epidemiology**: Nowadays, allergic diseases affect more than 25% of the world's population. Nevertheless, It is expected to reach 50% in two decades (especially due to the introduction of genetically modified organisms).

• **Mechanism**: This occur in three steps: sensitization (1-8), immediate reactions (9-10) and late and chronic reactions (11-14). These are shown in **figure 1**.

2 Cross-reactions and pan-allergens

• **Cross-reaction:** Allergic phenomenon whereby people respond against allergens even if they have not been previously sensitized to them.

• **Epidemiology**: In central and northern Europe 20% of people presents CR (specially to to plant-food allergens). However, in Spain and in the Mediterranean countries, this percentage increases to over 50%.

• **Pan-allergens**: They are allergens of non-directly related species that induce CR between them (e.g profilins, lipid transfer proteins or Bet v 1 homologues).

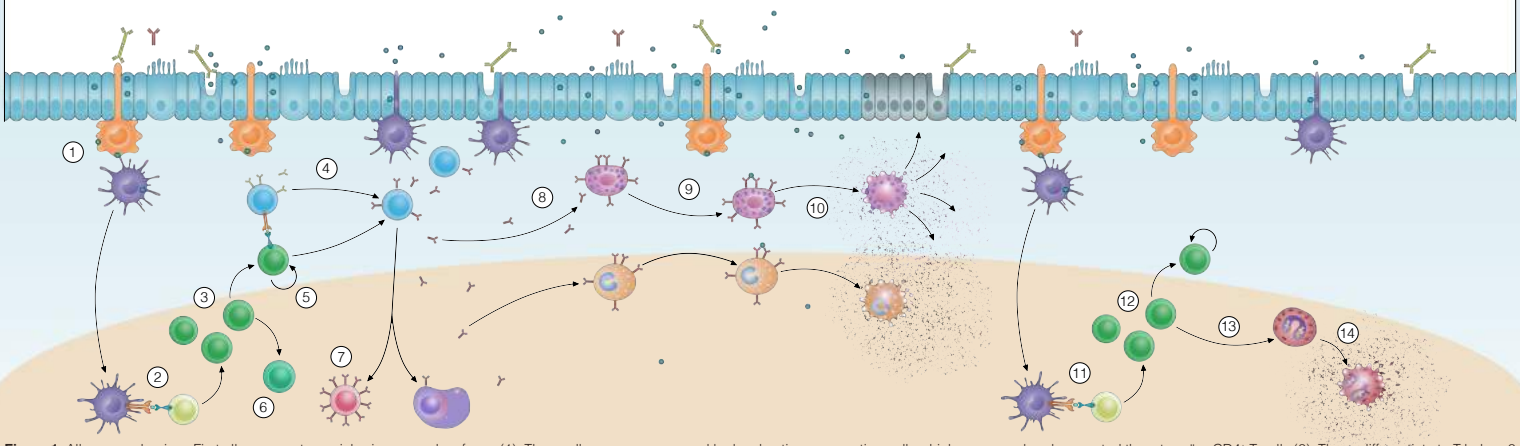

Figure 1. Allergy mechanism. First allergens enter mainly via mucosal surfaces (1). There, allergens are engaged by local antigen presenting cells which processed and presented them to naïve CD4⁺ T cells (2). These differentiate to T helper 2 (3), which secrete cytokines favouring B cells class switching to IgEs production (4) and clonal expansion of T cells (5). Memory T and IgE⁺ B cell pool are also generated (6)(7). Moreover, produced IgEs sensitize mast cells and basophils (8). If allergens re-enters the body, they will cross-link with the IgE–receptor complexes of sensitized mast cells and basophils (9), leading to their degranulation (10). If allergens persist in the body, they will be showed to T cells (11) resulting in their differentiation, proliferation and release of cytokines (12). These lead to tissue eosinophilia (13) and to the degranulation of these cells (14).

3 Lateral Flow ImmunoAssay Kit Design

• **Allergy diagnosis**: Can be conducted in vivo, such as Skin-prick tests, and in vitro, such as ELISA immunoassays. Nevertheless there is none as accurate and quick as Lateral Flow Immunoassays (LFIAs). These are based on the migration of a sample along a membrane by capillary forces. The analytes are captured by specific bio-recognition of the reagents that are immobilized on the surface of this membrane and marked by the addition of a second reagent allowing the visualization of the result.

• **Market analysis**: Nowadays, United States is the greatest power in the development of allergy diagnostic kits, but there is no market related to CRs due to the lack os many cases of these reactions there. Consequently, a great market opportunity appears in Europe and Mediterranean areas.

• **Product proposal**: Based on the background previously explained, the design of an LFIA Kit for the qualitatively and semi-quantitatively detection of Pan-allergens (specifically profilins, lipid transfer proteins and Bet v 1 homologues) and their respective CR is proposed. It is based on an improvement in the selection of reagents and materials, as well as in the strip case and operation of the kit, both explained in **figure 2** and **figure 3**.

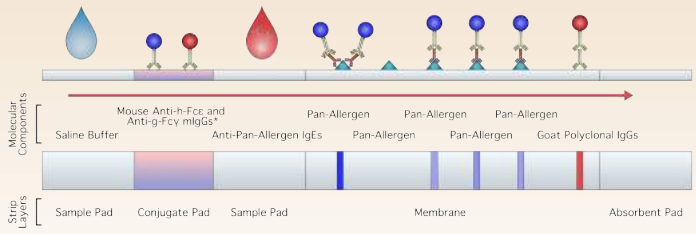

Figure 3. LFIA components and strip-molecule interactions overview. First, the Anti-pan-allergen IgEs contained in blood are released into the sample pad, which would bind the allergens fixed in the membrane. Next, the buffer would drag the lyophilized marked antibodies in the conjugate pad to the membrane. There, those anti-Fcε Abs will bind to the IgEs generating the blue positive bands. As for those specific to Fcγ, they will only bind to Abs fixed at the end of the membrane. This is used as a control mechanism to ensure the reagents reach the end of the strip. Finally, depending on the affinity of IgEs against the allergens, the bands will be more or less coloured, meaning allergy level.

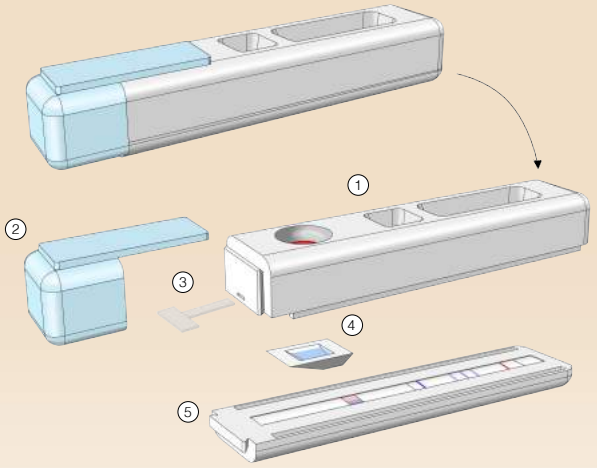

Figure 2. Strip case diagram. First, the user shoud deposit a blood drop (extracted by means of a small pointer that would come in the kit) into the smaller well of the two that can be seen at the top of the case (1). The blood would pass through a filter permitting only the serum to pass into the test strip. After about 5 minutes, it would be necessary to remove the blue lid (2), pulling slightly backwards, revealing a red button at the top, and a small plastic strip (3) at the back. This strip would then be pulled back until it was completely removed, and the red button would be consecutively pressed slowly. This strip is attached to a compartment (4) in which the buffer is contained. When removing this strip an aperture would be opened, and by pressing the red button the buffer would be released to the strip. After 5-10 minutes the results would be visible (5).

• **Materials**: The key materials in this kit are the ones that compose the different layers of the test strip. Although there are multiple feasible options for them, the most suitable ones might be: Cellulose for the sample and the absorbent pads, glass fibre for the conjugate pad and nitrocellulose for the membrane.

• **Product manufacturing**: Monoclonal antibodies could be produced in batch using hybridomas and polyclonal antibodies would be purified directly from the animal's blood. Allergens should be obtained recombinantly by *Escherichia coli* or similar.

Conclusion | I personally believe that the proposed LFIA kit would help to efficiently diagnose Allergic CR before they occur. In addition, by means of Its novel design, a more intuitive or user-friendly product would be obtained allowing families to use it without requiring the assistance of a medical specialist. However, since the proposal is only theoretical and hypothetical, it is not possible to be certain that this kit works correctly. There are still some gaps and an extrapolation to an R&D laboratory would be necessary to ensure the expected results. Moreover, other aspects, such as regulations and patents, should also be considered.

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