A high-throughput protein array-based approach for allergy screening & multiple parallel discovery & characterisation of IgE-binding proteins

Rachel Van Dyk (1)
Claudia Kirisits (2)
Paul Potter (3)
Fook Tim Chew (4)
Reinhard Hiller (1)

(1) Centre for Proteomic and Genomic Research (CPGR), Cape Town, South Africa (2) VBC-GENOMICS GmbH, Vienna, Austria (3) University of Cape Town Lung Institute, Cape Town, South Africa (4) National University of Singapore, Singapore.

Introduction -Allergy Research at the CPGR

Antibody-mediated symptoms of allergic diseases represent a severe challenge to health care today, with in excess of 700 million people suffering from one of the persistent symptoms of allergy or asthma worldwide. While previously labeled a disease of industrialized nations, recent findings point towards allergy as an emerging health problem in developing countries, including South Africa and Asia. In order to tackle this pandemic disease, more efficient approaches to specific antibody testing and discovery of novel allergens are needed. Here, a novel high-throughput microarray-based approach to the screening of allergen reactivities in crude biological extracts is presented using seafood allergens as an example. Workflows established at the Centre for Proteomic and Genomic Research (CPGR) permit the effective screening of hundreds of putative allergens in parallel using minute amounts of patient serum while at the same time constituting a cost-efficient allergen-specific antibody screening method applicable to routine diagnostic settings.

Assay Development – Automated High-throughput Serum Screening

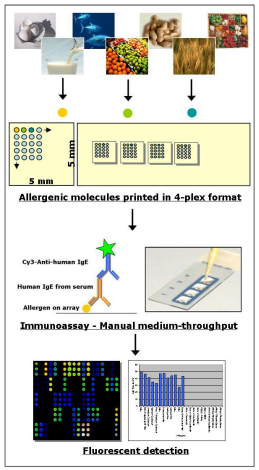


Figure 1

Figure 1: Standard Immuno Solid-phase Allergen Chip (ISAC) assay (VBC-Genomics, Vienna). Selected allergens were piezo-printed onto glass slides in a 4-plex format, each array being separated by a teflon mask. Incubation steps are performed statically in a humid chamber and washing is performed in staining dishes. Fluorescent scanning determines which antibodies were present in the patient serum thus revealing their individual sensitisation profile.

Figure 2a: Development of seafood extract arrays processed in a high throughput fashion. 75 seafood extracts and allergen molecules were printed (VBC-Genomics) in an 8-plex format on ultra-thin nitrocellulose slides from Gentel Biosciences corresponding spatially to the wells of a 96-well microtitre plate. A high-throughput assay has been developed in collaboration with Tecan using a Hydroflex plate washer adapted to process slides held in a Whatman FAST frame. All incubation and washing steps are performed with shaking to improve reaction kinetics. It is possible to assay 4 slides with up to 16 arrays per slide using this approach. The assay time is reduced significantly from 6 hours to 2 hours when compared to the manual slide handling.

Figure 2b: Further high-throughput assay development. Identical seafood allergen arrays as described above where printed in a 3-plex format corresponding spatially to the first 3 chambers of the Tecan HS4800Pro quad chambers. The HS4800Pro is an automated hybridisation station that performs all stages of sample incubation, slide washing and drying to maximise assay reproducibility. Incubation occurs with agitation to increase sensitivity and reduces assay time to 2.5hours.

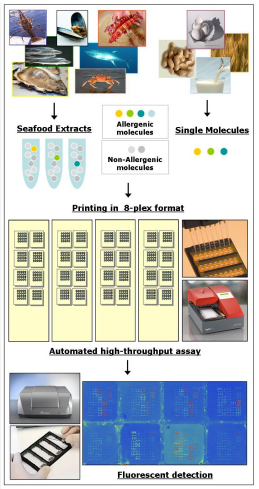


Figure 2a (above) and 2b (below)

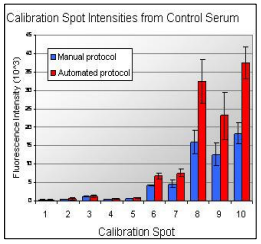
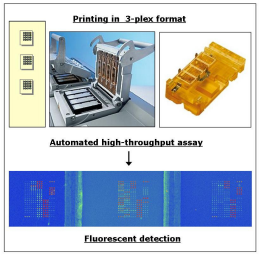


Figure 3

Figure 3: Graph comparing calibration data from the manual and automated protocol. The standard ISAC protocol was performed on the 8-plex slides held statically in the FAST frame and was compared to the assay performed in 8-plex on the Hydroflex. Data shown were averaged from triplicate spots per array and triplicate arrays per slide. These triplicate assays were performed across three different slides over two days to assess reproducibility. The results indicate a strong increase in signal intensity with no apparent loss in reproducibility. A similar pattern in test variability in the manual and automated assays indicates that further optimisation in protein array printing is needed.



Results – Microarrayed Antigenic Extracts as Allergy Screening Tools

Patient sera were provided (Paul Potter, Lung Institute, SA) for assay development of the seafood extract arrays. These serum samples have previously been screened for allergies using conventional solid phase immuno assays, screening against either single allergens or a defined mix of allergenic molecules. Selected sample data are shown below (Figures 4,5 and 6) to illustrate the information that can be gained from serum antibody profiling of allergic patients on arrays containing a mixed panel of allergen extracts and purified single allergen molecules. The main graphs depict antibody binding data obtained on the seafood arrays whereas the inset tables highlight the comparing data from state-of-the-art allergy diagnostic assays (UniCAP, PhaDia).

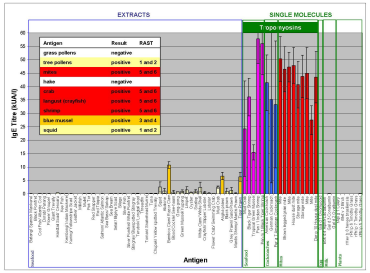


Figure 4

Figure 4: The array data are consistent with the previous tests in terms of allergen-specific antibody binding and the level of the response (signal intensity). However, the array data provides a comprehensive overview into the nature of the patient's allergenicity profile. In the example, a specific and string reactivity to the individual tropomyosin allergens has been revealed. Concomitantly, antibody binding to extracts derived form shellfish but not other seafood species indicates the presence of tropomyosins in the crude extracts, a major allergen in crustaceans and other shellfish species. The array data are confirmed by previous data suggesting that this patient is allergic to shellfish but not to fish.

Figure 5: The patient in this example has under gone only limited previous testing and has shown a negative response to mussel and langoustine. The array data are more comprehensive showing intermediate antibody reactivity to purified single shellfish and mite tropomyosins ands some grass pollen allergens. A low to medium allergic response is detected for most of the fish whole extracts but not to the shellfish and other seafood.

Figure 6: The patient has been previously tested for allergy to mussel and langoustine and has shown a low and medium response respectively. The array data shows very low (but not negative) response to most seafood, a consistent medium response to all the tropomyosins and a borderline response to other single molecule antigens, suggesting that the patient is specifically sensitised to tropomyosins.

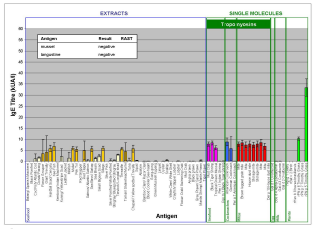


Figure 5

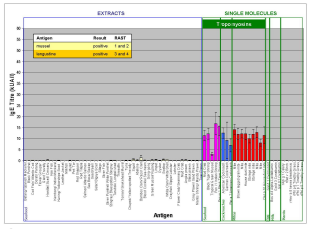


Figure 6

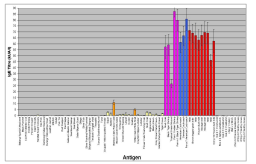


Figure 7: Highlights that results from assays obtained on the Hydroflex (Figure 4) and the HS4800Pro are comparable.

Further Developments

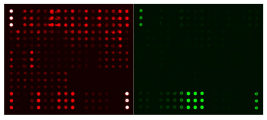


Figure 8: Antibody class comparison.

We are currently combining IgG detection (via anti-human IgG-Cy5) in dual colour assays with IgE detection (via anti-human IgE-Cy3), shown left and right respectively. We intend to also investigate IgG subtypes, IgM and IgA antibody classes and the different roles they play in allergic response.

Conclusion

Combined arrays of printed single allergen molecules and crude allergen extracts represent efficient screening tools for allergen discovery and characterisation in the context of diagnosis, immunotherapy and bio-safety. The use of automated liquid-handling platforms for running multiple arrays in parallel increases both throughput and assay performance, ultimately reducing the costs of large screening programs as well as in routine diagnostic settings.

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Centre for Proteomic and Genomic Research (CPGR)
Institute of Infectious Disease & Molecular Medicine
Lab S2.09, Wernher Beit South Building, University of Cape Town
Anzio Road, Observatory, South Africa, 7925

Contributions:
VBC Genomics – microarray production and hydroflex protocol
Tecan – hydroflex protocol development and conference sponsorship
Gentel – ultra-thin nitrocellulose protein arrays slides

Cape Biotech
PlantBio