

LABORATORY METHODS IN ALLERGOLOGY

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IDENTIFICATION AND QUANTIFICATION OF SPECIFIC IgG ANTIBODIES

Allergic diseases represent increasing health problems worldwide, and symptoms may not be easily distinguished from other disorders. The term hypersensitivity is defined as a reaction that induces reproducible symptoms and signs, initiated by exposure to a defined stimulus at a dose tolerated by normal subjects. Hypersensitivity can be differentiated into IgE and non-IgE allergic hypersensitivity as well as non-allergic hypersensitivity, which does not involve the immune system. Most patients are sensitised to more than one allergen which might trigger clinical symptoms, and often it is difficult to identify the major allergen. In addition, the presenting symptoms are dependent on current inflammation, presence of infection, physical and psychological stress and hormonal influences. Different tests may be used to distinguish between these conditions. An allergic hypersensitivity is usually IgE-mediated but may involve IgG antibodies. In this article we explore currently available tests and highlight their applications and limitations.

During an immune reaction to a foreign antigen, antibodies are produced as part of the body's complex defence mechanism. Antibodies of the IgE type are typical in Type I allergic reactions; however, high titres of antigen-specific IgG can also be observed. In autoimmune disorders, these antibodies are directed against self-antigens (autoantigens). The presence and level of specific IgG antibodies in serum can reflect the extent of exposure to that antigen. IgG antibodies can be quantified via the ImmunoCAP system and more recently also with the micro-arrays system. The IgG antibody response can be quantified to all available ImmunoCAP allergens; however, only a few allergens have been evaluated and respective cut-off values determined (Table I). A new tool for quantifying specific antibodies to allergens where no commercial validated test system is available has been developed. Researchers can now couple their own allergens to the **Streptavidin ImmunoCAP** using the normal UniCAP system.

Measuring specific IgG antibodies may provide valuable information in different areas:

Allergic diseases

- Marker for exposure in different lung diseases: including aspergilloma, aspergillosis and **allergic alveolitis** to bird allergens. The latter can be regarded as positive when values exceed 30 mg/l.¹

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Table I. Validated IgG ImmunoCAP tests using the UniCAP system

- alpha-Lactalbumin
- *Alternaria alternate*
- *Aspergillus fumigatus*
- beta-Lactoglobulin
- *Candida albicans*
- Casein
- *Cladosporium herbarum*
- Common wasp venom
- Common silver birch
- *Dermatophagoides pteronyssinus*
- *D. farinae*
- Egg white
- Gliadin
- Honey-bee venom
- Pigeon/parrot/budgerigar
- Rice
- Thyroglobulin
- Thyroid peroxidase
- Timothy grass pollen
- Wheat protein

Food allergy

- Presence of IgG is a sign of exposure (also of particular interest for cross-reactivity to foods which are not usually consumed by these individuals, e.g. kiwi, avocado).
- Diagnostic importance for certain food antigens, e.g. **milk** or **gliadin** in coeliac disease (see below).

Immunotherapy

- Monitoring success of immunotherapy with inhalant allergens and hymenoptera venoms (increasing IgG antibody titres indicate positive response to therapy).

Autoimmunity

- Elevated levels of antibodies to thyroid peroxidase (TPO) and thyroglobulin (TG).

Inhalation of large amounts of organic dust for long and intense periods can cause an inflammation of the lung parenchyma in the terminal bronchioles and alveoli, previously referred to as extrinsic **allergic alveolitis** now termed allergic alveolitis (AA).^{1,2} This condition can occur among 'bird fanciers' and farmers who keep animals or grow mushrooms. It involves specific antibodies predominantly of the IgG type as well as complex cellular mechanisms. The level of serum antibody against bird antigens reflects the level of antigen exposure, and higher levels are usually associated with more severe disease. AA to birds is diagnosed predominantly based on a characteristic clinical picture accompanied by the presence of IgG antibodies as detected by the visualisation of precipitating antibodies (Ouchterlony test) to serum antigens (Fig. 1). More recently this

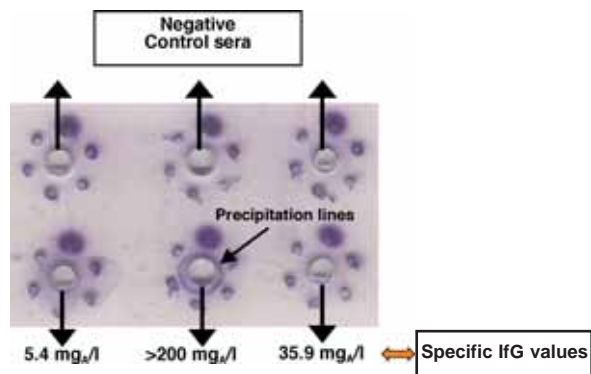


Fig. 1. Ouchterlony test to demonstrate the precipitation of pigeon specific IgG antibodies with pigeon serum antigens. The precipitation is visualised by staining with the protein dye Coomassie Blue. ImmunoCAP results of positive and negative sera are indicated with an arrow and IgG level expressed in mgA/l (mg antigen-specific antibody).

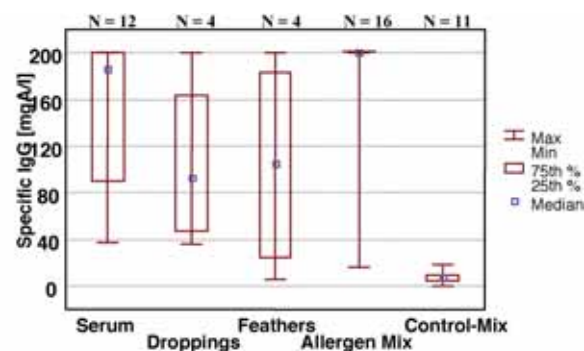


Fig. 2. Evaluation of specific IgG antibodies to different allergen sources of pigeons of symptomatic subjects with positive Ouchterlony. Specific IgG levels were quantified using the UniCAP system and specific immunoCAPs for pigeon serum, droppings and feathers, as well as the allergen-mix for all three allergens. Specific IgG antibody values are expressed in mgA/l and the median values calculated for each antigen and group (N = number of subjects).

method is being replaced with the sensitive quantification of specific IgG antibodies, which can in addition be applied to different antigen sources (Fig. 2).

Elevated IgG antibodies have also been detected in **cow's milk allergy (CMA)**, which is a very complex disease with diverse clinical manifestation and allergen recognition.³ Bovine milk contains about 3.5% protein of which casein constitutes 80% while whey proteins and minor allergens constitute 20%. Furthermore, casein can be divided into four fractions while the major whey proteins are alpha-lactalbumin and beta-lactoglobulin. The latter is acid-stable and likely to remain intact even after passage through the stomach, explaining its role as an IgG-binding allergen in CMA.

Increased levels of specific IgG antibodies, particularly to food proteins, vary considerably according to exposure rates and eating habits, but are not directly linked to an allergic disease. However, elevated levels may indicate increased exposure due to damage to the intestinal mucosa, which is frequently seen in **coeliac disease**.⁴ The measurement of elevated levels of antibodies to gliadin, specific IgA but also IgG in patients with IgA deficiency, is highly sensitive and specific. During elimination diets decreases in specific antibody titres are also used to monitor gluten avoidance. Suggested cut-off values for gliadin specific IgG and IgA antibodies are about 18 mg/l and 2.0 mg/l respectively, but should be validated with normal healthy individuals for each geographic area.

Declaration of conflict of interest

The author declares no conflict of interest.

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