

Allergy profiles from bloodstains

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Summary

The radioallergosorbent test (RAST) has been adapted to measure specific IgE antibodies in bloodstains. By incubating several allergen discs simultaneously with one stain extract, an allergy profile can be readily obtained from less than 50 μ l of dried blood.

Introduction

The radioallergosorbent test (RAST) has been shown to be a useful tool in the diagnosis of atopic allergy. Previous studies have shown that good agreement exists between RAST results and conventional clinical tests (Johansson, Bennich & Berg, 1971; Foucard, 1972; Ahlstedt *et al.*, 1975; Bryant, Burns & Lazarus, 1975).

Filter paper techniques for the collection of samples with a view to subsequent antibody detection are well documented. Techniques have been adapted for epidemiological studies of antibodies to various organisms (Brody, 1963; Cohen, Hatgi & Wisseman, 1969; Wolf & Hudson, 1974). The work presented here describes the use of the Phadebas RAST to detect IgE antibodies in bloodstains.

Method

Bloodstains were collected by finger-prick and subsequently tested for IgE antibodies to the following allergens: *Dactylis glomerata* (cock's foot grass pollen), *Dermatophagoides pteronyssinus* (house dust mite) and cat epithelium. For each test, approximately 1 cm² of bloodstained cotton cloth or filter paper was extracted by the method of Baxter & Rees (1974). Each sample was extracted three times: a 15 min soak in 50 μ l PBS followed by 10 min centrifugation at 650 g. The pooled extract was then taken through the RAST procedure similar to that recommended by Pharmacia GB Ltd, with beneficial modifications described by Gleich & Jones (1975). In addition, the total activity of added anti-human ¹²⁵I-IgE was measured for each tube during incubation. This procedure effectively eliminates the error due to sampling of labelled antibody.

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A 5 μ l sample of the pooled bloodstain extract was used to determine total protein by the Lowry auto-analyser method (Lowry, 1970). Antibody activity (x) in the bloodstain eluate was expressed as

$$x = \frac{\text{Counts/100 sec} \times 10^4}{\text{Protein concentration (mg/ml)} \times \text{Total Activity Added}}$$

Bloodstains from twenty-six individuals were screened for IgE antibodies; any specific allergy reported by the donor was noted. Duplicate bloodstains from six individuals were prepared to demonstrate the reproducibility of the method. Six bloodstains from hypersensitive individuals were extracted and incubated with three allergen discs simultaneously. Each disc was subsequently incubated separately with 50 μ l anti-human ^{125} I-IgE.

Results

Of the twenty-six individuals tested, seven were found to be positive to *D. glomerata*, eight were positive to *D. pteronyssinus*, four were positive to both of these allergens and two individuals were positive to *D. glomerata*, *D. pteronyssinus* and cat epithelium.

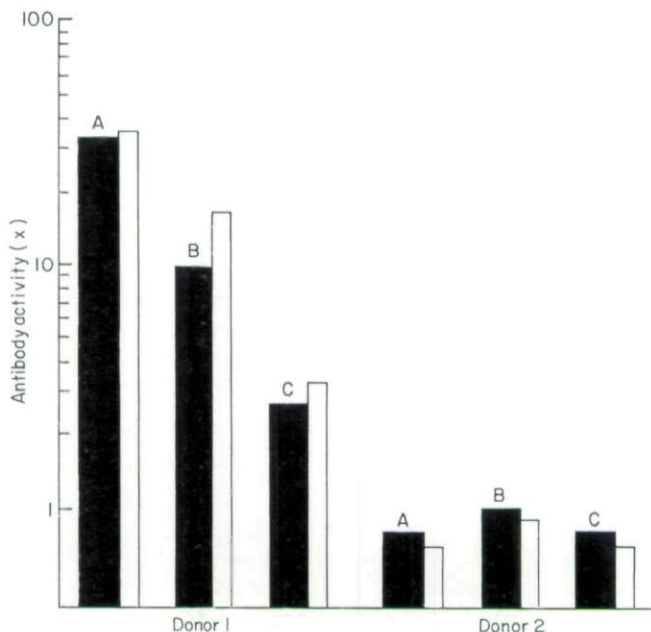


Fig. 1. Allergy profiles of a multiple-hypersensitive donor (1) and a non-atopic donor (2). A, *D. glomerata*; B, *D. pteronyssinus*; C, cat epithelium. A single extract from a 1 cm² bloodstain from each donor was incubated simultaneously with three Phadebas RAST allergen discs. The open bars show the reproducibility from a duplicate experiment.

All of the positive RAST results agreed with the donors' reported allergy status. The results were expressed in the form of allergy profiles (Fig. 1). The reproducibility of the technique is also illustrated by Fig. 1. Blood donors who reported no hypersensitivity for the three allergens described had very low antibody activity ($x < 2$).

It was found that one stain extract was sufficient for all three allergen discs. There

was no significant reduction in activity due to the simultaneous incubation. Similarly, no significant difference was noted for bloodstains prepared on either cotton cloth or filter paper.

Discussion

The use of the RAST with bloodstains under clinical circumstances, e.g. epidemiological surveys of remote areas, is considered below; its use in forensic serology will be described elsewhere (Werrett, King & Whitehead, 1975).

The absorption and subsequent elution of blood from cotton cloth or filter paper has been shown not to affect the diagnostic value and reproducibility of the RAST. The stability of IgE antibodies in dried blood is considered to be adequate for most purposes. [The 'half life' of soluble antibody in dried blood is about 6 weeks (Werrett, King & Whitehead, 1975)]. Diagnoses can be made by comparison of the test results with similarly prepared standards. The determination of the total protein content obviates the need to use known volumes of blood. Finally, a small bloodstain, corresponding to 50 μ l whole blood is sufficient to diagnose multiple-hypersensitivity if several allergen discs are incubated simultaneously.

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