

IgE testing in capillary blood

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Serologic IgE testing is generally performed using serum, obtained by venepuncture. We tested whether paper-absorbed and eluted capillary blood, obtained by a less invasive method (finger prick) could be used for allergy testing in young children. This was performed by comparative IgE testing, using paper-absorbed blood/serum and serum. Practical applicability of the procedure was tested by assaying paper-absorbed and eluted blood, obtained from 640 children with complaints of prolonged coughing, for IgE to airborne allergens. We found that IgE testing, using paper-absorbed/eluted material and serum yields virtually identical results (mean ratio for positive samples: 1.01, 95% confidence interval: 0.58–1.75). Blood spot testing revealed that sensitization to inhalant allergens is not uncommon in preschool children (13% positive radioallergosorbent test [RAST] tests), which means that this procedure is a useful method for assaying allergic sensitization in children.

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Serologic IgE-testing for detection of allergic sensitization has gained increasing appreciation over the last decades, since many tests can be performed with limited amounts of serum without risk for the patient.

One reason for reluctance toward *in vitro* IgE testing, however, is that obtaining blood by venepuncture is in some instances less acceptable, e.g., in the case of young children. A less invasive method for obtaining blood, for instance by finger prick (1) would be preferable in such cases, especially as the latter technique can also be performed by unskilled personnel.

We aimed to test whether capillary blood, obtained by finger prick and absorbed on to filter paper, can be used for IgE testing in preschool children.

Material and methods

Absorption and elution of blood proteins

To evaluate the capillary blood spot procedure, we compared IgE results obtained with paper-absorbed and eluted whole blood/serum with results using routine serum procedures. Whole blood was obtained by venepuncture or by finger prick, using an automated lancet (Glucolet 2 Automatic Lancing Device in combination with Ames

Minilet Lancets; Bayer Diagnostics, Puteaux, France), and applied to filter paper (Schleicher & Schuell 2294, K120258). After sample application, filter paper was left to dry for at least 16 hours and stored at room temperature for up to 14 days. Absorbed material was cut out (c. 3 cm² of paper for 50 µl of absorbed material) and eluted by incubation with 1 ml of phosphate-buffered saline/Tween-20 [PBS-T, 0.1% (v/v)] for 16 hours under constant rotation. The same absorption/elution procedure was performed using serum samples from our routine department. Eluates were separated from filter paper by centrifugation.

Serum albumin as internal standard

Paper-absorbed whole blood spots obtained by finger prick represent an unknown amount of serum. To assess this amount for volume correction of test results, we measured the concentration of serum albumin in the eluted samples by nephelometry (BN2[®], Behring Werke, Freiburg, Germany). This method seemed convenient as only very small amounts of serum sample are needed (< 1 µl per test). Albumin was chosen as a reference protein as this compound shows only little variation among donors (2, 3), and albumin is efficiently eluted from blood dried on filter paper (see Results). Measuring total protein

content may be less appropriate in view of possible variations in the leakage of hemoglobin from red blood cells.

Serum albumin was measured in 100 serum samples from subjects in different age groups, using the same method.

IgE testing

Determination of total IgE and specific IgE in serum, eluted serum or whole blood was performed essentially as previously described (4, 5), using Sepharose-immobilized reagents and ^{125}I -labeled anti-human IgE, raised in sheep. Minor technical modifications (increased amounts of added radiolabel and prolonged radioactivity counting) were applied in order to render these RAST tests more accurate for assaying small quantities of IgE. All IgE test results [U/ml; one unit representing 2.4 ng of IgE (6)] were corrected for the estimated amount of serum used in the tests.

In order to test the validity of the elution and albumin volume correction procedures, we compared IgE results obtained with serum spot eluates, blood spot eluates, and serum obtained by venepuncture. This was performed as follows:

(1) Total IgE was measured in both serum eluate and serum, using 40 patient samples sent to our laboratory for routine allergy testing: after adsorption of 50 μl serum on to filter paper, spots were eluted with 1 ml elution buffer, serum albumin was measured in the eluate and 100 μl of eluate was tested. Total IgE results, obtained after test volume correction using 41.9 g/l of serum albumin as a reference value (see Results), were compared with total IgE as measured in serum *per se*.

(2) Total IgE was measured in capillary blood spots and in venepuncture-obtained serum samples from 11 subjects: paper-absorbed blood spots (of unknown volume) were eluted, and assayed for total IgE, using 100 μl of eluate. The amount of plasma used in these whole blood tests was determined on the basis of 41.9 g/l of albumin. IgE values were compared with those found in venepuncture-obtained serum samples from these subjects.

(3) Allergen-specific IgE was measured by RAST, comparing serum and eluate from 50 subjects: 50 μl of serum from patients who had a RAST test > 0.5 U/ml for either house dust mite, cat dander, dog dander, grass pollen, birch pollen, hen's egg or cow's milk (50 samples) was applied on filter paper. After elution, 200 μl of eluate (corresponding to *c.* 10 μl of serum) was tested on either a mixture of mite, cat and dog

allergosorbent, on pollen mixture (grass and birch), or on a mixture of hen's egg and cows' milk allergosorbent. Results were compared with those obtained by assaying 50 μl of venepuncture-obtained serum, according to our standard RAST procedure.

For IgE testing of blood spot samples from preschool children 100 μl of eluate was used for total IgE determination. For measuring specific IgE to house dust mite, cat dander and dog dander 200 μl of eluate per test was applied. RAST results were considered positive when values were higher than 0.0015 U per test.

Test cut-off values

Cut-off values for blood spot assays were determined by 10-fold testing of IgE-negative samples and adding $2 \times \text{s.d.}$ to the observed mean values, as well as by spiking of IgE-negative samples with serum dilutions containing known amounts of IgE.

Patient samples

In a study on the development of inhalant allergy in preschool children we tested whether the blood spot procedure could be useful in daily practice. Seventy-two general practitioners collected capillary blood from 640 children, aged 1–5, who presented with complaints of prolonged coughing (Eysink PED, Bottema BJAM, ter Riert G, Aalberse RC, Stapel SO, Bindels PJE, personal communication) by finger prick, as described above. Blood spots were left to dry and sent to our institute in a plastic envelope. Parents gave their informed consent and the study were approved by the Medical Ethics Committee of the Academic Medical Center, University of Amsterdam.

Statistics

The ANOVA was used for comparing albumin contents in serum samples. Geometric mean ratios with 95% confidence intervals (CI) were calculated for albumin-corrected results for eluted material and results for direct testing.

Results

Elution procedure

Assaying for albumin and total IgE in whole blood eluates and serum from 11 subjects revealed that not only albumin, but also IgE, present in paper-absorbed blood spots, eluted

efficiently from the filter paper (elution recovery for both components: > 95%).

Human serum albumin as a reference protein

Results of albumin measurement in 100 sera (Table 1) indicate that there is no major age effect (ANOVA: $p = 0.18$) in the albumin

Table 1. Human serum albumin (HSA) in different age groups

Age (months)	n	HSA (g/l)
1–100	19	40.9 ± 3.9
101–200	15	44.0 ± 4.2
201–300	11	43.0 ± 4.5
301–400	14	41.8 ± 4.5
401–500	14	40.4 ± 3.8
>500	27	41.3 ± 5.4

A total of 100 serum samples from different age groups (indicated in months) were tested for serum albumin (g/l), as described in Material and Methods.

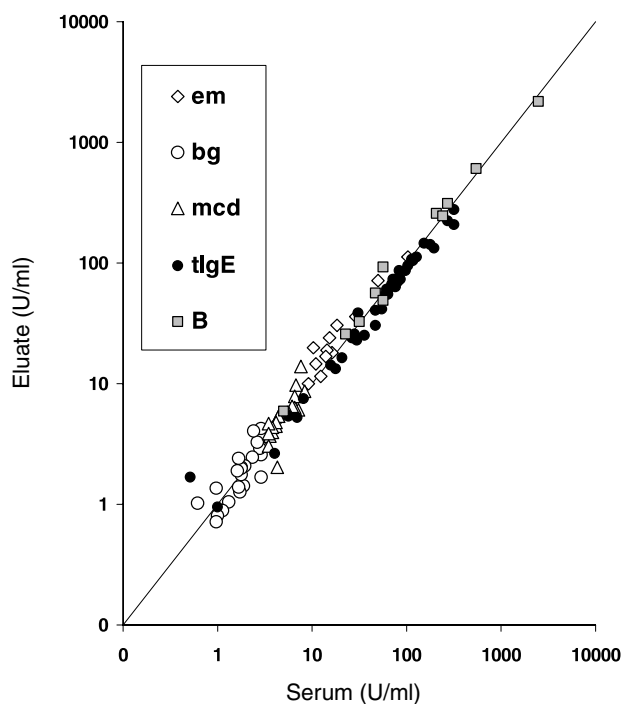


Fig. 1. Comparative IgE testing in spot eluates and serum total IgE (tIgE): 50 µl aliquots from 40 serum samples were adsorbed on filter paper. Blood (b): capillary blood, obtained from 11 subjects, was absorbed on filter paper and eluted. After elution, eluates were assayed for total IgE. Specific IgE: 50 µl aliquots from 50 serum samples, containing IgE to either egg, milk, mite, cat, dog, birch or grass were absorbed on filter paper. Test results (U/ml), obtained with eluted material, were, after albumin correction (using 41.9 g/l as reference value), compared with values obtained by using serum testing according to routine procedures. em, egg/milk-positive sample; mcd, mite/cat/dog-positive sample; bg, birch/grass-positive sample.

concentration (mean value and s.d.: 41.9 ± 4.1 g/l).

IgE assays

Fig. 1 shows comparative results for total IgE and specific IgE testing, using paper-absorbed/eluted serum, eluted whole blood, and serum *per se*. As can be seen, albumin-corrected results for eluted material are in agreement with the results obtained by direct testing (geometric mean ratio for positive samples: 1.01, 95% CI: 0.58–1.75). Deviation between the tests did not significantly depend on total or specific IgE levels.

Cut-off values

By assaying negative serum samples and serum dilutions, the cut-off value for our IgE assays was found to be 0.0015 unit (3.6 pg of IgE) per test.

Patient samples

Eighty-three (13%) of 640 patient samples tested (children aged 1–4 years) in this procedure showed a positive RAST to one or more allergens, when 0.5 U/ml is used as cut-off value. Sixty-four of these children had one positive RAST, 15 showed two positive RAST-scores and four children were IgE-positive for three allergens.

Discussion

We investigated whether assaying for total IgE and allergen-specific IgE in eluted blood spot material is a feasible technique for allergy testing.

Albumin values in 100 sera were in accordance with previously reported observations (2, 3), and elution efficacy of this protein was high, which renders it a suitable parameter to be used for volume correction of test results.

As can be seen in Fig. 1, IgE values obtained with serum and with paper-absorbed/eluted serum were similar. This indicates that the correction of test results, using the observed mean serum albumin value of 41.9 g/l (Table 1) as internal standard, is valid. It must be noted, however, that variations observed for albumin values in test sera will also be reflected in albumin-corrected IgE results.

Although serum albumin amounts in venepuncture serum samples and finger prick samples can be different: 40.6 ± 2.36 g/l and 41 ± 2.14 g/l, respectively (7). These differences will not result in relevant differences in albumin-corrected IgE-values.

Results of comparative total IgE testing, using serum and eluted capillary whole blood, point

out that serum values and eluate values are similar when the latter are corrected on the mean reference albumin value [Fig. 1, blood spots from 11 patients (a), see Material and Methods].

If the clinical cut-off value of the RAST test is taken to be 350 mU/ml serum, we need at least $1.5/350 = 0.0043$ ml serum equivalent per test. Routinely, we use 200 µl of the eluate per RAST assay, which is one-fifth of the total eluate volume. For an assay with the commonly used cut-off value of 0.35 U/ml we therefore need five times 0.0043 ml serum equivalent per blood spot, i.e., 21.4 µl serum equivalent (or *c.* 50 µl whole blood per spot). Smaller blood spots will, obviously, result in assays with a higher cut-off value. For example, if a blood spot sample containing the equivalent of 10 µl serum is spotted (of which one-fifth is used per RAST), the assay cut-off will be 1.5 mU per 2 µl serum, or 750 mU/ml. In this situation a negative test result would be interpreted as 'less than 0.75 U/ml', which would not exclude a weakly positive RAST. Testing 500 µl of the eluate might be an option, but this would obviously diminish the number of tests that can be performed.

The observation that 83 of 640 (13%) tests performed with paper-absorbed blood samples from children younger than 5 years were positive for inhalant allergens, indicates that sensitization to these allergens is not uncommon in this age group, as has been observed earlier (8).

On the basis of these results we conclude that RAST screening, using paper-absorbed capillary blood (absorbed samples being stable for at least 2 months, results not shown), is a

convenient and valid method for IgE testing in young children.

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