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# Immunocapillarymigration

## Use of Fluorescein- and Enzyme-Labeled Antibodies to Quantify C-Reactive Protein

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## Abstract

The method is based upon the attachment of antibodies to a porous insoluble support and the subsequent capillary migration of the antigen-containing solution in the support. The antigen-covered area thereby obtained was visualized with fluorescein- or horseradish peroxidase-labeled antibodies. The height of this area increased with increasing antigen concentration. The method was used to quantitate C-reactive protein.

**Index Entries:** Immunocapillary migration; migration, immunocapillary; fluorescein-labeled antibodies, in C-reactive protein analysis; enzyme-labeled antibodies, in C-reactive protein analysis; antigen, immobilized; antibodies, immobilized; C-reactive protein, immunoassay of; immunoassay, of C-reactive protein.

## Introduction

During the last 50 years a large number of immunochemical techniques for the quantitation of proteins has been developed. Methods like single radial immunodiffusion (*1*), electroimmunoassay (*2*), nephelometry (*3*), and radioimmunoassay (*4*) are today used in most laboratories. They are distinguished by simplicity, rapidity or extremely high sensitivity, and by the process used to


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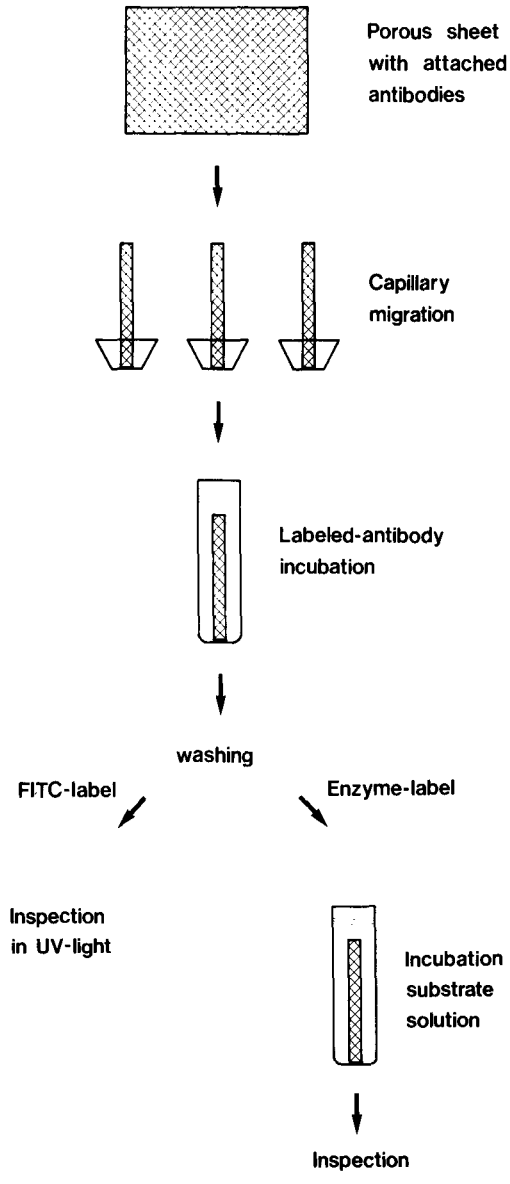


Fig. 1. (a) The immunocapillarymigration procedure.

bring about the necessary interaction between antigen and antibody. Immunocapillarymigration (*5–7*) was developed to bring about the antigen–antibody interaction utilizing the capillary force of a porous material.

## Experimental Procedure and Discussion

In Fig. 1 a and b the immunocapillarymigration procedure and principle are shown. Antibodies were attached to sheets of porous materials such as filter paper, cellulose acetate, or polyvinyl chloride sheets with silica gel as a filler (microporous

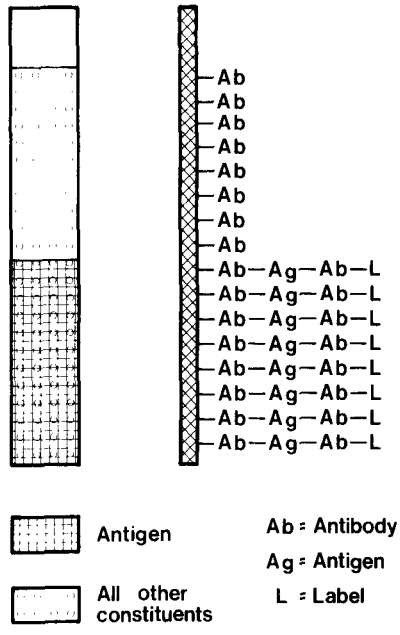


Fig. 1. (b) During the migration the antigen is delayed in its migration in comparison to all other constituents in the sample. The antigen-covered area obtained is visualized by labeled antibodies.

plastic sheets A 40, Amerace Erna Corporation, New York, USA). The attachment methods used were either covalent linkage with CNBr (*8*) or crosslinkage with glutardialdehyde for filter paper and cellulose acetate, and adsorption from buffered solution, pH 8.0, for polyvinyl chloride sheets. The sheets were dried and cut into strips 5 × 70 mm. Each strip was dipped into a cup, 9 mm in diameter, containing 0.3 mL of a sample dilution, and the cups were placed in a humidified chamber. The sample was allowed to migrate to a height of 60 mm. Areas covered with antigen were visualized by incubating the strips in a solution of fluorescein-labeled antibodies or in a solution with horseradish peroxidase-labeled antibodies. Excess labeled antibodies were washed off under running tap water. Strips that had been incubated in fluorescein-labeled antibodies were then inspected in utltraviolet light (wavelength 254 or 350 nm) and the fluorescent areas were marked with a pencil. Strips incubated in peroxidase-labeled antibodies were incubated in substrate solution (20 mg 3-amino-9-ethylcarbazoe/2.5 mL dimethylformamide made up to 50 mL with 0.05 mol/L acetate buffer, pH 5.0, containing 0.025 mL 30% $H_2O_2$), resulting in the development, within 5 min, of dense-colored areas where enzyme-labeled antibodies were bound. The heights of the fluorescent or colored areas were measured and compared with corresponding heights obtained from strips treated with a serial dilution of a standard, and then developed as described above. For sheets of the same material containing the same amount of attached antibodies, the heights of these areas were found to increase with increasing amount of antigen in the sample (Fig. 2).

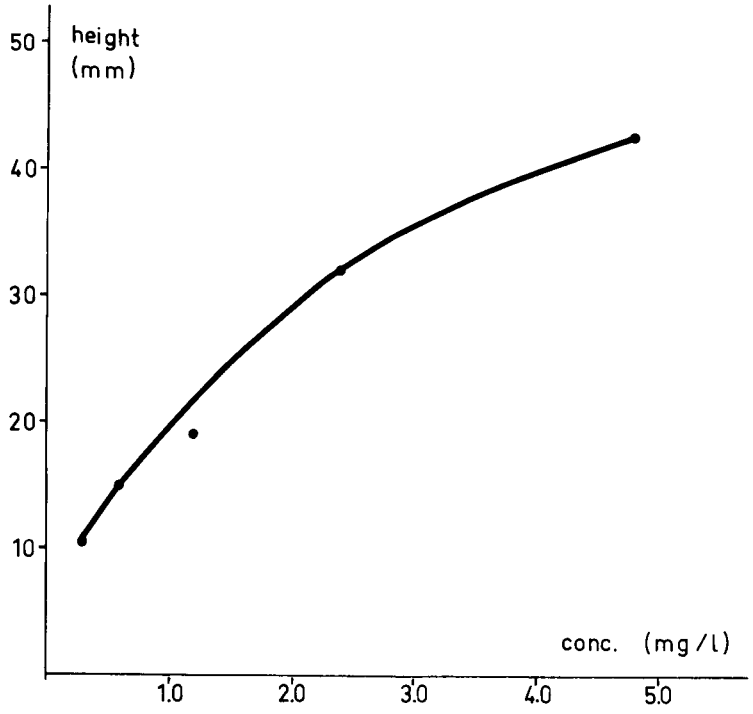


Fig. 2. Standard curve relating the heights of the colored areas obtained on strips with antibodies against C-reactive protein attached and the C-reactive protein concentration of the sample.

A comparison of the porous materials used concerning antibody attachment procedures, non-specific binding of labeled antibodies, migration rate and fragility was undertaken. The antigen–antibody systems used were transferrin–goat antitransferrin antibodies and C-reactive protein–rabbit anti-C-reactive protein antibodies. *Polyvinyl chloride sheets* were easy to handle; antibodies could be attached by adsorption, but the migration rate was relatively slow (60 mm/45 min); peroxidase-labeled antibodies could not be used because of unspecific binding. *Cellulose acetate sheets* were fragile, antibodies had to be covalently coupled, and the migration rate was as slow as for polyvinyl chloride sheets. But although fluorescein-labeled antibodies were unspecifically bound, peroxidase-labeled antibodies could be used with good result. *Filter paper* showed a relatively high migration rate, but was found to bind both fluorescein- and peroxidase-labeled antibodies unspecifically. Hence, polyvinyl chloride sheets were used with fluorescein-labeled antibodies and cellulose acetate sheets with peroxidase as the antibody label.

When the antibody activity of the immunoglobulin fraction attached to the materials was decreased, lower concentrations of antigen in the sample could be measured, but concomitantly the intensity of the fluorescence or the density of the color decreased. The lowest protein concentration that could be clearly differentiated from zero was about 40 mg/L when flourescein was used as the label and 0.30 mg/L when peroxidase was used.

A comparison of immunocapillarymigration when peroxidase-labeled antibodies were used and electroimmunoassay (*2*) for the quantitation of C-reactive protein in 19 human samples provided a correlation coefficient of 0.92. The standard deviation of the immunocapillarymigration procedure calculated from duplicate determinations was found to be 10% of the mean with fluorescein-labeled antibodies and 15% of the mean with peroxidase-labeled antibodies. The comparatively lower precision obtained when enzyme-labeled antibodies were used may be related to the rather low enzyme activity of the conjugate.

Immunocapillarymigration is a rapid quantitative method, with a sensitivity comparable to that obtained with most immunochemical methods used for quantitation of plasma proteins. Since a minimum amount of technical equipment is needed, immunocapillarymigration is suited for analysis under field conditions and in acute situations.

## Acknowledgments

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