MICROQUANTITATIVE DETERMINATION OF α_1-ANTITRYPSIN BY A UNIDIMENSIONAL COUNTERIMMUNODIFFUSION TECHNIQUE IN CAPILLARY TUBES

V. S. Repin and Yu. V. Balabanov

UDC 577.152.344.042.2.087.4

To determine microquantities of α_1-antitrypsin the immunoprecipitation test was carried out in a length of agarose gel in a capillary tube (length of gel 7-8 mm, diameter 0.6 mm). Microvolumes (about 1 µl) of antigen and antiserum were applied to the surface of the opposite ends of the gel by a microsurgical instrument. Precision of control of the manipulations with the microvolumes was ensured by means of the ocular micrometer of the MBS-2 binocular loupe. The quantity of immunoprecipitate formed in the gel was calculated from the total optical density after staining with Amido black, recorded by two-wave microspectrophotometry. The sensivity of the method is 0.3-1 ng α_1-antitrypsin. The amount of α_1-antitrypsin is a linear function of the quantity of precipitate formed over the range of 1-25 ng protein. The error of the method is about 10%.

KEY WORDS: *micromethod; microimmunodiffusion in capillary tubes; α_1-antitrypsin.*

The maximal sensitivity of the usual methods of counterimmunodiffusion is 1-12 µg/ml protein [1, 2], which is quite insufficient for work with small samples of cells, when determinations must be made on samples containing 10^{-7}-10^{-9} g protein. Routine immunodiffusion macromethods cannot be used when the analysis is limited by the volume of the sample (microhomogenates, microexudates, biopsy material, and so on). For these cases, several variants of countermicroimmunodiffusion in agar gel with a sensitivity of 10^{-9} g protein per sample have recently been suggested [5]. These methods are not strictly quantitative, for scanning the stained arc of immunoprecipitate presents certain technical difficulties. To obtain a precipitate in the form of a microdisk suitable for scanning, the writers suggested that counterimmunodiffusion be carried out in a capillary tube filled with a short length of agarose gel. The principle of unidimensional counterimmunodiffusion in glass tubes was first suggested by Preer [6] for approximate calculations of macroquantities of antigen on the basis of the rate of migration of the immunoprecipitate in the gel. In the microvariant suggested by the present writers, 10^{-7}-10^{-9} g antigen protein in a sample is determined from the quantity of immunoprecipitate formed, calculated from the total optical density measured by two-wave microspectrophotometry [3]. A sample of 1% agarose, made up in 0.9% NaCl solution with 0.1 M potassium phosphate buffer, pH 7.1, was liquefied in a water bath and drawn into a glass capillary tube 35 mm long and 600 µ in diameter, in separate portions so that the length of the agarose column was 7-8 mm. The melted agarose was positioned in the middle of the capillary tube, which was placed on the bottom of a Petri dish containing water, in which it remained overnight at 4°C. The antigen and antiserum were introduced into the capillary tube by means of a type MM-1 micromanipulator under the control of the MBS-2 binocular loupe. The capillary tube was held horizontally in the special holder of the manipulator. A layer of 1.3 µl antiserum (measured with the ocular micrometer) was applied to the surface of the agarose at one end of the gel from a polyethylene micropipet with an outlet 20-25 µ in diameter. A layer of antigen in a volume of 0.6-1.3 µl was applied to the opposite end of the gel 3 h after application of the antiserum. The capillary tube to which

Laboratory of Memory, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Ioffe.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 4, pp. 506-508, April, 1977. Original article submitted June 3, 1976.

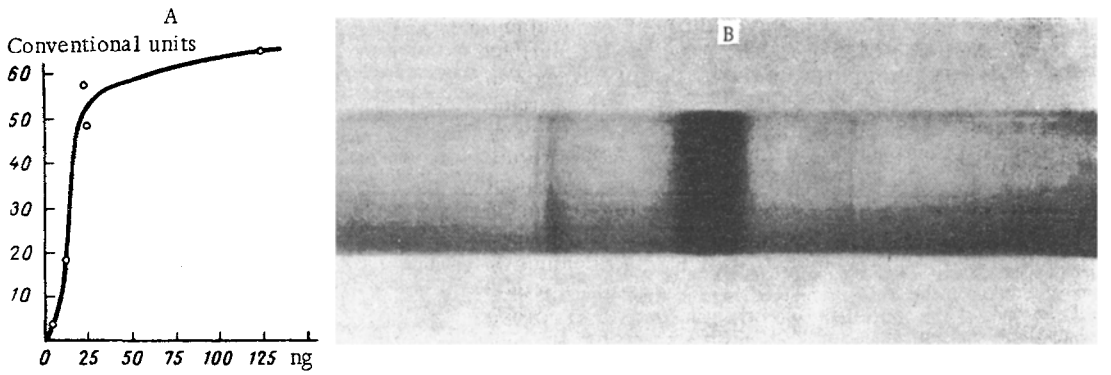

Fig. 1. Determination of microquantities of α_1-antitrypsin by capillary counterimmunodiffusion. A) Graph showing quantity of immunoprecipitate as a function of quantity of α_1-antitrypsin; ordinate, quantity of immunoprecipitate formed as total optical density of disk in gel, measured at E_{620}^{470} by two-wave capillary microspectrophotometry (in conventional units); abscissa, quantity of α_1-antitrypsin (in ng); B) general view of microimmunoprecipitate in length of gel after staining with Amido black (44×).

the antigen and antiserum had been applied was placed on the bottom of a Petri dish containing distillate and kept for 48 h at room temperature until the immunoprecipitation band had completely developed. The gel was then removed from the capillary tube by means of a syringe and placed in cold physiological saline to remove unreacted proteins. The washed gel was transferred to a 0.1% solution of Amido black in 7% acetic acid and stained for 4 h. The excess of dye was removed with 7% acetic acid. The column of gel with the stained zone of immunoprecipitate was drawn into a glass capillary tube 650-700 μ in diameter, after which the gel was scanned on a two-wave capillary microspectrophotometer (Experimental Workshops, Novosibirsk Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR [3]).

The main characteristics of the method can be illustrated by an example of microquantitative determination of α_1-antitrypsin. A preparation of highly purified α_1-antitrypsin, isolated by Crawford's method [4] from human blood serum, was used to immunize rabbits. Preparations of α_1-antitrypsin and monospecific antiserum against human antitrypsin were obtained in the Department of Microbiology of the Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, by A. K. Vais and Yu. N. Zubzhitskii, and were generously placed at our disposal.

In the experiments with α_1-antitrypsin, the quantity of antigen was found to be a linear function of the quantity of immunoprecipitate formed within the range from 1 to 26 ng protein for the volumes of antigen and antiserum chosen (Fig. 1A). The length of the linear region can be increased by increasing the volume of antiserum in the capillary tube. The maximal sensitivity of the method is of the order of 0.3 ng α_1-antitrypsin. The error of the method was calculated by measuring the identical quantity of protein in a series of parallel measurements and was found to be about 10%. In all the experiments the immunoprecipitate was shaped like a regular disk with clear symmetrical edges (Fig. 1B). The shape of the immunoprecipitate did not depend significantly on the quantity of antigen tested.

By the suggested method it was possible to detect and calculate the quantity of α_1-antitrypsin in a solubilisate of 500-600 mammalian ova (of the order of 1-2 ng).

The method of unidimensional counterimmunodiffusion in capillary tubes as suggested above is thus characterized by high sensitivity and reliability of quantitative measurement of protein within the range from 10^{-9} to 10^{-7} g and by relative simplicity of the measurement procedures.

LITERATURE CITED

1. L. A. Zilber (editor), Immunochemical Analysis [in Russian], Moscow (1968).
2. E. A. Kabat and M. Mayer, Experimental Immunochemistry, C. C. Thomas, Springfield, Ill. (1961).
3. L. S. Sandakhchiev, in: Ultramicroanalysis of Nucleic Acids [in Russian], Moscow (1973), pp. 77-95.

4. J. R. Crawford, Arch. Biochem., 156, 215 (1973).
5. V. Neuhoff, Micrometh. Mol. Biol., 14, 174 (1973).
6. J. R. Preer, J. Immunol., 77, 52 (1956).

USE OF A HIGHLY SENSITIVE ELECTROPHORESIS—PRECIPITATION METHOD TO DETECT VIRUS HEPATITIS B ANTIGEN IN HUMAN BLOOD SERUM

S. A. Grannikova and P. Z. Budnitskaya

UDC 616.36-002.14-022.6-078.734:543.545

A modification of the highly sensitive method of electrophoresis—precipitation in polyacrylamide gel for the detection of virus hepatitis B antigen (HBsAg) and antibodies against it is described. The method completely preserves the specificity of immunodiffusion in gel, but is 1000-2000 times more sensitive for the detection of HBsAg. The method has given good results with the sera of patients with various diseases. It detects HBsAg more efficiently than other methods used previously.

KEY WORDS: *1 antigen of hepatitis B (HBsAg); electrophoresis—precipitation method.*

The antigen of virus hepatitis B (Australian antigen HBsAg) is nowadays universally tested for in blood service establishments in order to detect carriers among donors, and in clinical and diagnostic laboratories, hemodialysis units, and so on.

The sensitivity of the methods used to detect HBsAg (double immunodiffusion in gel and counterimmunoelectroosmophoresis) is too low to guarantee completely the absence of HBsAg in the sera tested. The development of highly sensitive methods for the determination of HBsAg and of antibodies against it is thus an urgent matter.

In this paper a modification of the method of electrophoresis precipitation in polyacrylamide gel (EPPG) for the determination of HBsAg is described.

The EPPG method suggested by Abelev [1] combines the resolving power and specificity of the immunodiffusion method with the sensitivity of the radioimmunological test. The method is based on concentration of the test antigen in an interrupted buffer system followed by its detection by a precipitating test system, whereby the samples detected can be identified. The sensitivity of the method for α-fetoprotein lies between $3 \cdot 10^{-4}$ and $6 \cdot 10^{-4}$ μg/ml.

In the present writers' variants the method cannot be used to detect HBsAg because of the large size of its molecules: High-molecular-weight HBsAg will not penetrate into finely porous polyacrylamide gel; the method had therefore to be modified by using agarose gel instead of polyacrylamide gel.

The method consisted of two stages: concentration of HBsAg, development of the antigen with an immunological diagnostic serum for HBsAg, detection of invisible precipitates by ^{125}I-labeled antigammaglobulin, followed by autoradiography of the precipitate. Changes were introduced into the first and second stages: concentrations and immunodiffusion detection of the antigen, in which the polyacrylamide gel was replaced by 0.8 and 1% agarose.

The reaction is carried out in the flat chamber (30 × 90 × 1 mm) of an apparatus for vertical electrophoresis in gel, full details of which were described earlier [1]. For

Scientific Experimental-Production Group, Laboratory of Cellular Immunity, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 4, pp. 508-509, April, 1977. Original article submitted July 29, 1976.