Radioimmunoassay, Complement Fixation and Counter-Immunoelectrophoresis in the Laboratory Diagnosis of Hepatitis B

By

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Summary

Three hundred and fifty specimens from a selected group of hospital patients were tested for the presence of hepatitis B antigen (HB-Ag) by radioimmunoassay (RIA), micro-complement fixation test (CFT) and counter-immunoelectrophoresis (CIEP).

RIA detected 82 (23.7 per cent), CFT 63 (18 per cent), and CIEP only 43 (12.2 per cent) HB-Ag positive sera.

All sera found positive by CFT and CIEP were also positive by RIA. No serum was found positive by CFT or CIEP that was negative by RIA.

The problem of anticomplementary activity was solved by absorbing the sera with fluorocarbon instead of guinea pig complement.

For our selected group, RIA was the most sensitive method for the detection of HB-Ag in human sera, followed by CFT and CIEP.

1. Introduction

The presence of HB-Ag in the blood (serum or plasma) of an individual with hepatitis B can be detected by established laboratory techniques with varying degrees of sensitivity (HOLPER and JAMBAZIAN, 1971; KELEN, HATHAWAY and McLEOD, 1971; ABU-ZARRA et al., 1971). At present, the most commonly used laboratory tests for detecting HB-Ag with different degrees of sensitivity are the counter-immunoelectrophoresis (CIEP) and complement fixation test (CFT). Recently, a sensitive simplified method of radioimmunoassay (RIA) was developed

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by Abbott Laboratories (Miller and Mordecai, 1972). We studied these three methods in order to compare their sensitivity and usefulness in a Diagnostic Laboratory.

2. Materials and Methods

2.1. Antisera

The antisera used in this study were bought from Abbott. These antisera were prepared in guinea pigs by injection of purified and concentrated HB-Ag by a process similar to that employed by Gerin, Purcell, Hoggan et al. (1969). Specificity of these sera was confirmed (Holper and Jambazian, 1971).

2.2. Counter-Immunoelectrophoresis (CIEP)

CIEP was performed by the method used by Gocke and Howe (1970) and modified as follows: Double columns of wells of two different sizes were cut in agarose glass plates of a size 8 x 10 cm. The large wells (3.0 mm in diameter) were cut for the antigen and the smaller wells (2.5 mm in diameter) for the antiserum. The wells were filled with a fine drawn Pasteur pipette.

The smaller wells were always placed towards the anode since the antiserum, as a gamma globulin, will migrate towards the cathode. One hundred and ten Volts were applied for 30 minutes, resulting in a current of approximately 25 milliamperes per 8 x 10 cm plate.

2.3. Complement Fixation Test (CFT)

The micro-method of Sever (1962) was followed using two units of complement, four units of antigen, four units of antibody and overnight fixation. A titer of 1:8 or higher was considered positive. As quality control, several dilutions of the test samples were examined in order to rule out the prozone effect that sera with very high titers may exhibit.

2.4. Removal of Anticomplementary Activity (AC)

The problem of AC activity in our laboratory was solved by treating the sera with either fluorocarbon (FC) or guinea pig (GP) complement (Kouroupis and Leers, 1971; Hummeler and Ketler, 1958). The FC treatment consisted of one volume of dichlorodifluoromethane (FC) to one volume of specimen serum. After thorough mixing, the serum was incubated at 37°C in a water bath for two hours and thereafter refrigerated at 4°C overnight. The following morning, the serum was left at room temperature for 30 minutes and was then inactivated at 56°C for an additional 30 minutes. The serum was then ready to be used.

Guinea pig (GP) complement treatment was performed in the same way, except that three volumes of complement were used instead of one.

2.5. Radioimmunoassay Test (RIA)

The two step direct solid-phase radioimmunoassay technique (Austria-125, Abbott) was employed for the detection of HB-Ag.

A pool of guinea pig HB antibody labelled with 125I, and polypropylene test tubes coated with non-radioactive HB antibody were used. The method is based upon the principle of solid-phase radioimmunoassay reported by Catt and Treager (1967). The steps in this procedure are as follows: 0.1 ml of patient's test serum was pipetted into a test tube coated with non-radioactive HB antibody and incubated at room temperature for 16 hours. Then the contents were aspirated and the tubes washed five times with 2 ml of 0.01 M Tris-(hydroxymethyl) aminomethane buffer, pH 7.1. 125I-labelled HB antibody (0.1 μCi) was then added in 0.1 ml amounts to each tube. After incubation at room temperature for 90 minutes, the contents were aspirated and the tube washed five times with Tris-buffered solution.

The counts per minute (CPM) of the tested sera were carried out in a well-type gamma ray scintillation detector Logic III-B (Abbott) with seven negative and three

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Abbott Laboratories, North Chicago, Illinois, U.S.A.