

## A RAPID SLIDE TEST FOR HETEROPHILE ANTIBODY IN INFECTIOUS MONONUCLEOSIS

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SINCE the discovery by Paul and Bunnell<sup>1</sup> in 1932 of the presence of heterophile antibodies in the sera of patients with infectious mononucleosis, there has been a great deal of investigation into the nature and production of anti-sheep red cell agglutinins.<sup>2</sup> With the advent of the Rh factor and the subsequent discovery of blocking<sup>3</sup> (incomplete,<sup>4</sup> or hyperimmune<sup>5</sup>) antibody new avenues of approach were opened to many perplexing problems concerning red cell antigenicity.

In 1945, Levine and Gilmore<sup>6</sup> reported the discovery of a blocking antibody in the serum of a patient with infectious mononucleosis. At this time attempts by one of us (W. C. M.) to disclose heterophile blocking antibody, using sheep and goat cells, were unsuccessful.<sup>7</sup> However, this work was carried out in England while in the Army Medical service and comparatively few cases were studied; moreover, the proper breed of goat was not obtainable. When knowledge of Diamond's slide method for Rh testing became available in June 1945, a modification of this test was employed to further search for the presence of blocking antibody in the sera of cases of infectious mononucleosis and other diseases. For the past three years this work has been carried out more extensively in civilian practice and since the slide method may have practical applications, its use is reported in this paper.

### METHODS AND MATERIALS

The test was carried out by mixing 0.1 cc. of defibrinated sheep blood on a glass slide with 0.2 cc. of serum to be tested. Tests were considered positive *only if 3 plus to 4 plus macroscopic clumping occurred within 30 to 60 seconds*. The heterophile antibody test was carried out on the same sera using the Paul-Bunnell method.<sup>1</sup> A serum dilution of 1:128 was considered the lowest positive level. The sheep cells were preferably used fresh but defibrinated sheep blood kept at 5 C for two weeks gave reliable tests. Citrated, phenolized and 50 per cent saline sheep cell suspensions also gave good results with *strongly* positive sera. However, to avoid factors which might interfere with blocking antibody, only defibrinated sheep blood was used in the slide tests reported in this paper. Serum was obtained in the usual fashion, inactivation was carried out in a number of cases but for practical purposes this was found to be unnecessary. Sera kept in the icebox lost potency slowly, while if stored in the deep freeze the heterophile antibody content was well preserved for long periods. The amounts of serum and cells used in the test made a definite difference in the agglutination reaction. A 2 to 1 proportion of serum to cells was found to give the most clear-cut test. All slide tests were carried out at room temperature. As described below, the heterophile antibody in infectious mononucleosis is active at 37 C as well as at lower temperatures. In certain cases of cirrhosis and patients with hemolytic syndromes, the antibody which gave a positive slide test at room temperature was inactive when the test was carried out at 37 C.

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## RESULTS

Tests were carried out on the sera of 473 individuals with various diseases and in this group were included a number of normal controls.

*Infectious Mononucleosis.* In this group there were 41 patients with definite hematologic and clinical evidence of the disease (see table 1).

Of these 41 cases, 34 were serologically positive by the Paul-Bunnell test at some time during the course of the illness. There were 6 cases in which repeated heterophile antibody titers were 1:8 or below and the slide tests were also negative in these individuals. In one case the heterophile antibody titer was 1:64 and at the same time the slide test was positive, otherwise the remaining 34 cases had titers of 1:128 or above with strongly positive slide tests. It was observed that in

TABLE 1.—*Infectious Mononucleosis*

Diagnosis	Total cases	Positive slide test	Positive Paul-Bunnell test
Infectious Mononucleosis . . . . .	41	35	34

TABLE 2.—*Diseases of the Liver*

Diagnosis	No. of cases	Positive slide test	Positive Paul-Bunnell test
Cirrhosis (Laennec) . . . . .	43	6*	0
Cirrhosis (biliary) . . . . .	5	0	0
Cirrhosis (post infectious hepatitis) . . . . .	5	1*	0
Acute infectious hepatitis . . . . .	21	0	0
Total . . . . .	74	7	0

\* Positive at room temperature, negative at 37 C.

following the disease along, as the heterophile titer in saline fell below 1:128 the slide tests became negative.

*Diseases of the Liver.* The sera of 53 patients with cirrhosis of the liver and 21 patients with acute infectious hepatitis were tested (see table 2).

In 6 cases of cirrhosis of the Laennec type and one of cirrhosis following infectious hepatitis, positive slide agglutinations of sheep cells occurred. However, in none of these cases were sheep cell agglutinins by the Paul-Bunnell method present in a dilution of 1:8 or above. On carrying out the slide test at 37 C, the agglutination disappeared. This is in contrast to the sheep cell agglutinins found in infectious mononucleosis which are active at 37 C as well as lower temperatures. It was concluded that the sheep cell clumping observed on the slide at room temperature in these cases was due to nonspecific cold agglutination.

Although most of the cases of infectious mononucleosis in this series gave positive cephalin-cholesterol flocculation, thymol turbidity and ZnSO<sub>4</sub> turbidity tests, there was no apparent correlation between the presence of heterophile anti-

body (either by slide test or by the Paul-Bunnell method) and the occurrence of these tests which indicate an alteration of the serum proteins. In keeping with the observation of others, positive heterophile antibody tests were not found in the cases of infectious hepatitis.

*Malignant Diseases.* The sera of 58 patients with a variety of neoplastic disorders were examined for heterophile antibodies (see table 3).

In only one case was a positive slide test observed. This occurred in a patient with multiple myeloma but subsequently repeated tests on the same patient were negative. Sera from other patients with multiple myeloma have shown no increase in heterophile antibodies nor have slide tests been positive.

TABLE 3.—*Malignant Diseases*

Diagnosis	No. of cases	Positive slide test	Positive Paul-Bunnell test
Cancer.....	40	0	0
Malignant lymphoma.....	5	0	0
Leukemia.....	7	0	0
Multiple myeloma.....	6	1*	0
Total.....	58	1	0

\* Nature of antibody not known.

TABLE 4.—*No mal Pregnancy and Cord Blood*

Diagnosis	No. of cases	Positive slide test	Positive Paul-Bunnell test
Pregnancy.....	95	0	0
Cord blood.....	23	0	0
Total.....	118	0	0

*Normal Pregnancy and Cord Blood.* The sera of pregnant women may show various positive flocculation tests. However, in 95 patients during pregnancy and in 23 cord blood specimens there were no positive heterophile antibody tests (see table 4).

*Hemolytic Disorders and Iso-immunized Women.* This group of patients deserves special consideration and further studies are being carried out (see table 5).

In 7 patients with acquired hemolytic anemia there were 2 cases which gave positive slide tests. These 2 individuals had no increase in heterophile antibody by the Paul-Bunnell test. When the slide test was carried out at 37C, no agglutination occurred. These two patients had very strong cold autohemagglutinins. Both had undergone splenectomy without improvement and subsequently one patient died and was found to have a bizarre myeloblastic leukemia. The other patient survived but has continued to show hemolytic anemia and no further underlying disease has been disclosed to date.

In the sera of 10 women heavily immunized in pregnancy by the Rh factor there were no heterophile antibodies found. There were 3 women strongly immunized

by fetal A<sub>1</sub> cells and one woman with anti-B agglutinins giving a positive serum dilution of 1:60,000. In none of these women were there positive heterophile antibody tests. However, a patient who is still under investigation has been of considerable interest. After this woman gave birth to her second baby the infant developed moderately severe hemolytic disease. The mother was O, Rh positive, the father was also Rh positive, A<sub>1</sub> A<sub>2</sub>—and the infant was A<sub>2</sub> O, Rh positive.\* The mother developed an Anti A<sub>2</sub> agglutinin which reached a positive dilution of 1:50,000. This serum also gave a positive slide test for heterophile antibody which did not disappear at 37 C and the Paul-Bunnell test showed a borderline

TABLE 5.—*Hemolytic Syndromes and Iso-immunized Women*

Diagnosis	No. of cases	Positive slide test	Positive Paul-Bunnell test
Acquired hemolytic anemia . . . . .	7	2*	0
Anti Rh agglutinins . . . . .	10	0	0
Anti A <sub>1</sub> agglutinins . . . . .	3	0	0
Anti B agglutinins . . . . .	1	0	0
Anti A <sub>2</sub> agglutinins . . . . .	1	1	1†
Total . . . . .	22	3	1

\* Became negative at 37 C.

† Border line positive dilution.

TABLE 6.—*Miscellaneous Diseases and Controls*

Diagnosis	No. of cases	Positive slide test	Positive Paul-Bunnell test
Miscellaneous . . . . .	50	0	0
Controls . . . . .	110	0	0
Total . . . . .	160	0	0

positive dilution of 1:64. On absorption tests the antibody was absorbed by guinea pig kidney but not by boiled beef cells. This antibody was apparently related to the Forssman type rather than the variety of sheep cell agglutinins found in infectious mononucleosis.

*Miscellaneous Diseases and Controls.* Tests were carried out on the sera of patients with a variety of diseases. In this group were included 3 cases of serum sickness. None of these had positive heterophile tests although it should be expected that if strong enough, the heterophile antibodies of the Forssman type would give positive slide tests. Unfortunately, the only tests on these three cases were carried out on the 1st or 2nd day of the illness and later specimens of serum were not obtained for testing (see table 6).

In the sera of 110 normal individuals, there were no false positive tests.

\* The genotypes and specificity of the anti-A sera were kindly determined by Dr. William Boyd of Boston University Medical School.

## SUMMARY AND CONCLUSIONS

The sera of 473 individuals were examined for sheep cell agglutinins both by the slide test and the Paul-Bunnell method. In this group there were 46 patients with positive slide tests and 35 of these individuals also had a diagnostic serum dilution test for heterophile antibody. In 11 cases the slide test was positive but the Paul-Bunnell test gave very low serum dilution values. However, when the slide test was carried out at 37 C, it was negative in 9 of the 11 cases. In the remaining 2 instances, one patient had a Forssman type of antibody which gave a 1:64 titer in saline and the slide test was positive at 37 C. In the other case no studies were made on the effect of temperature and the nature of the agglutination reaction was unfortunately not determined.

Using human and bovine albumen, sheep serum and human AB serum absorbed with sheep cells as a diluent no evidence for blocking or hyperimmune antibody was discovered in the cases of infectious mononucleosis studied in this series. Moreover, of the 6 patients with negative serology but with strong clinical and hematological evidence for the disease, no blocking or hyperimmune antibody was disclosed by the slide test or by the use of absorbed human AB serum. The conclusion seems justified that blocking, incomplete or hyperimmune heterophile antibody must be rather uncommon in infectious mononucleosis.

In the use of the rapid slide test it has been pointed out that cold agglutinins, (which may be abolished by warming to 37 C) and Forssman antibodies (which may be absorbed by guinea pig kidney) can give positive results. However, diseases in which cold agglutinins are strong enough to give a positive slide test are relatively rare and the occurrence of Forssman antibodies of a strength likely to give a positive slide test would be decidedly uncommon. In any event unless further experience reveals more serious discrepancies, the rapid slide test as described in this paper seems to offer a practical *screening* test to detect clinically significant amounts of heterophile antibody in cases of infectious mononucleosis.

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