

A comparison of the Monospot with the Paul-Bunnell test in infectious mononucleosis and other diseases

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SYNOPSIS The Monospot is a spot test designed for the diagnosis of infectious mononucleosis and its efficacy has been compared with that of the standard Paul-Bunnell test. Three out of 210 (1.4%) sera from normal persons and persons suffering from diseases other than infectious mononucleosis gave 'false' positive results when compared with the Paul-Bunnell test. Using 38 sera from patients with strong clinical and haematological evidence of infectious mononucleosis no false negative results were found with the Monospot test. The sera of 37 patients gave positive results with the Paul-Bunnell test: the one negative result was positive using serum taken a few days later.

Sheep red cells have been widely used as an aid to the diagnosis of infectious mononucleosis ever since the description of heterophile antibodies by Paul and Bunnell in 1932. However, sheep red cells are by no means the only animal cells that will react with these antibodies. It has been shown, for instance, that horse, goat, and ox red cells are also agglutinated by the same antibody.

In 1964 Wilkinson and Carmichael demonstrated that in the course of infectious mononucleosis the agglutinin titres in the serum were higher with horse red cells than with sheep red cells. The same observation had been made 28 years earlier by Beer (1936). Lee, Davidsohn, and Slaby published further work on horse red cell agglutinins in 1968. They confirmed the findings of Wilkinson and Carmichael (1964) but they showed that there was considerable overlap in the agglutinin titre against horse red cells when the reactions of sera from patients suffering from infectious mononucleosis were compared with the reactions of sera from normal people. However, if absorptions with guinea-pig kidney and ox red cells were done the expected results were obtained, *ie*, only the ox red cells removed the antibody against horse red cells from the serum of patients suffering from infectious mononucleosis, while both absorbants removed the antibody from the serum of patients suffering from other diseases. Thus the two groups of sera could be clearly differentiated. The horse red cells, moreover,

being more sensitive, would detect antibody earlier in the disease than sheep red cells.

With the observation that citrated red cells remain usable for many months it became unnecessary to treat the cells with formalin, and in 1968 Lee, Davidsohn, and Panczyszyn introduced a spot test for the diagnosis of infectious mononucleosis. This test uses citrated horse red cells for the final agglutination and a very fine suspension of guinea-pig kidney and ox red cells for the preliminary absorptions. The absorption suspensions are so fine that they do not obscure the agglutination and the patient's serum can be absorbed and then tested without centrifugation.

The test is available commercially and is called the Monospot test. This investigation was designed to assess the sensitivity and specificity of this test.

MATERIALS

SERA FROM PATIENTS KNOWN TO BE SUFFERING FROM INFECTIOUS MONONUCLEOSIS The diagnosis had been made on clinical and haematological criteria and was confirmed by a positive result with the conventional Paul-Bunnell test. The unabsorbed serum agglutinated sheep red cells to titres of 40 to 1,280 and the expected results were obtained after absorption.

SERA FROM NORMAL PEOPLE Sera from 100 normal people were used. They were mainly taken from members of the hospital staff, and also from blood donors.

SERA FROM PATIENTS WITH OTHER DISEASES Sera from

60 patients suffering from diseases known to be associated with abnormal antibody production were used. Table I gives the individual diseases. Sera from 50 children with cervical adenitis but without atypical lymphocytes and with a negative Paul-Bunnell reaction were also used.

TABLE I

PATIENTS WITH OTHER DISEASES

Disease Tested with Monospot	No. of Patients
Systemic lupus erythematosus	3
Lymphosarcoma with high anti-i titre	3
Autoimmune haemolytic anaemia of the warm antibody type	15
Cold haemagglutinin disease due to:	
(a) <i>Mycoplasma pneumoniae</i> infection	3
(b) Chronic cold haemagglutinin disease	5
Myeloma IgG(K)	2
Hodgkin's disease	11
Lymphosarcoma	7
Reticulum cell sarcoma	3
Chronic myeloid leukaemia	2
Chronic lymphatic leukaemia	5
Serum hepatitis	1
Total	60

METHOD OF TESTING

THE MONOSPOT TEST The reagents were used exactly as instructed. It is particularly important not to move the slide during the test otherwise agglutination may occur both with the serum absorbed with guinea-pig kidney and the serum absorbed with ox cells, rendering the test unreadable. This may be due to detaching the antibody against horse cells from the absorbants.

THE PAUL-BUNNELL TEST This was done by a standard technique (Dacie and Lewis, 1963).

RESULTS

PATIENTS SUFFERING FROM INFECTIOUS MONONUCLEOSIS

The results of testing 38 infectious mononucleosis sera are shown in Table II. All the sera gave positive results within one minute using the Monospot test.

NORMAL CONTROLS The results of testing sera from normal people or from patients with diseases other than infectious mononucleosis are shown in Table III. The serum from one of the normal people gave a clearly positive result with the Monospot test after 40 seconds. A second person gave a doubtful result at 60 seconds. Both these sera were from members of the hospital staff. In the first case there was a history of acute febrile upper respiratory infection three weeks before complicated by sinusitis. At the time the blood was taken the blood picture showed 9,000 white blood cells per cmm with 82% neutrophils. No lymph nodes were palpable at any stage of the disease but it is difficult to rule

TABLE II

SERA FROM KNOWN CASES OF INFECTIOUS MONONUCLEOSIS

Case No.	Monospot	Time (sec)	Sheep Cell Agglutinins to Unabsorbed Serum (titre)
1	+ve	5	320
2	+ve	3	640
3	+ve	3	1,280
4	+ve	5	320
5	+ve	10	320
6	+ve	10	160
7	+ve	5	640
8	+ve	40	80
9	+ve	3	1,280
10	+ve	3	320
11	+ve	5	320
12	+ve	30	80
13	+ve	20	320
14	+ve	10	320
15	+ve	30	160
16	+ve	10	640
17	+ve	20	320
18	+ve	40	40
19	+ve	30	160
20	+ve	30	80
21	+ve	10	160
22	+ve	3	640
23	+ve	5	640
24	+ve	5	320
25	+ve	5	320
26	+ve	5	1,280
27	+ve	20	320
28	+ve	5	160
29	+ve	5	320
30	+ve	5	1,280
31	+ve	10	320
32	+ve	5	320
33	+ve	3	320
34	+ve	3	640
35	+ve	3	320
36	+ve	3	320
37	+ve	5	320
38	+ve	3	640

TABLE III

KNOWN NON-INFECTIOUS MONONUCLEOSIS SERA

	No. of Cases	Positive	Doubtful	Negative
Normal controls	100	1	1	98
Various diseases	110	1	—	109

out infectious mononucleosis entirely. The Paul-Bunnell test was negative and the subject's serum agglutinated sheep red cells to the following titres: unabsorbed serum 160, absorbed with guinea-pig kidney 20, absorbed with ox cells 20. In the second case there was no history of illness in the previous six months. The Paul-Bunnell test was again clearly negative and the agglutinin titres were as follows: unabsorbed serum 80, absorbed with guinea-pig kidney 10, absorbed with ox cells 10. Three months later, serum from the first subject again gave a positive result with the Monospot test and a negative with the Paul-Bunnell test. Serum from the second subject gave a negative result with both tests when the test was repeated three months later.

PATIENTS WITH OTHER DISEASES One serum from a patient suffering from serum hepatitis gave a positive result with the Monospot test within 20 seconds. The Paul-Bunnell test was negative and agglutinated sheep red cells to the following titres: unabsorbed serum 320, absorbed with guinea-pig kidney 40, absorbed with ox cells 80 (Table III).

DISCUSSION

A firm diagnosis of infectious mononucleosis still requires the detection of specific heterophile antibodies in addition to the clinical and haematological findings associated with this disease. The Monospot test for the detection of these antibodies has the advantages of simplicity and speed. It can be done by staff with very little training provided the instructions are followed exactly. It gave clear-cut positive results in all those patients who were known to have a positive Paul-Bunnell test, including one patient whose serum gave, initially, a negative

test. The serum from this patient, who had strong clinical and haematological evidence of infectious mononucleosis, gave a positive Paul-Bunnell test five days later.

Only three out of 210 control sera (1.4%) gave 'false' positive results with the Monospot test when compared with the Paul-Bunnell test. No 'false' negative results were obtained. The horse cells still gave satisfactory results after two months' storage.

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