

Horse agglutinins in infectious mononucleosis¹

III Criterion for differential diagnosis

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SYNOPSIS One hundred infectious mononucleosis and the same number of non-infectious mononucleosis sera were studied to evaluate the sensitivity and specificity of horse erythrocytes in the diagnosis of infectious mononucleosis. Titres of horse agglutinins in infectious mononucleosis sera ranged from 28 to 7,168 with a geometric mean of 550, whereas the corresponding sheep agglutinin titres ranged from less than 7 to 3,584, with a geometric mean of 126. Horse agglutinin titres of non-infectious mononucleosis sera ranged from less than 7 to 896, with a geometric mean of 59.

Infectious mononucleosis sera tested with horse erythrocytes before and after absorption with guinea pig kidney and beef erythrocytes showed patterns different from those of non-infectious mononucleosis sera, even when sheep agglutinin titres were too low for satisfactory evaluation.

After absorption with guinea pig kidney, horse agglutinin titres of infectious mononucleosis sera were uniformly higher than in the corresponding serum absorbed with beef erythrocytes. This was not the case for non-infectious mononucleosis sera.

Results confirm our previously expressed view that horse erythrocytes are preferable to sheep erythrocytes in the serological diagnosis of infectious mononucleosis.

In a previous study of 27 infectious mononucleosis and 26 non-infectious mononucleosis sera, we found that horse erythrocytes are highly specific for diagnosis if sera are tested before and after absorption with guinea pig kidney and beef erythrocytes (Lee, Davidsohn, and Slaby, 1968). We also confirmed the observation of others (Stuart, Griffin, Wheeler, and Battey, 1936; Beer, 1936; Barrett, 1941; Wilkinson and Carmichael, 1964) that horse erythrocytes are more sensitive than sheep erythrocytes. To evaluate our preliminary findings, 100 each of infectious and non-infectious mononucleosis sera were tested with horse erythrocytes. Based on the analysis of these results, we established in this report a simple criterion for the differential diagnosis of infectious mononucleosis.

MATERIALS AND METHODS

INFECTIOUS MONONUCLEOSIS SERA One hundred sera from 75 patients with clinical, haematological, and serological findings compatible with the diagnosis of infectious mononucleosis were studied. Since we were particularly interested in sera with low titres, not all specimens with high sheep agglutinin titres were included in the study; hence, the 100 sera examined do not represent random samples.

NON-INFECTIOUS MONONUCLEOSIS SERA One hundred sera were taken from three sources: 34 from sera submitted for serological testing for infectious mononucleosis and found to be negative in the differential tests with sheep erythrocytes; 29, including 13 plasma specimens and 16 sera from random samples of 300 each, negative in the spot test (Lee, Davidsohn, and Panczyszyn, 1968) but showing agglutination of horse erythrocytes within one minute; 37 from 500 random specimens with horse agglutinin titres of 56 or higher in the screening test.

All other materials and the methods used were essentially the same as previously reported (Lee *et al.*, 1968).

¹Two previous papers 'Horse agglutinins in infectious mononucleosis' by C. L. Lee, I. Davidsohn, and R. Slaby, and 'Horse agglutinins in infectious mononucleosis, II, The spot test' by C. L. Lee, I. Davidsohn, and O. Panczyszyn, were published in *Amer. J. clin. Path.*, **49**, 3-11 and 12-18, 1968.

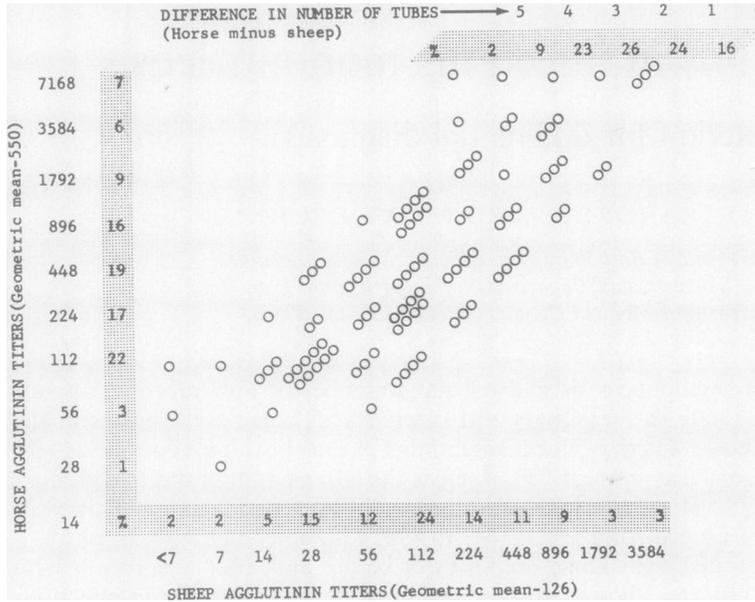


FIG. 1. Comparison of horse and sheep agglutinin titres of 100 infectious mononucleosis sera. Each O represents one serum orientated with its horse agglutinin titre on the ordinate and sheep agglutinin titre on the lower abscissa. Identical titres of horse agglutinins are plotted at the same horizontal levels, and their total numbers (= %) are entered on the shaded vertical column. The titres of sheep agglutinins are arranged vertically and their total numbers are in the shaded lower horizontal zone. The differences in titres (horse minus sheep) are expressed in number of tubes; all sera having the same tube difference are distributed along the same oblique line, and their total numbers are listed in the shaded upper zone.

RESULTS

ONE HUNDRED INFECTIOUS MONONUCLEOSIS SERA All sera were tested with sheep and horse erythrocytes before and after absorption with guinea pig kidney and beef erythrocytes. Horse and sheep agglutinin titres before absorption are listed in Figure 1. Horse agglutinin titres ranged from 28 to 7,168 with a geometric mean of 550. The one serum with a titre of 28 was taken four months after the patient recovered from his illness; only three sera had a titre of 56; the remaining 96 sera had titres of 112 or higher. The corresponding sheep agglutinin titres ranged from less than 7 to 3,584 with a geometric mean of 126 which is less than one-fourth of that of the horse agglutinin titres; twenty-four had titres of 28 or lower. Horse agglutinin titres were equal to sheep agglutinin titres in 16 sera, between one to three tubes higher in 73 sera, four to five tubes higher in 11 sera, and none was lower.

Both horse and sheep agglutinin titres decreased by not more than three tubes after absorption with guinea pig kidney; they decreased by four or more tubes after absorption with beef erythrocytes, except in sera having titres of three tubes or less before absorption in which a complete absorption was the rule. This pattern is essentially the same as that established with sheep erythrocytes (Davidsohn, 1938). When horse erythrocytes were used, titres after absorption with guinea pig kidney were always

higher than titres after absorption with beef erythrocytes, regardless of the titres before absorption.

In Table I are listed horse and sheep agglutinin titres of 18 sera from eight patients with infectious mononucleosis. During the course of their illness each patient had once or twice sheep agglutinin titres of 14 or lower which is not diagnostic; at other times

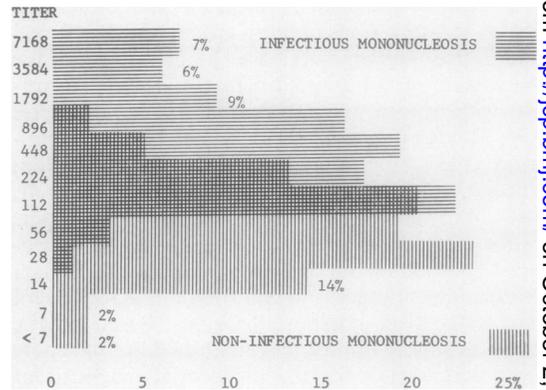


FIG. 2. Distribution of horse agglutinin titres of 100 each infectious and non-infectious mononucleosis sera. Titres between 28 and 896 are found in both types of sera; only 22 infectious mononucleosis sera had titres higher than 896 and only 18 non-infectious mononucleosis sera had titres lower than 28. The 200 sera selected for the study of differential absorption were not random samples.

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TABLE I
HORSE AGGLUTININ TITRES OF SERA HAVING SHEEP
AGGLUTININ TITRES OF 14 OR LOWER

Patient's Initials	Date of Specimen	Horse RBC			Sheep RBC		
		TBA ¹	TAAGPK ²	TAABE ³	TBA	TAAGPK	TAABE
<i>Infectious Mononucleosis</i> ⁴							
D.B.	14-2-66	56	7	<7	<7	<7	<7
	22-2-66	1,792	896	28	224	112	<7
B.H.	10-11-61	224	112	<7	28	14	<7
	27-11-61	112	56	<7	<7	<7	<7
H.S.	13-6-66	112	28	<7	7	<7	<7
	20-6-66	3,584	896	7	896	448	<7
C.L.	24-4-63	7,184	7,184	28	3,584	3,584	28
	24-5-63	896	448	<7	896	448	<7
	5-11-63	28	14	<7	7	<7	<7
R.C.	1-2-66	112	56	<7	14	7	<7
	7-2-66	896	896	56	448	224	7
P.D.	9-3-66	224	112	<7	14	7	<7
	16-3-66	224	112	<7	112	56	<7
G.M.	13-4-66	56	28	<7	14	<7	<7
	20-4-66	112	56	<7	14	7	<7
	2-5-66	56	28	<7	56	7	7
T.S.	5-8-66	112	112	<7	112	56	<7
	16-8-66	112	112	<7	14	<7	<7
<i>Non-infectious Mononucleosis</i>							
A.C.	10-6-66	112	<7	56	<7	<7	<7
B.H.	7-7-66	56	7	28	<7	<7	<7
C.U.	3-11-66	14	<7	<7	<7	<7	<7
C.G.	22-7-67	14	<7	7	<7	<7	<7
C.S.	14-8-67	112	56	112	7	<7	<7
E.A.	14-6-66	56	<7	28	7	<7	<7
F.G.	4-11-66	112	7	56	7	<7	<7
M.E.	27-6-67	224	<7	14	7	<7	<7
M.N.	8-12-67	14	<7	<7	7	<7	<7
T.F.	9-8-67	28	<7	<7	14	<7	<7
I.L.	12-4-60	56	7	14	14	<7	7
	17-4-60	28	<7	7	14	<7	<7
	22-4-60	28	<7	7	14	<7	14
K.A.	10-10-66	112	7	56	14	<7	<7
J.R.	11-5-66	56	7	56	14	<7	<7
T.S.	7-8-67	112	14	56	14	<7	<7

¹Titres before absorption

²Titres after absorption with guinea pig kidney

³Titres after absorption with beef erythrocytes

⁴Titres of nine sera from the same eight patients with sheep agglutinin titres higher than 14 were included to show that the sheep differential test was definitely positive at other times (except patient G.M.).

apparently closer to the peak of antibody response, the sheep differential test was definitely positive with the exception of patient G.M. The differential absorption test with horse erythrocytes was positive in each of these 18 sera.

ONE HUNDRED NON-INFECTIOUS MONONUCLEOSIS SERA
Horse agglutinin titres of 100 sera ranged from less than 7 to 896, with a geometric mean of 59. Distribution of these titres and those of 100 infectious mononucleosis sera are listed in Figure 2. Titres from 28 to 896 were found in 78% of infectious and 82% of non-infectious mononucleosis sera.

When formalin-fixed horse erythrocytes were used, they were agglutinated only by sera with titres of 112 or higher, infectious or non-infectious mononucleosis.

Changes in horse agglutinin titres of non-infectious mononucleosis sera after absorption with guinea-pig kidney and beef erythrocytes were evaluated in two ways. (1) By comparing the titres of the same serum before and after absorption with guinea-pig kidney and beef erythrocytes; non-infectious mononucleosis sera did not follow the pattern found in infectious mononucleosis sera, *ie*, three-tube maximum after guinea-pig kidney and four-tube minimum after beef erythrocytes absorption. Instead, 97 non-infectious mononucleosis sera exhibited a reduction of less than four tubes after absorption with beef erythrocytes. (2) When the titres of only the absorbed sera were compared, it was found that, in contrast to infectious mononucleosis sera, not a single non-infectious mononucleosis serum had, after absorption with guinea-pig kidney, a titre higher than that

after absorption with beef erythrocytes.

Horse agglutinin titres of 16 non-infectious mononucleosis sera with sheep agglutinin titres of 14 or lower are compared with those of infectious mononucleosis sera in Table I. The pattern of horse agglutinin titres in each of these 16 sera was in accord with the criterion described above for non-infectious mononucleosis sera, and different from that in infectious mononucleosis sera listed in the same table. This does not apply to the corresponding sheep agglutinin titres.

DISCUSSION

Our data confirm previous observations that horse erythrocytes are agglutinated by higher dilutions of infectious as well as non-infectious mononucleosis sera than sheep erythrocytes (Barrett, 1941). Barrett gave up his attempt to use horse erythrocytes for the diagnosis of infectious mononucleosis because of the high titres seen also in non-infectious mononucleosis sera. Horse erythrocytes treated with formalin continued to be agglutinated unselectively by infectious and non-infectious mononucleosis sera with titres of 112 or higher. The fact that they were no longer agglutinated by sera with titres of 56 or lower was due not to increased specificity but to a reduction in sensitivity. To overcome this difficulty, we applied absorption with guinea-pig kidney and beef erythrocytes, used in the differential test with sheep erythrocytes (Davidsohn, 1938), to the diagnosis of infectious mononucleosis with untreated horse erythrocytes. Our results indicate that by combining the sensitivity of horse erythrocytes with the specificity of absorption, it is possible not only to differentiate infectious from non-infectious mononucleosis serum but also to do so at an earlier and at a later stage of illness than with sheep erythrocytes.

In the differential absorption tests with sheep erythrocytes, interpretation of results is based on differences between titres of unabsorbed serum and of serum absorbed with guinea-pig kidney and beef erythrocytes. When the sheep agglutinin titre of unabsorbed serum is 56 (four tubes) or higher, a reduction of a three-tube maximum after absorption with guinea-pig kidney and four-tube minimum after absorption with beef erythrocytes is the criterion for a positive test for infectious mononucleosis (Davidsohn, 1938). When the sheep agglutinin titre of unabsorbed serum is 28 or lower (24% of infectious mononucleosis sera in our series),

this criterion can no longer be applied. However when horse erythrocytes were used, the same criterion could be employed in 99% of infectious mononucleosis sera, missing only one serum with titre of 28. In order to achieve differentiation between 100 infectious and 100 non-infectious mononucleosis sera without exception, the following criterion recommended:

$$\begin{aligned} \text{TAAGPK} > \text{TAABE} &= \text{infectious} \\ &\text{mononucleosis} \\ \text{TAAGPK} \leq \text{TAABE} &= \text{non-infectious} \\ &\text{mononucleosis} \end{aligned}$$

- (1) TAAGPK = titre after absorption with guinea-pig kidney
 (2) TAABE = titre after absorption with beef erythrocytes

This criterion is easy to remember. It conforms with that used in our spot test which is based on the same principles and has been evaluated with a much larger number of sera (nearly 400 infectious and 700 non-infectious mononucleosis sera in our laboratory alone). It makes the testing of the unabsorbed serum superfluous, particularly since we found that the height of titres in infectious mononucleosis has no bearing on the diagnosis or prognosis of the disease. It is also consistent with the data obtained with the sheep differential test on those having titres of 28 and higher.

The spot test is adequate for routine use, because of its sensitivity, specificity, simplicity, and speed. In cases in which results of the spot tests are doubtful or not compatible with clinical and/or haematological findings, a check with the differential test in tubes is desirable. Since sheep erythrocytes may not be agglutinated by low titre sera, the higher sensitivity of horse erythrocytes prompted us to use them instead of sheep erythrocytes.

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