Quantitation of a slide test (Monotest) for infectious mononucleosis

P. KENNETH CARTER¹, IRWIN SCHOEN, AND TERU MIYAHIRA

From the Division of Pathology, Cedars-Sinai Medical Center, Mount Sinai Hospital Division, Los Angeles, and Student Health Service Clinical Laboratory, University of California at Los Angeles, California, USA

SYNOPSIS A slide test for infectious mononucleosis using formalinized horse erythrocytes (Monotest²) was quantitated and compared with standard differential heterophile (Davidsohn) titres performed on the same specimens. The Monotest titre parallels the standard presumptive heterophile (antisheep cell) titre in the degree of elevation, with a ratio of Monotest to heterophile titre of approximately 1 to 56. The simplicity of the quantitative slide test recommends it as a routine test for infectious mononucleosis.

A number of new slide tests using horse erythrocytes for infectious mononucleosis have recently been introduced. These have been shown to have a high degree of sensitivity and specificity, whether the horse erythrocytes are citrated (Monospot, Ortho) (Lee, Davidsohn, and Pan-czysyn, 1968) or formalin-treated (Monotest) (Hoff and Bauer, 1965; Davidsohn, 1967; Sinay, Schoen, and Miyahira, 1968; Lowell and Kazakaitis, 1969). None has mentioned quantitation of the test, and we elected to compare quantitations of the Monotest with standard differential heterophiles performed on the same serum samples.

Methods

Doubling dilutions of patients' sera in saline were prepared using 0.25 ml volumes of serum and saline and a single 1 ml plastic disposable pipette. The Monotest was then performed according to the instructions of the manufacturer. A drop of serum dilution and a drop of Monotest reagent were mixed on a glass slide and gently rotated for two minutes. A coarse 4+ agglutination was considered positive.

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¹Please send requests for reprints to Dr Carter at Mount Sinai Hospital.

²Monotest, Wampole Laboratories.

No agglutination or a finely granular pattern was considered negative. Any pattern between these two was considered weakly reactive (1+, 2+, or 3+). It was found that the last tubes in a titration proceeded from positive to negative in a manner similar to quantitative VDRLs, that is, usually after the last positive tube there was a weakly reactive tube followed by negative tubes. The titre was arbitrarily chosen as the last tube showing at least a 2+ agglutination. The tests were performed at random intervals from November 1968 to May 1969, using Monotest batch numbers 58, 64, 66, 67, 68, and 69. The differential heterophile tests were performed the same day as the Monotest, using the well known standard method of Davidsohn and Nelson (1969).

The sera studied included hospital specimens submitted for infectious mononucleosis serology at Mount Sinai Hospital; specimens from students presenting at UCLA Student Health Service with symptoms suggestive of mononucleosis; a few specimens from Loma Linda University Clinical Laboratory; and two commercial specimens of desiccated positive control sera, rehydrated according to the manufacturers' directions (Hyland, BBL). The patients' sera were all tested within 24 hours or were frozen within 24 hours of collection and thawed immediately before testing.

No attempt was made to correlate the results

No. of Specimens	Monotest Titre	Differential Heterophile Titre ¹		
		Sheep Cell	Guinea-pig Kidney	Beef Erythrocytes
23	Neg.	14	0	7
7	1:1	28	14	0
9	1:2	112	56	0
9	1:4	224	224	14
21	1:8	224	112	0
22	1:16	448	224	0
27	1:32	896	896	7
5	1:64	1792	1792	14
2	1:128	3584	3584	56
1	1:256	1792	3584	28

Table I Comparison of quantitative Monotest and differential heterophile titres in 126 specimens from patients

¹Reciprocal of most frequent titre value.

of the tests with the clinical status of the patients; our primary consideration was a comparison of the quantitative slide test with the titres of the standard method of Davidsohn and Nelson (1969).

All of the sera were inactivated at 56°C for 30 minutes before testing.

When performing differential heterophiles, we routinely use as reagent controls a positive control mononucleosis serum and a negative control commercial 'Forssman antibody' (serum sickness) serum. The latter is used to demonstrate the ability of the guinea pig antigen reagent to absorb heterophile antibodies other than those of infectious mononucleosis. Several lots of such commercial 'negative control' sera were included in the Monotest/Davidsohn comparison, and because of results to be described, the negative control sera were also tested with the Monospot test, performed according to the manufacturers' instructions (Monotest batch no. 73, Monospot batch no. IM-161).

Results

Table I presents a summary of the results. In all cases the Monotest and differential heterophile titres agreed regarding the diagnosis.

The most significant finding is that the Monotest titre generally parallels the heterophile titre. Comparison of the Monotest titre with the presumptive heterophile (antisheep cell) titre reveals a ratio of approximately 1 to 56, that is, the Monotest titre multiplied times 56 approximately equals the sheep cell titre. This value is selected, although the correlation is not perfect at all high and low titres, to enable an easy comparison of the Monotest titre values with the well known Davidsohn titre values, such that the approximate equivalent Davidsohn titre value may be reported to the physician as well as the Monotest titre, if desired.

Most of the heterophile titres were clustered

about a most frequent titre +1 dilution at each level of Monotest titre, both for the unabsorbed sheep cells and after absorption with guinea-pig kidney and beef erythrocytes. In only three instances was any titre more than two dilutions from the most frequent titre. Each of the three patients had differential test titres concordant with the Monotest result.

Table II details the 'weakly reactive' Monotest reactions of undiluted (1:1) sera observed in seven of the 126 patients. None of these seven undiluted (1:1) sera showed a 4+ reaction. Results cannot be adequately interpreted, as the clinical state of the patients was not studied in depth. However, it is apparent that occasional 'doubtful' reactions occur, as in previous studies, suggesting that the test be repeated later to demonstrate a rising (or falling) titre (Sinay *et al*, 1968).

Monotest	Heterophile	Guinea-pig Kidney	Beef RBC	Davidsohn Interpretation
3+	1:28	0	1:7	Neg.
2+	1:28	1:28	0	Pos.
3+	1:56	1:14	1:7	Pos. ¹
3+	1:56	1:28	1:14	Neg.
2+, 1+	1:14	0	0	Neg.
3+	1:28	1:14	0	Pos.
2+	1:28	1:14	0	Pos.

Table II 'Weakly reactive' Monotests

Atypical beef cell absorption perhaps due to decreased antigenicity of the beef erythrocytes.

Results of the 'negative control' sera and two batches of 'positive control' commercial sera are listed in Table III.

Both the Monotest and the Monospot tests were in agreement with the differential heterophile interpretation using these sera, with one exception. The BBL serum sickness control serum, which was negative for Monospot and Davidsohn tests, revealed a fairly strong agglutination with Monotest (1:2 and 1:4, with two batches of the control serum). The reason for this is unexplained and warrants further study, as it may represent decreased specificity of the Monotest formalinized horse cells.

Batch No.	Control Serum	Mono-spot	Monotest	Pre-sumptive	Guinea-pig Kidney	Beef RBC	Davidsohn Interpretation
1518320	Difco infectious mononucleosis-positive serum	Pos.	Pos. (1:16)	1:448	1:224	0	Pos.
804174	BBL positive control serum	Pos.	Pos. (1:8)	1:224	1:112	0	Pos.
9041701	BBL serum sickness control serum	Neg.	Pos. (1:2)	1:28	0	0	Neg.
9051711	BBL serum sickness control serum	Neg.	Pos. (1:4)	1:28	0	0	Neg.
8031731	BBL high titre heterophile normal serum	Q.N.S.	Neg.	1:56	0	1:56	Neg.
9051721	BBL high titre heterophile normal serum	Neg.	Neg.	1:56	0	1:112	Neg.
528219	Difco heterophile Forssman antiserum	Neg.	Neg.	1:56	0	1:28	Neg.

Table III Slide tests for mononucleosis and Davidsohn differential test with commercial reagent control sera

Discussion

For diagnostic problems that may be resolved by demonstration of rising titres, or occasionally falling titres, the quantitation of infectious mononucleosis serology could be significant. Many clinicians request quantitations of titre as an aid to management of the patient, eg, when to send the patient back to school. Previously, the recommendation has been made that when the Monotest is positive the quantitative differential absorption test might be necessary 'for obtaining titres of positivity when these are required for comparative purposes' (Davidsohn, 1967). The simple quantitation of the Monotest described here appears to be equally satisfactory if such comparative data are requested.

The results in our series indicate an apparent sensitivity and specificity of a degree similar to previous reports. As a result of these studies we now routinely perform only the Monotest, and, if positive, quantitate the specimen. We report the Monotest titre and the approximately equivalent presumptive Davidsohn (antisheep cell) titre. The differential test is reserved for special instances, as for example when the clinical status of the patient is discordant with the Monotest result,

or serum sickness is considered in the differential diagnosis.

No attempt was made to test the sera without inactivation because of previous experience showing occasional false-negative reactions on unheated sera (Sinay *et al*, 1968). Other investigators are also inactivating their test sera (Lowell and Kazakaitis, 1969). The directions for Monotest state that the test may be performed on fresh or inactivated serum.

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