A Quick Method of Performing the Paul-Bunnell Test

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The routine method of performing the Paul-Bunnell test is time-consuming, and the final reading is not available until the following morning.

The technique described here gives reliable results 20 minutes after the test has been set up. Also a difference in titre is noted which may be useful as a diagnostic procedure in cases of glandular fever where the symptoms have been present for some time.

Method

The test is put up in duplicate.

(1) Patient's serum, 2 ml., is inactivated in a water-bath at 56° C. for 30 minutes.

(2) A rack containing two rows of 7.5 by 1 cm. rimless tubes is prepared.

(3) In the first tube of each row is placed 0.9 ml. of 0.85% sodium chloride.

(4) In the remaining tubes of each row is placed 0.5 ml. of 0.85% sodium chloride.

(5) To the first tube of each row 0.1 ml. of serum is added and mixed thoroughly.

(6) Then 0.5 ml. of the diluted serum is carried over into tube 2 and so on until tube 10, the last 0.5 ml. being discarded.

(7) To each tube 0.5 ml. of washed fresh sheep cells is added, thus giving a final serum dilution of 1:20 up to 10,240.

(8) One row of tubes is now placed in a separate rack. The separate rows of tubes are now treated as follows:

A. Orthodox Technique.—One rack is incubated at 37° C. for four hours, after which it is stored overnight in the refrigerator at 2 to 4° C. The following morning the rack of tubes is re-incubated at 37° C. for one hour, after which macroscopic agglutination is looked for after gently inverting the tubes twice.

B. Quick Method.—The other rack of tubes is placed on an automatic (Kahn) shaker, set at 275 oscillations per minute. The rack is shaken for five minutes, after which it is allowed to stand for 15

minutes. At the end of this time the result is read as described in A above. Two-hour readings were taken in the last five tests of this series.

Results

The sera of 150 patients, suspected on clinical grounds of having glandular fever, were tested. In all cases the blood picture was also investigated.

The test was completely negative, i.e., no agglutination, in 124 cases. In only one of these was the blood picture suggestive of glandular fever.

The results in the remainder of the cases in which agglutination occurred is shown in Table I.

TABLE I

No.	Conventional Method	Shaking Method		Blood Picture
		½ hr.	2 hr.	
1	1/80	1/80		Suggestive
2	1/20	1/20		Normal
3	1/40	0		
4	1/40	1/40		Typical
5	1/80	1/80		"
6	1/160	1/160		"
7	1/80	1/80		"
8	1/160	1/320		"
9	1/20	0		Normal
10	1/320	1/320		Typical
11	1/320	1/320		"
12	1/20	1/20		Old glandular fever
13	1/160	1/160		Typical
14	1/20	1/80		Typical test done in second week
15	1/40	1/80		Typical " " " " "
16	1/40	1/40		"
17	1/640	1/640		"
18	1/20	1/20		Normal
19	1/40	1/20		"
20	1/320	1/320		Typical
21	0	1/40		Very suggestive
22	1/40	1/40	1/160	Old glandular fever
23	1/20	1/40	1/40	Normal
24	1/160	1/80	1/160	Typical
25	0	1/40	1/80	Suggestive, clinically typical but test done 2 weeks after onset
26	0	1/40	1/80	"

Titres varying from 1/40 to 1/320 were obtained in 12 cases in which the blood picture was typical of glandular fever. In 10 of these cases the titre by both methods was identical. In one the titre by the shaker method was double that of the conventional method, while in the other the reverse was true.

Cases 14 and 15 were cases of glandular fever in which the test was done at the end of the second week. In both these cases the shaker method gave a considerably higher titre.

Cases 25 and 26 were very suggestive clinically of glandular fever, but again the test was done two weeks after the onset of symptoms. In both these cases the conventional method gave a negative result, while the shaker method gave, in both instances, a titre of 1/40 at quarter hour and 1/80 at two hours.

Case 22 was one of old glandular fever, and here the shaker method gave a titre of 1/160 at two hours as against 1/40 given by the conventional method.

Conclusion

A quick method of performing the Paul-Bunnell reaction is described which is as sensitive as the orthodox method. When read at two hours the rapid test appears to be definitely more sensitive than the orthodox method. When the rapid method is performed in the later stages of glandular fever a much higher titre is obtained than by the orthodox method. This might prove to be of diagnostic importance in such cases, particularly if they have not been investigated in the early stages. The validity of this point is being investigated further.

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A Simple Method for Desalting Biological Fluids for Paper Chromatography of Amino-acids

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Paper chromatography of biological fluids, especially urine, is difficult, owing to the presence of salts and other interfering substances, which prevent a satisfactory separation of the amino-acids in chromatograms.

Consden, Gordon, and Martin (1947) desalted the specimens electrolytically and used them for chromatography. Ion exchange resin was used by Phillips and Pollard (1953). Baliga, Krishnamurthy, Rajagopalan, and Giri (1955) used alcohol for desalting. A very simple and easy method for desalting urine and other biological fluids, which can be carried out in any ordinary clinical laboratory, is now described.

Procedure

Filtered urine, 25 ml., is evaporated to dryness in a dish over a water-bath. If proteins or phosphates are precipitated on heating, they are filtered off and the filtrate is evaporated to dryness over the water

FIG. 1.—Protein hydrolysate. 1, Aspartic acid; 2, glutamic acid; 3, serine; 4, glycine; 5, threonine; 6, alanine; 7, histidine; 8, lysine; 9, arginine; 10, tyrosine; 11, valine; 12, leucine and isoleucine.