Elimination of the False Positive Reaction With Human Sera in Complement Fixation Tests[1]

GORDON C. BROWN

Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor

In a recent paper DeBoer and Cox (*5*) reported that complement fixation tests for the diagnosis of eastern and western equine encephalomyelitis employing infected chick embryo antigens as described by the author (*1*) or the mouse brain antigens of Casals (*4*) were not truly specific in that these tissues fixed complement with known positive human syphilitic sera. This phenomenon had been described previously by Wertman, who used normal yolk sacs or chick embryos (*7*). DeBoer and Cox described a procedure for extraction of the antigens in the lyophilized state with benzene, toluene, or dichlorethylene, which eliminated the so-called false positive reactions. Twenty-two individual and 14 pools of syphilitic sera were tested. The authors failed to mention the temperature of inactivation for these human sera, merely referring to the method of Kolmer and Boerner (*6*) for the technique of the complement fixation test in which 55° C. for 20–30 minutes is recommended.

TABLE 1

EFFECT OF INACTIVATION TEMPERATURE ON COMPLEMENT FIXATION TESTS

Serum	No.	Titer with irradiated chick embryo antigens								
		56°C.—30 min.	60°C.—15 min.							
			0	1/4	1/8	1/16	1/32	1/64	1/128	1/256
Pos. syphilitic.....	6	1/64	5	1						
" "	14	1/128	9	4		1				
" "	8	1/256	3	2	2	1				
Neg. "	3	1/16	2	1						
" "	2	1/32	2							
" "	1	1/128					1			
WEE immune.....	4	1/256							4	
EEE immune.....	1	1/64				1				
"	2	1/128						1	1	
"	1	1/256						1		

Casals (*2, 3, 4*), using infected mouse brain antigens, has repeatedly stressed the importance of different temperatures of inactivation according to the species of serum under test and recommends 60° C. for 20 minutes with human sera. In this laboratory, complement fixation tests employing irradiated antigens derived from infected chick embryos have proved satisfactory in the detection of antibodies for eastern and western equine encephalomyelitis viruses provided the sera are properly inactivated. Although temperatures of 55° or 56° C. are acceptable for some animal sera, they are definitely unsatisfactory for human sera. In a series of tests with 34 human sera obtained from the syphilis clinic, false positive reactions were obtained in titers ranging from 1/16 to 1/256 when the sera had been inactivated at 56° C. for 30 minutes. When these same sera were treated at 60° C. for 15 minutes, 21, or 61 per cent, were completely negative, and the titers of the remainder were reduced from 16- to 64-fold. On the other hand, sera containing antibodies for eastern or western equine encephalomyelitis viruses either retained their original titers or dropped only 2-fold or 4-fold after being inactivated at 60° C. Table 1 presents these results. Obviously, the vast difference in the capacity of specific sera to resist this higher temperature of inactivation provides a qualitative diagnostic criterion. This remarkable difference of only 4° C. is best illustrated by the following example: A pool of positive syphilitic human sera heated at 56° C. gave a false positive reaction with the chick embryo antigen to a titer of 1/64 after as long as 120 minutes at that temperature. When this identical pool was subjected to a temperature of 60° C., the titer was reduced to 1/16 after 5 minutes, 1/8 at 10 minutes, and was completely negative after 15 minutes inactivation.

The simple expedient of inactivating human sera at 60° C. for 15 minutes instead of 56° C. for 30 minutes, when used for complement fixation tests with antigens derived from chick embryos, makes unnecessary the elaborate techniques suggested by DeBoer and Cox for purifying virus antigens.

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Apparatus for Continuous Yeast Culture

JOHN G. B. CASTOR

Enology Laboratory, Division of Viticulture, University of California, Davis

T. J. B. STIER

Department of Physiology, Indiana University, Bloomington

A simple batch type of continuous culture apparatus which provides a daily series of freshly grown yeast cultures for experimental use was developed and used during studies on yeast metabolism (*7, 9*). This apparatus is useful in reducing to a minimum daily, time-consuming culture manipulations. A rubber stopper-glass tubing joint permits the rapid and aseptic withdrawal of part of all of the culture and assures its continuation by immediate renewal and automatic reinoculation of the medium.

The apparatus is inexpensive and easily constructed, from glassware and supplies available in most laboratories, to suit various capacity requirements. It is easily dismounted, cleaned, resterilized, and returned to operation, and is adaptable to various types and shapes of culture vessels.

The apparatus should prove useful in the continuous serial batch cultivation of any aerobic organism capable of multiplying when suspended in a liquid medium. Agitation of the suspension sufficient to distribute nutrients and oxygen and remove metabolic end-products is considered important to high