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

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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

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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

crop yield (*1, 8*). While air-flow agitation alone does not always establish optimum aeration conditions in this type of culture, addition of vigorous mechanical agitation tends to do so (*1, 3*). Pure oxygen flow alone, however, would result in conditions more nearly equivalent to those existing at the surface of an agar plate (*7, 8*). The apparatus described here fulfills such

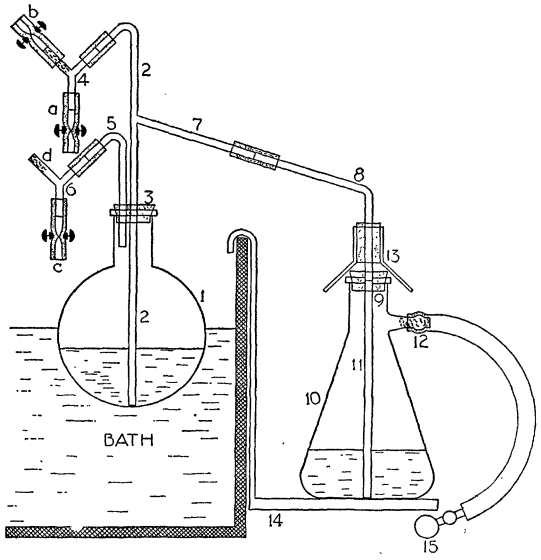

FIG. 1

requirements at an intermediate aeration level in a simple form. It avoids the disadvantage in methods of the type described by Moor (*6*) and Klem (*4*), in which cells are permitted to sediment in a thick layer, and it is simpler and less expensive for small-scale cultures than the mechanically agitated apparatus described by Feustel and Humfeld (*3*). Other simple culture vessels which provide extensive aeration agitation are described by Magoon and Brunstetter (*5*) and Stier and Stannard (*10*).

Fig. 1 shows a diagram of the apparatus set up with an ordinary boiling flask as the culture vessel. The parts required for one unit are one culture vessel, glass Y- or T-connections, a glass powder funnel, a supply of filter flasks fitted with one-hole rubber stoppers, spring-type pinchcocks, glass tubing, and rubber stoppers and tubing of suitable type and size.

In preparing the apparatus the parts may conveniently be handled as three separate units. The openings of the vessels and the intravessel tubing are covered with heavy paper. Several filter flasks, 10, each containing a predetermined amount of medium and equipped with a rubber stopper, 9, glass tube, 11, and a cotton air filter in or attached to side tube 12, are covered with paper caps. The three units are then autoclaved.

A series of cultures is begun by assembling the units with ordinary aseptic precautions. The medium in one filter flask is inoculated. The paper cap is removed from the powder funnel, 13, and the free end of tube 8 is inserted *halfway* into the rubber stopper to contact tube 11. This is the rubber stopper-glass tubing joint mentioned above. The joint has proven stable with a tight friction fit and may be improved by cementing tube 11 into the stopper with a latex preparation.

Connection to a source of washed and moistened air or oxygen is made through side tube 12. Slight gas pressure forces the inoculated medium through the aeration-siphon tubes, 8, 7, 2, into the culture vessel. Exhaust gases are vented through tubes 5 and 6. Cotton air filters at 4b and 6d prevent airborne contamination of the culture during siphoning operations. Pinchcocks at 4a and 4b prevent loss of medium through the upper limb of tube 2 during transfer from flask 10 to the culture vessel. Moisture traps and drains are provided at 4a and 6c to prevent soaking of the cotton filters by moisture condensing in tubes 4 and 6.

Powder funnel 13 protects the joint in stopper 9 from airborne contamination. The funnel is held on tube 8 by a piece of rubber tubing which is fastened into the funnel by use of liquid latex. The funnel should slide on tube 8 to permit a snug fit on different rubber stoppers. The movement required is slight. The rubber tubing supporting the funnel is prevented from adhering to tube 8 by lubricating with glycerol or powdered graphite before autoclaving.

Samples or the whole culture may be withdrawn at will by using tubes 2, 7, and 8 as a siphon. The siphon is started by removing the gas-supply tube and applying slight suction at side tube 12 or slight gas pressure at 6d. Siphon action can be instantly stopped by opening pinchcock 4b, thus "breaking" the liquid column at the junction of tubes 2 and 7.

Reinoculation is accomplished by "breaking" the siphon before all of the culture is withdrawn. Amounts of inoculum varying in size from the few drops of liquid left on the walls of culture vessel and siphon tubes to any larger amount desired may be obtained. A very small amount of the initial inoculated medium or sterile renewal medium may fail to transfer from the filter flask to the culture vessel. This amount is usually negligible. However, substitution of a dry, sterile filter flask unit after transfer of the medium prevents any difficulty at this point. After withdrawing samples of culture and before attaching a flask of fresh medium, the end of tube 8 is flamed.

Rubber parts made of neoprene give better service than natural rubber. Plastic tubing such as Tygon (S22-1), which is somewhat transparent, should be an improvement over rubber. The apparatus is sufficiently rigid so that only the culture vessel and filter flask need support. The joint between tubes 7 and 8 was included to give the aeration siphon flexibility when changing filter flasks. A gas manifold, 15, attached under the shelf as shown is convenient for operating parallel cultures.

A reservoir for sterile culture medium attached to the aeration siphon may be substituted for the supply of medium-containing filter flasks (*2*).

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