ELECTROPHORETIC POTENTIAL AS AN AID IN IDENTIFYING STRAINS OF THE B. COLI GROUP

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The object of this paper is to show that the electrophoretic potential (P.D.) can be accurately determined for a given strain of the *B. coli* group by a simple technic and that the P.D., as determined by this method, is constant for that strain. It follows from this that, since strains differ considerably in their potentials, the P.D. can be used to assist in demonstrating the similarity or dissimilarity of two cultures of the coli group in a manner similar to the use of blood grouping in relation to paternity. In most of our strains, dissociants have given similar potentials but we have observed a few strains, the dissociants of which have given different potentials, each one being constant for its dissociant. As we have shown in another paper, this potential bears a direct relationship to the virulence of *B. coli.*

The most convenient apparatus for this work is the capillary cell type described by Falk (1928). The vital element of this cell is the capillary tube. The bore of commercial tubes varies considerably in spite of advertised tolerances. However, the bore is secondary in importance to the cleanliness of the tubes. All our tubes are freed from moisture by suction, filled with dichromate-sulfuric cleaning fluid, boiled in this fluid and washed with fat-free distilled water. They are then dried with a current of air.

Since the P.D. is influenced by differences in hydrogen-ion concentration, the presence of various ions, previous washing of the bacteria, the age of the culture, dehydration of the culture medium and some other factors, it was essential to study these variables and to formulate a standard technic in order that the

results obtained in one laboratory could be comparable with those from any other.

Our first problem was to control the pH of the growth. We attempted to do this by means of the usual amounts of phosphate buffer salts but found that they were hopelessly inadequate. When, however, the phosphate content was increased to 15 grams of each per liter, there was only a slight change in reaction in four hours. If the cultures were incubated for a longer period, the results became inconsistent and wide differences in pH were often observed in five to six hours. Since there was enough growth in three hours to make 1 or 2 cc. of suspension, and as the amount of metabolic by-products in this short incubation period would be slight, rendering washing unnecessary, we decided upon this as the optimum growth period. The medium finally adopted had the following composition:

Agar	15 grams
Peptone	10 grams
Meat extract	3 grams
Mono sodium phosphate	15 grams
Di-sodium phosphate	15 grams
Bromthymol blue	0.08 grams
Water to make	1000 cc.

The reaction after sterilization is pH 6.75.

After three hours incubation on this medium, the growth is suspended in fat-free distilled water and diluted to a concentration of about 500 millions per cc. as judged by opacity. A capillary tube is filled immediately with the suspension and put in a Falk cell which has been connected to an E.M.F. of 45 volts. The speed is determined, preferably, by means of the new Jaquet precision stopwatch. Five determinations are made in one direction and the polarity of the cell is then reversed. Another five determinations are made which should compare favorably with the first set. The tube is then replaced by a second capillary filled from the suspension and the speed determined as with the first one. The two sets of determinations should agree within 4 per cent. In case of a disagreement, it is necessary to repeat the tests with other capillaries or to make fresh suspensions.

When reasonable checks have been obtained, it is desirable to convert the observed speeds into standard units. We prefer to use microns per 1000 volts per second since, with this method, the P.D. falls between 25 and 100. This is also convenient since it is sometimes desirable to use other than 45 volts. For rapid calculation, tables can be prepared for converting the observed speeds into standard units.

Having rigidly controlled the technic, it was next important to show that the P.D. is constant under these conditions and that it is constant for a given strain over a period of time. Two strains having quite dissimilar indices were tested a number of times on different plates of media and the P.D. was found to be contant for each. They were then plated daily for a month with very consistent results. Finally, they were put into daily use over a period of six months and the results obtained at the end of each two weeks were as follows:

Strain 1	48, 47, 48, 50, 50, 49, 48, 48, 47, 48, 48, 50	Av. 48
Strain 2	87, 91, 82, 92, 87, 89, 86, 90, 83, 83, 87, 90	Av. 87

Considering differences that might exist in culture media and in other experimental conditions, these results are quite constant. Some of the variations which did appear were found to be due to storage of the culture medium.

When these two standard strains are run on the same plates as unknown strains, we obtain rigid checks on the condition of the culture medium and the resulting growth. If the two standard strains do not give the correct P.D. that has been found from the average of a large number of determinations, it is evident that the unknown strains will also give unreliable results. In such instances, the tests are always repeated until satisfactory results are obtained. With these safeguards, we believe that the P.D. of the *B. coli* group can be accurately measured. In order to assist other laboratories, we will gladly send cultures of our two standard strains on request. Transplants are being offered to the American Type Culture Collection to perpetuate this standardization.

We have used this method to identify a given strain that has

been repeatedly isolated from the same person's stools; to investigate the possibility of the same strain existing in the stools of various members of a family; to determine if a given strain of *B. coli* has been successfully implanted in the intestinal tract; to determine if several colonies are identical, as, for instance, in the isolation of bacteria from water or feces; and to assist in identifying strains of the same organism isolated on different culture media.

These studies have been confined to the group of Gram-negative intestinal bacilli since their conditions of growth are admirably adapted to the above technic, but it is possible that, with modifications, the method may be of value in other groups of bacteria.

SUMMARY

1. A standard technic has been described for the determination of the electrophoretic potential (P.D.) of the coli group.

2. This potential is shown to be constant for each strain.

3. The value of the P.D. as an aid in comparing strains of the coli group is pointed out.

REFERENCE

FALK, I. S. 1928 Jour. Bact., 15, 444.