References and Notes

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Virus Neutralization Test in a Capillary Tube

Abstract. A simple and accurate virus titration method which requires extremely low numbers of cells per test vessel is described. Inhibition of metabolism was the criterion for 50 percent end point analysis when capillary tubing was used as the cell culture container. Virus neutralization titers were obtained within 24 hours.

Cell culture techniques have evolved to the extent that they may be used routinely in diagnosis of certain virus diseases. The most widely used virus neutralization tests are based either on cytopathic changes or inhibition of metabolism (*1*). Currently, these tests have the disadvantage of requiring special facilities for carrying cell lines, or if cells are purchased from commercial sources, tests are limited for economic reasons. The technique described here requires only a small number of cells in each test vessel and allows a neutralization titer to be made with cells harvested from only one or two tube cultures (*2*).

The culture vessels were prepared from capillary tubing of outside diameter 1.3 mm, wall thickness 0.2 mm, and length 40 mm. They were sterilized by autoclaving without prior cleansing.

Stock cell cultures were grown in screw-cap test tubes (15 by 125 mm) in Eagle's medium with 10 percent calf serum (*3*). Incorporation of antibiotics in all media, checks for media contamination, and constant use of normal precautionary measures to maintain asepsis resulted in a low incidence of contamination of the test vessels. The occasional contaminated capillary tube could be identified by appearance alone. Suspensions of cells were prepared by trypsinization of the cell layer; for this purpose, 0.06 percent trypsin in Hanks balanced salt solution, in which there was no added calcium or magnesium, was used. The number of viable cells was determined by a hemacytometer count of neutral red stained preparations, and total number of cells with an electronic counter. The cells were then suspended in Eagle's medium with 2 percent calf serum, at *p*H 7.6, so that each 0.02 ml of final volume contained 600 cells. The number of cells determined the length of time which was required before the phenol red in the medium changed to yellow.

If the titer of the virus was to be determined, tenfold serial dilutions were made in Eagle's medium, and aliquots of each dilution were mixed with equal proportions of the cell suspension. The mixture of cell and virus was then drawn by capillary action into the capillary tubes. This was facilitated by preparing the mixture in Wassermann tubes so that the suspension could then be brought to the lip of the tube to charge the capillary. Each end of the filled capillary tube was then sealed, by capillary action, with a 2-mm plug of mineral oil. The tubes were kept horizontally at 36°C in a dry atmosphere incubator until the control tubes reached the proper *p*H for end point readings. A simple tube holder was devised by stapling white adhesive tape to cardboard so that the tubes could be stuck to its surface in dilution groups.

Virus titers and serum neutralization titers could be obtained with an accuracy comparable to that obtainable by other methods which depend upon a 50-percent end-point analysis. The color difference between tubes with viable cells and those with cells destroyed by virus was marked, so that the end points were distinct. This color difference was enhanced because the medium became more basic as CO_2 was gradually lost through the oil seal in tubes which did not contain metabolizing cells.

The greater the number of metabolizing cells the more rapid was the conversion to an acid reaction. In fact, conversion could be observed in about 6 hours if there were 2000 cells in each tube. Large numbers of cells both caused a rapid appearance of end points and were responsible for a concurrent decrease in the sensitivity of the test. Thus, by careful adjustment of the number of cells, fairly accurate end points could be obtained in 24 hours. The most accurate titers were obtained when there were 300 cells in each tube and there was a lapse of 3 to 6 days before final readings were made.

In titrations of type III poliovirus, using HeLa cells grown in 15- by 125-mm tubes, the end points based on cytopathic cell response compared favorably with titers obtained by the capillary tube method. A sample of type III poliovirus antiserum indicated approximately the same titer by each method.

This technique, because of its speed and economy, could be used with the ordinary equipment of a bacteriological diagnostic laboratory to increase the use of virus neutralization tests in the diagnostic laboratory (*4*).

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