A Capillary Tube Method of Rh Typing Using Albumin Agglutinating Anti-D Serum

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The capillary tube technique of Rh typing (Chown, 1944; Chown and Lewis, 1946) is a very useful rapid method which, however, depends on using sufficiently potent saline agglutinating anti-D serum, the supply of which is restricted. A method using Chown's technique with albumin agglutinating anti-D serum diluted with 20% bovine albumin has been described (Tuck, 1951), but it has been found that the method described below, which differs from Chown's technique in a number of points, and uses 30% bovine albumin, gives more conclusive results. The albumin agglutinating anti-D serum need not be especially potent; that used for routine tube testing has been found to be satisfactory. The method also has the advantage that no special preparation of serum, test cells, or bovine albumin is necessary.

Method

The test is performed in capillary tubes 90 mm. $\times$ 1 mm. (lymph tubes supplied by Baird and Tatlock have been found satisfactory). Small batches of these tubes should be placed in test-tubes, which are then plugged with cotton wool, and sterilized in the hot-air oven before use.

The anti-D serum is probably best kept in 1 ml. quantities in tubes, rather than in bulk in bottles, and care should be taken to avoid handling the end of the capillary tube which is dipped into the serum, in order to minimize any risk of contamination.

The specimen of clotted blood is shaken so that a 25%–50% suspension of cells in serum is obtained. If necessary the cell serum suspension must be centrifuged and the serum decanted in order to obtain a cell suspension of sufficient strength.

The capillary tube should be held horizontally throughout the filling process. The end of the tube is first dipped into the anti-D serum until 1.5 to 2 cm. is filled with the serum. After wiping any excess serum off the outside of the tube on the edge of a piece of blotting paper, the same end of the tube is dipped into the cell serum suspension, and an amount equal to that of the anti-D serum is allowed to enter. Care should be taken to avoid small clots entering, or air locks between the cells and serum. After again wiping the outside of the tube on the edge of the blotting paper to remove any cells, the same end is dipped into the 30% bovine albumin, and an amount equal to the combined

volume of cells and anti-D serum is allowed to enter, once more avoiding air locks between the albumin and the cells and serum. Having wiped any excess albumin off the outside of the end of the tube, still holding the tube horizontally, the end opposite to that used for filling the tube is gently inserted into a block of "plasticine," and, as soon as the end is blocked, the tube is elevated into the vertical position, so that the anti-D serum is below, the cells are above this, and the bovine albumin is uppermost.

The test is placed in a 37° C. incubator and in most cases the result can be read after five minutes or less, and in all cases a final result can be read in 10 to 15 minutes.

The tests may be read with the naked eye, or with the assistance of a low-power lens in a good light against a white background. Rh-positive tests show large clumps of agglutinated cells extending down the tube; in Rh-negative tests the cells extend down the tube in a slightly rosy but continuous column, which tends to remain distributed throughout the length of the tube, in marked contrast to the positive tests, where the clumps of agglutinated cells settle rapidly into the lower half of the tube. If the tests are allowed to stand on the bench for an hour after being read, the contrast between positives and negatives becomes even more marked, as in positives the agglutinated clumps pack down to the bottom of the tube, whereas in negatives the cells still remain distributed in a smooth suspension.

If a test is observed with a lens immediately after setting up, it will be seen that the albumin, being heavier than either the cells or serum, descends rapidly, pushing the cells into the serum, and these, having mixed, rapidly ascend to occupy the upper part of the tube, while the albumin takes up the lower position (this was actually shown to happen by carrying out tests with dyed albumin). Next, if the cells are Rh positive they are agglutinated, and the cells go into clumps which fall downwards into the lower part of the tube. If, however, the cells are Rh negative, they are not agglutinated, and descend more slowly.

The above method has been used in testing over 1,000 Rh groups in parallel with a standard method of Rh typing; and in no case has a test been read as positive which in fact proved negative by the standard technique. In three tests a negative result was read when the tests were actually positive by the standard method, but in each case the cell suspension was too weak, and the tests proved positive on being repeated with a stronger cell suspension. A number of D^us were encountered in the series, and all gave a negative result with the test.

It should be emphasized that the test as described is primarily a D screening test, and all specimens giving a negative result must be further investigated by the recommended standard techniques.

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