Single Radial Immunodifusion Using Labeled Antigen

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Antigen quantitation with single radial immunodiffusion (SRID) and its theoritical consideration were indicated by Mancini. However this method is limited to measure relatively high concentration of antigen. Application of radioisotope to single radial immunodiffusion is divided into two categories. The first method is using labeled antibody, either first antibody or second antibody. The second is using labeled antigen, which is our object to present paper for measurement of α-fetoprotein (AFP).

Line of small well with 2.5 mm in diameter was punched out on the Agarose immunoplate containing appropriately diluted antibody of AFP. In these wells 1 ul of I-125 AFP with high specific activity was applied. After that 5 ul of standard AFP or Sample serum was applied. Then the plate is incubated at room temperature for two days. to form precipitation ring. Autoradiography was performed to measure the diameter of these rings. Five ul of I-125 AFP can make exposure time shorter without any influence on the result.

Two standard curves were obtained to cover 50 to 10000 ng/ml of AFP or 50 to 2000 ng/ml. Therefore dilution of AFP-high serum samples is not required in the present method.

However high diluted portion of antigen, break of standard curve was observed, when dilution of standard was performed with saline. Five percent of albumin saline solution could make the break less prominent. With standard solution diluted with rabit serum or normal human serum, the break was disappeared and the standard curve became straight. The importance of the presence of protein for precipitation was stressed.

Advantages of this method are as follows. Antibody of AFP is kept stable in immunoplate for more than 6 months. One can examine any single case when it becomes necessary. Only 5 ul of serum samples and very small activity are needed. No radioactive soluble waste. The procedure is very simple. No special instrument is required. Application of the method to other immunological assay is possible when precipitation antibody and labeled antigen are available.