RAPID MICROMETHOD FOR ASSAY OF ANTIBIOTICS OF THE TETRACYCLINE GROUP (BIOMITSIN, TSIKLOMITSIN AND RIOMITSIN) IN BIOLOGICAL FLUIDS

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The work of Schneierson (S. S. Schneierson, Proc. Soc. Exp. Biol. Med., 1951, v. 74, No. 1, p. 106 - 108) on a rapid method for the assay of aureomycin in the blood was published in 1951. This method is based on the ability of B. proteus OX_{19}, growing on a medium containing urea, to decompose it to ammonia; the consequent pH rise is registered by the color change of the medium, to which phenol red was added as an indicator. The sensitivity of the method is 0.8 μg/ml.

However, assay of an antibiotic in blood by this method requires large quantities of serum (about 3 ml), which in a number of cases hampers the work. Moreover, the method is not sufficiently standardized; in particular, the number of bacteria necessary for seeding is indefinite.

Using Schneierson's method as our basis, we developed a micromethod for the rapid assay of antibiotics of the tetracycline group – biomitsin (synonyms: aureomycin, chlorotetracycline), tsiklomitsin (synonyms: deschlorobiomycin, acromycin, tetracycline) and riomitsin (synonyms: terramycin, hydroxytetracycline) in biological fluids. We changed the ratios of the components used in the reaction so that small quantities of biological fluids (0.2 ml) could be used for the assay of the cited antibiotics. The latter feature is of great importance since it permits the assay of tetracyclines (and, evidently, of other antibiotics that act on B. proteus OX_{19}) not only in the blood but also in the lymph and cerebrospinal fluid, which are not obtainable in large quantities. Moreover, the composition of the culture medium was simplified and the number of bacteria necessary for seeding was determined. The composition of the culture medium:

Phenol red	10 ml*
Na_2HPO_4	1.9 g
KH_2PO_4	1.82 g
Urea	4 g
Distilled water	190 ml

By means of a 10% solution of NaOH, pH 7.0 is established. The medium does not require sterilization and may be kept at room temperature for a long period. In case a precipitate forms, the medium must be filtered through filter paper.

A culture of B. proteus OX_{19} is grown for 6 hours on the usual beef-peptone broth or on agar with pH 7.1 - 7.3. A smear from the agar or an undiluted broth culture may be kept at 4° for up to 10 days. Before the experiment, the culture is diluted with urea medium to 40 million bacteria per 1 ml of medium (by optical standard). When the experiment is carried out with a lesser number of bacteria, the results are less clear and

* The solution of phenol red is prepared as follows: 5.7 ml 1/20 N solution of NaOH is poured into a sterile 50-ml flask and to this is added 0.01 g phenol red and sterile distilled water to make 50 ml.

they may be read only after 12-16 hours.

When B. proteus OX_{19} is seeded on the urea medium with the indicator in the absence of antibiotics, the urea decomposes and shifts the pH of the medium toward the alkaline side, and its color goes from orange to red. When an antibiotic is present in the medium, the growth of the bacteria is inhibited and the color of the medium does not change. In assay of antibiotic, the last test tube with medium of unchanged color is considered.

The titration is always carried out simultaneously with the titration of a standard solution of the antibiotic which is being tested. Standard biomitsin, tsiklomitsin or riomitsin is diluted to a concentration of 20 μg/ml: the antibiotic may be diluted with distilled water.

Determination procedure. Two series of test tubes are used — 12 in each series. 0.2 ml of the B. proteus OX_{19} culture diluted according to the standard is run into each test tube. 0.2 ml of the biological fluid under investigation is added to the first test tube of the first series, mixed and 0.2 ml is transferred to the 2nd test tube, mixed again and 0.2 ml is transferred to the third test tube, etc., until the eleventh test tube, from which 0.2 ml liquid is poured off. The last test tube is the control.

The same dilutions are carried out in the test tubes of the second series, adding 0.2 ml of a standard solution of the antibiotic under investigation to the first test tube. Thus, the first test tube of the second series will contain 10 μg/ml antibiotic, the second will contain 5 μg/ml, the third will contain 2.5 μg/ml, etc. The test tubes are placed in an incubator at 37° for 4-4.5 hours and then the results are read. In each series, the last test tube with medium of unchanged color is considered.

To calculate the concentration of the antibiotic in the liquid under investigation, the concentration of the standard antibiotic that inhibited the growth (the last test tube of the second series with medium of unchanged color). Thus, if the standard solution of biomitsin is observed to inhibit growth in the test tube containing 0.31 μg/ml, and the investigated liquid, up to 16-fold dilution, then the concentration of biomitsin in the investigated liquid is $0.31 \times 16 = 4.86$ μg/ml.

The sensitivity of B. proteus OX_{19} to the standard solutions of tetracyclines is: to biomitsin, 0.38 μg/ml, to tsiklomitsin, 0.95-1.15 μg/ml: to riomitsin, 0.95-1.15 μg/ml.

Utilization of the described micromethod permits rapid (4-4.5 hours) assay of antibiotics of the tetracycline group under non-sterile conditions employing small volumes of the fluids under investigation.