THE COMPLEX METHOD FOR DETERMINATION OF INTESTINAL MICROFLORA COMPOSITION OF LABORATORY ANIMALS

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Determination of qualitative and quantitative composition of intestinal microflora of the laboratory animals, namely albino rats is important for conduct of the study regarding assessment of the impact of probiotic preparations for the restoration of intestinal microbiocenosis of animals under the experimental dysbiosis. There is no single common method to establish this composition. Normative documents establish methods for the quantitative determination of the content of specific groups of microorganisms [1-4], such as *E. coli*, staphylococci, proteus, pathogenic enterobacteria. However, the research work should simultaneously determine different groups of microorganisms in one sample of the intestinal content to establish the overall picture of infringements or correction of the composition of intestinal microbiocenosis of the laboratory animals.

The aim of the work was to develop and provide generalizing method for establishment of qualitative and quantitative composition of microflora of the intestinal content in laboratory albino rats.

Preparation of glassware and selective media. Each sample requires preparation of sterile weighing bottle (for entering sample of the intestinal content of 1 g), sterile glass rod, 7 sterile spatula, 3 sterile test tubes, 1 mL (6 pcs.) and 10 mL (for dilutions) sterile pipettes, 8 sterile Petri dishes.

All glassware is sterilized in a steam sterilizer for 30 minutes at a pressure of 0.75 atm. and temperature of 120°C.

Selective media are prepared and sterilized according to the instructions of the manufacturer and poured into sterile Petri dishes. Therefore, the following is prepared: 3 dishes with Endo medium, 1 dish with bismuth-sulphite medium, 1 dish with Sabouraud medium, 1 dish with blood agar, 1 dish with Baird-Parker medium. Blaurock medium is prepared as semi-liquid in accordance with recipe [5], poured into 2 test tubes of 4.5 mL and autoclaved with cotton stoppers. Sterile slope agarized Olkenytskyi and Simons media (10 of each for

each test tube) are prepared to identify individual species of enterobacteria. Test tubes with slope medium can be stored in the refrigerator for 30 days.

For microscopic examination of the samples from Blaurock medium and suspicious colonies from other media, set of reagents for Gram staining and microscope are used.

Selection and preparation of samples. Samples of rat intestinal content (commonly from the rectum) are taken immediately after slaughter. For this purpose, a region of intestine, the content of which will be investigated is cut with sterile scissors, and placed into a sterile Petri dish. Further, applying aseptic regulations, intestine is cut and its content is removed. 1 g of content is placed in the sterile weighing bottle and 9 mL of sterile normal saline solution is added. Thus, ten-fold dilution (10^{-1}) is obtained.

It is not infrequent that the intestinal region has less content than one gram, especially when working with sick animals. Then maximum possible number of content is weighed, and volume of normal saline solution to be included in the weighing bottle is recalculated for 10-fold dilution. For example, when the sample weight is 500 mg, we are adding 4.5 mL of solution, and in case of 300 mg – 2.7 mL of solution.

Weighing bottles with samples are not keep, and immediately used for inoculation of media. Before preparing dilutions, content of the weighing bottle is thoroughly mixed with sterile glass rod.

Preparation and inoculation of dilutions.

To prepare the required dilutions, three sterile test tubes are used in which 9.9 mL of sterile normal saline solution is added.

Sample 0.1 mL of solution from the weighing bottle with sterile pipette and transfer to a test tube No. 1 with 9.9 mL of normal saline solution, and then shake. Thus, we obtain 10^{-3} dilution. Transferring 0.1 mL of the solution from this test tube to the next test tube (tube No. 2), we obtain 10^{-5} dilution, and so on under the scheme (Fig. 1).

After preparation of all dilutions,

withdraw 0.1 mL of the solution from each test tube using sterile pipette and place into the Petri dish with a corresponding selective medium, increasing each dilution for another 10 times. The solution is rubbed over the surface with a sterile spatula until its complete absorption with agar. Place 0.5 mL of the solution with certain dilutions into the test tubes with Blaurock medium, and at the same time dilution degree is increased ten times.

Thus, according to the scheme (see. Fig. 1), withdraw 0.1 mL of solution from the weighing bottle and place into the Petri dish with Endo medium and bismuth-sulphite agar, getting a 10^{-2} dilution. From the test tube No. 1 place 0.1 mL in the dishes with Endo medium, blood agar, Baird-Parker and Sabouraud agar. This resulted in a 10^{-4} dilution. From the test tube No. 2 place 0.1 of solution into the dish with Endo medium and 0.5 mL of solution into the test tube with Blaurock medium obtaining 10^{-6} dilution. Withdraw 0.5 mL of the solution from test tube No. 3 into the test tube with Blaurock medium and obtain 10^{-8} dilution.

Leave inoculated dishes and test tubes on the table for 15 minutes, and then put in a thermostat at 37°C . Dishes with Endo medium, blood agar and bismuth-sulphite agar are controlled 24 hours after inoculation, dishes with Baird-Parker and Sabouraud medium – after 48 hours, and Blaurock medium undergoes microscopy at day 4 of growth.

Evaluation of results. Determination of qualitative and quantitative composition of intestinal microflora of laboratory animals makes it possible to judge the health of animal abuse dysbiotic infringements in the composition of intestinal microflora and their correction with probiotics.

Thus, according to our studies [7-11], normal value of the total number of *E. coli* in laboratory rats is within 10^6 - 10^8 CFU/g, while the number of weak-fermenting strains does not exceed 25%. There are isolated lactose negative strains. Among haemolytic microorganisms,

representatives of staphylococci, streptococci, and *E. coli* are predominant. Number of staphylococci is within 10^4 - 10^6 CFU/g, and the number of pathogenic strains does not exceed 10% of the total number of coccal forms. The most numerous group of microorganisms is bifidobacteria and lactobacilli, which generally account for about 99% of the total number of microorganisms in the intestine of rats. Their number is 10^8 - 10^{10} CFU/g for bifidobacteria and 10^6 - 10^8 CFU/g for lactobacilli. Reduction of their number by two decades (100 times), at the background of the growing number of opportunistic groups of bacteria indicates the occurrence of a serious intestinal dysbiosis. Number of fungi of the genus *Candida* ranges within 10^4 - 10^5 CFU/g. Increase in their number by decade or more suggests occurrence of fungal dysbiosis. Development of Proteaceae dysbiosis is evidenced by the rise in the number of proteus cells from 10^2 - 10^3 CFU/g to 10^4 - 10^5 CFU/g. Total number of other lactose negative opportunistic enterobacteria ranges from 10^2 - 10^3 CFU/g.

It should also be noted that study of intestinal microflora of laboratory rats, which are fed with probiotic supplements allows to predict increase in the total number of bifidobacteria and lactobacilli, therefore, it is required to make additional dilution (test tube No. 4), and inoculate Sabouraud medium with 10^{-10} dilution. In case of dysbiosis modelling by administration of antibiotics, we predict decrease in the total number of *E. coli* or staphylococci (depending on the type of antibiotic), and bifidobacteria and lactobacilli. Therefore, inoculation on selective medium for these types of microorganisms should be done at lower dilutions.

Thus, we have proposed the complex method for determination of intestinal microbiocenosis of laboratory animals, which generally takes 4 days, but makes it possible to determine the qualitative and quantitative composition of microflora content of rat rectum and diagnose its infringements.