

## A POSSIBILITY FOR CRYOCONSERVATION OF MICROBIOLOGICAL SPECIMENS IN MINICRYOTUBES

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The preservation of frozen samples in culture collections is commonly connected with a great number of questions including organisation ones. For example, the application of cryotubes with a volume of 2 cm<sup>3</sup>, in which 0.5÷2 ml microbiological suspensions are conserved, is widely spread for storage in liquid nitrogen (LN<sub>2</sub>). Practically, the "sufficient" quantity for the preservation of a microbe strain is 0.1 ml in 99 % of the cases. Obviously, it is an economically unjustified form for preservation because of the area occupied from cryotubes and the expense for maintaining the required temperature (LN<sub>2</sub>). Furthermore, a risk of hermeticity disturbance of the cryotubes exists resulted in contamination of the specimen as well as of environment - a container with thousand other microorganisms. These problems in some collections were solved by means of suspension distribution in pieces of straws with a length of 2.5÷2.7 mm, used for drinking of juices and cocktails. Depending on their diameter 3÷4 straws are placed in a cryotube. In order for applying these straws as minitubes pressing in the one end should perform a thermal sticking before the suspension placement followed by heating and pressing the opposite end. The risks, which

bring this way for package, are closely related with the impossibility for quality control – hermeticity of the tube ends, danger of cracking during freezing, storage and thawing. In the same time, minicryotubes with a length of 65 mm and diameter of 3 mm are applied for preservation in LN<sub>2</sub> of spermatozoids used in artificial insemination. These mini cryotubes are closed hermetically with small glass or metal balls (globules) (Fig. 1).



Fig. 1. Standard cryotube (4.5 cm<sup>3</sup>) and standard minitube (65 mm) with globules for closing.

The manufacturer guaranteed that the materials, from which these straws or minitubes and globules are made, are resistant to great changes in the temperature. They stand freezing with speed of 0.1°C.min<sup>-1</sup> to 400°C.min<sup>-1</sup> and could be also sterilised by autoclaving at 1.2 atm.

Since there were no available data for preservation of microorganisms in minicryotubes, we carried out experiments for freezing some yeast strains in such way. Standard minitubes with a length of 65 mm as well as a smaller part of them with a length of 27 mm were applied (Fig. 2). Six minitubes were filled with a suspension of 3 ml and were placed in a cryotube with a volume of 4.5 cm<sup>3</sup> (Fig. 1). The pieces contained 0.1 ml of a culture and 6 of them were also placed in cryotube with a volume of 2 cm<sup>3</sup> (Fig. 2).

After one-year observation it was established that the samples were preserved sterile and no cracking or other hermeticity disturbances were found at any stage of the work. It gave a reason to use this possibility in the collection activity in the National Bank of Industrial Microorganisms and Cell Cultures. Over 500 yeast strains have been conserved (by 2 minitubes filled with 0.1 ml in 2 cryotubes) in LN<sub>2</sub> and stored for a period of 15 years.



Fig. 2. Standard cryotube (2 cm<sup>3</sup>) and minitube (27 mm) with globules for closing.

The performed extensive investigation allowed us to recommend the application of mini cryotubes closed with glass globules for conservation of microorganisms in the collection activity.