Mem. Natl Inst. Polar Res., Spec. Issue, **49**, 184–191, 1994

EQUIPMENT AND METHODS OF MICROBIOLOGICAL SAMPLING FROM DEEP LEVELS OF ICE IN CENTRAL ANTARCTICA

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Abstract: We report on the equipment, methods and results of microbiological sampling from deep levels of ice in Central Antarctica. The equipment includes a special microbiological drill laboratory (MBU) thermodrill (TELGA 14M), unit for sterile sampring from the ice core (USL) and borehole sampling unit (PSM-152).

We report the methods of sterile sampling of ice core with USL-unit and results of investigations of ice core from deep levels of ice on Vostok Station, accomplished in Institute of Microbiology Russian Academy of Science (RAN) and new borehole sterile sampling with PSM-152-unit carried out in 1991 on the Vostok Station.

1. Introduction

Microbiological research of ice deciphers the history of microorganisms settling around the Earth, and at the same time, it gives a new perspective to the study of anabiosis, the duration of which is highly controversial.

BECQUEREL (1951) confirms that protoplasm in anabiosis after drying and freezing can keep its vitality indefinitely. But this opinion lacks experimental corroboration. Such an experiment in laboratory conditions has no practical significance because of its duration. The glaciers of Antarctica and Greenland, however, are unique natural laboratories for the study of anabiosis in low temperatures (ABYZOV *et al.*, 1979, 1989; LLANO, 1971; MCLEAN, 1919)

Ice cores from different levels of ice can be sampled by thermodrilling, but for microbiological studies high purity is required in every step of the sampling process.

For solution of this problem a set of drilling and sampling equipment has been developed in St. Petersburg Mining Institute jointly with the Institute of Microbiology RAN.

The research has been carried out at Vostok Station in Antarctica at the pole of cold of our planet. The climate conditions are most favorable to keep microorganisms frozen — the average monthly temperature in August is –68°C; the absolute minimum is –89.2°C, and the maximum –13°C. The thickness of ice here is more than 3500 m. The rate of accumulation during the last 5000 years is about 2.4 cm/year. The temperature of ice increases, from –57.13°C at 48 m depth, approximately 1°C per 100 m (KOROTKEVITCH, 1972; KUDRYASHOV *et al.*, 1977; SHUMSKY, 1955; THOMPSON *et al.*, 1975).

The atmosphere circulation above Antarctica with meridional circulation of air

transfers microorganisms and promotes their inclusion in ice.

2. Equipment

The set of equipment, making sterile handling of microbiological samples possible includes a microbiological drilling laboratory (MBU), thermodrill (TELGA), unit for sterile sampling of ice core (USL) and borehole microbiological sample unit (PSM-152). This set allows to perform the whole cycle of work — from drilling a borehole and sterile sampling from the ice core to microbiological study (ABYZOV *et al.*, 1979; KUDRYASHOV, 1972).

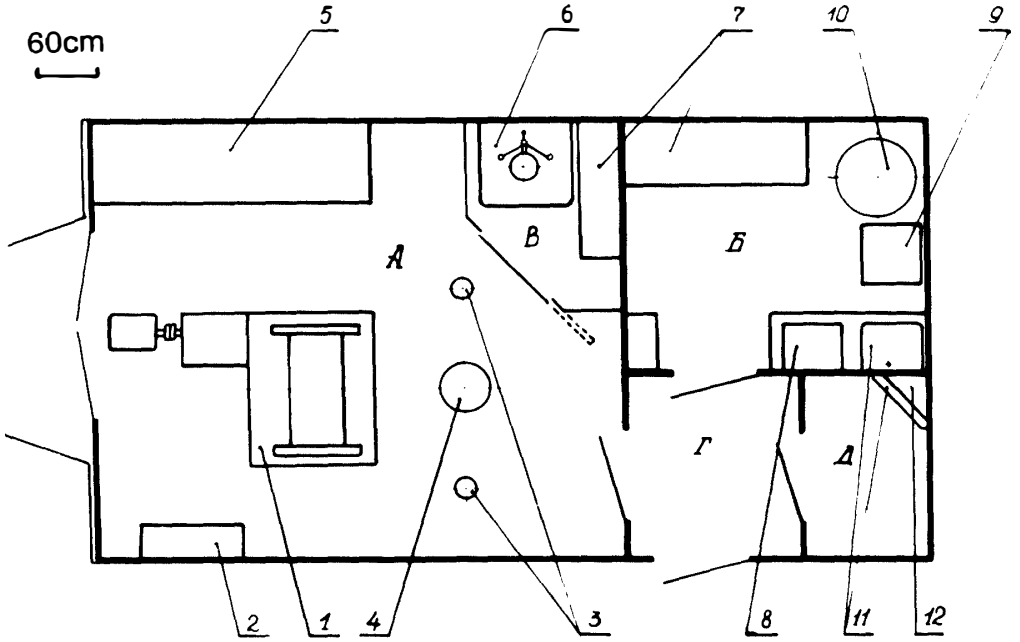

Fig. 1. Mobile microbiology drilling laboratory (MBU).
A-Drilling lab.; Б, B-Microbiological lab.; Г-Tambor. 1-Winch with cable; 2-Control panel; 3-Mast; 4-Borehole; 5-Joiner's bench; 6-USL-14M unit; 7-Tables; 8,9-Thermostatic boxes; 10-Sterilizing box; 11-Wash stands; 12-Water tank.

The mobile microbiological drilling laboratory (MBU) (Fig. 1) is a hut (9×3.7×2.5 m), mounted on a sledge, including drilling lab. (A), microbiological lab (Б ,/B) and a tambor (Г).

The drilling lab has a 7 m high mast, winch with cable and control panel. All rooms are equipped with UV lamps for sterilization of air and equipment surfaces.

The thermodrill (TELGA) is intended to drill "dry" boreholes (without antifreeze liquids). It was sterilized thermally and chemically before each insertion in to the borehole.

The TELGA-14 M thermodrill is shown in Fig. 2.

Technical data on thermodrill TELGA-14 M

–Diameter of hole, mm	180+2
–Diameter of heater, mm	178
–Diameter of core, mm	125
–Total power, kW	4.5–5.0

–Power of heater, kW	3.0–3.5
–Power of tubes warming, kW	0.7–1.0
–Power of vacuum compressor, kW	0.6
–Length of core tube, mm	2000
–Capacity of water tank, *l*	30
–Water lifting	by vacuum in water tank
–Length of drill, mm	6000
–Weight, kg	250

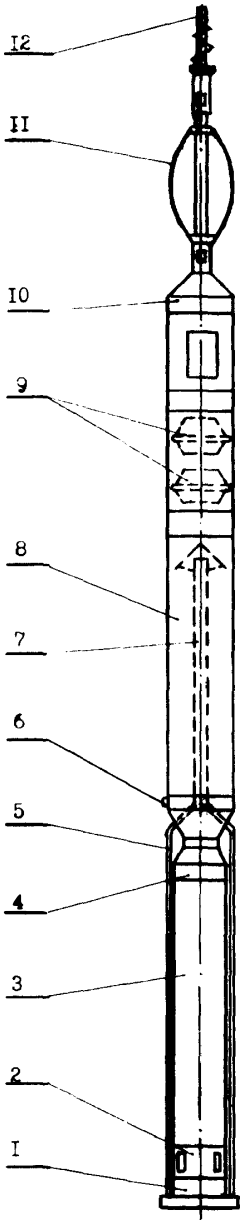

Fig. 2. The thermodrill TELGA-14M. 1-Heater; 2-Core catcher; 3-Core tube; 4-Transition bundle; 5-Water lifting tubes; 6-Pour-off stopper; 7-Inner tube; 8-Water-tank; 9-Compressor; 10-Transition bundle; 11-Anti-torque; 12-Cable.

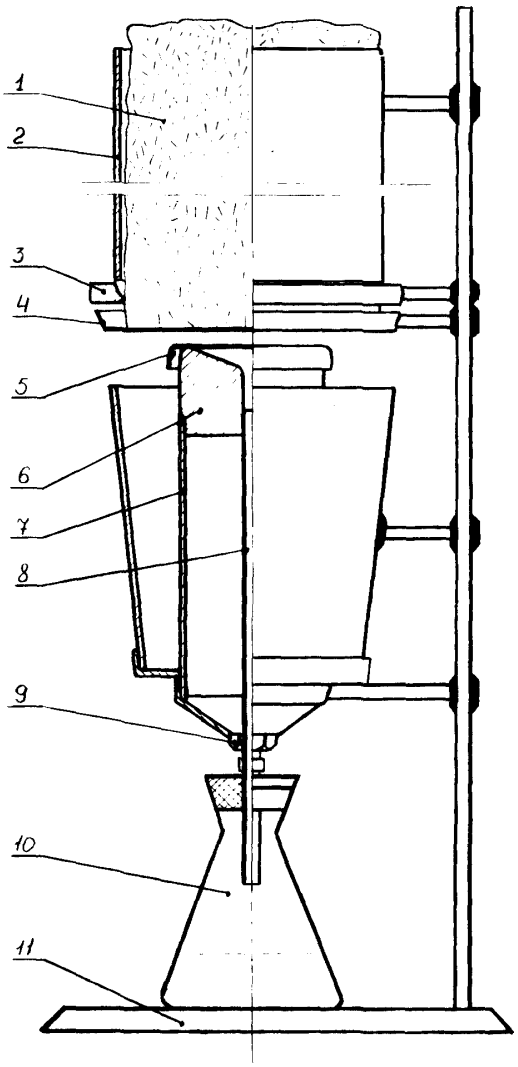

Fig. 3. USL unit for sterile sampling of ice core. 1-Ice core; 2-Guide tube; 3-Core-splitter; 4-Saucer; 5-Heater lid; 6-Heater; 7-Tube; 8-Water drawing tube; 9-Nut; 10-Receiving reservoir; 11-Stand.

Microbiological sampling from ice was carried out by sterile redrilling of the ice core with the USL unit. The main part of this unit is a heater. Its working surface is a concave cone. The heater together with a water drawing tube is fastened on the cartridge-case. Around it, there is a container for melting water from the external part of core. Above the heater there is a saucer where the piece of ice is placed before redrilling, a core-splitter and the guide tube.

Below the heater with water drawing tube a receiving reservoir is situated. The USL unit is shown in Fig. 3.

Technical data of USL unit

–Diameter of ice core, mm	120–90
–Diameter of redrilling in ice, mm	90; 70
–Thickness of external part of core, protecting sample from the atmosphere, mm	5
–Heater power, kW	0.7–1.0
–Heater voltage, V	220
–Unit size, mm	340×385×900

Before work, the room, all equipment and tools should be treated with 5% phenol solution and alcohol, then by UV radiation.

Sterilization of parts of the drill which are in contact with the ice core during drilling (heater, core-catcher, core-tube) must be done very carefully.

Directly before every lowering into the hole, the drill must be singed with flame.

Sterile sampling must be carried out immediately after lifting the drill to the surface.

The lower part of the core is separated with a core-splitter to obtain a clean part of the ice core surface. The sample is taken from the inner part of the core through this clean part of the core surface.

Then the core is placed with its split surface on the sterile heater. The inner part of the core is melted and the water is collected in the receiving reservoir through the water drawing tube. After sampling, the reservoir is soldered and then separated from the unit.

From one piece of core, obtained from one drilling cycle, (1.5 m) 10–12 samples (500 m*l*) can be taken. One sampling takes approximately 4 min.

Microbiological research has been carried out by the Soviet Antarctic Expeditions since 1980 to the present and includes ice thickness to 2405 m in depth from the surface (ABYZOV *et al.*, 1989).

Viable microflora was found in the ice from 0 to 2405 m in depth, represented only by sporeforming types. The age of the deepest level-2305 m-where a viable spore forming bacterium "Bacillus subtilis" was found is more then 180000 years. But we had no information about the total quantity of microorganisms in ice. Because of this, in 1989 microbiological research on ice from deeper than 1500 m was carried out. Besides the seeding on nutrient media, epyfluorescence microscopy and scanning electron microscopy methods were used. This was done to take into account the total quantity of cells found in the ancient ice levels.

The water samples (100–200 m*l*) for scanning electron microscopy were filtered through nuclear filters with 0,19 μm pore diameter. Filters were treated by 2% glutaraldegyde for 6 hours and then placed successively in 20, 30, 50, 70, 90, 96 ethanol for 10 min and acetone for gradual dehydratation in soft conditions (KUZNETSOV and

DUBINIA, 1989; LLANO, 1971).

Then the preparates were powdered with gold and studied with the scanning electron microscope "JEOL-JSM-T-3000" (Japan) with 5000–20000 magnifications.

The morphological variety of microbial cells was represented by bacilli-like and coccus-like forms, sometimes grouped in small accumulations (Fig. 4).

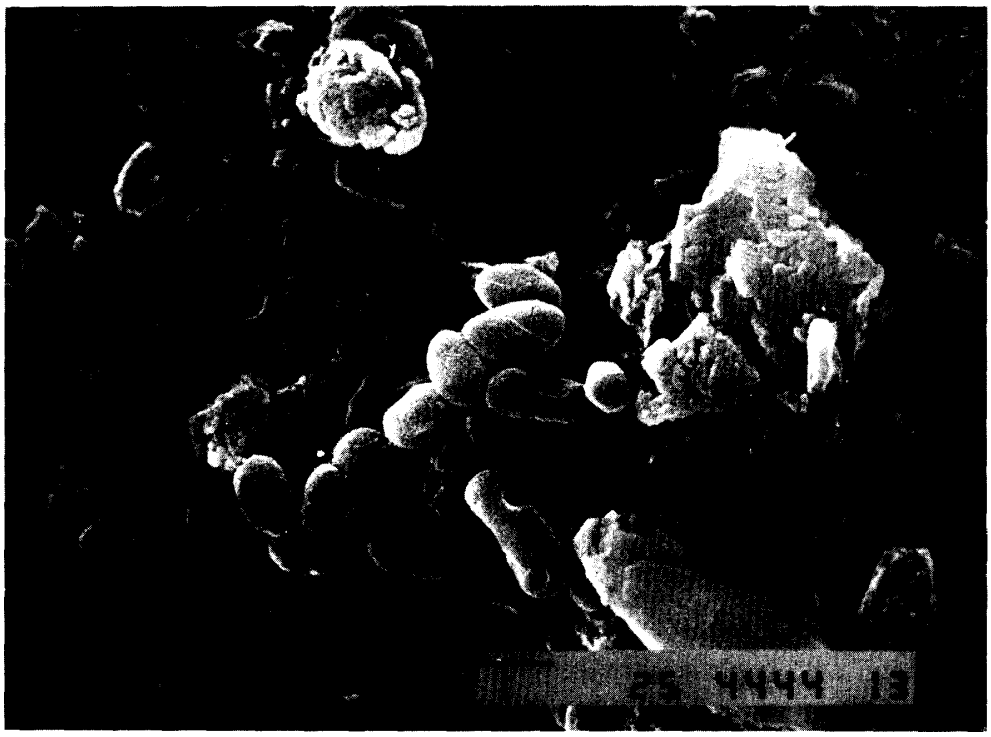

Fig. 4. Microorganisms discovered in ice from depth 2395 m (enlarged×14000).

The result of the direct count of microorganisms by SEM is shown in Table 1.

Comparative studies of deep levels of ice in Central Antarctica showed that most cells were discovered directly, bewer by seeding on nutrient media.

Obviously most microorganisms died during the long residence in ice or cannot grow on nutrient media.

As seen from Table 1, the quantity of viable microorganisms in ice is very low. Only 3% of samples from 1500–2405 m contain them. This is a result of not only extremely low microorganism contents in the ice but also the very small volume of sample taken away from the ice core (500–1000 m*l*).

Statistical validity of ice research demands increasing sample volume. By enlarging the ice-core diameter it is possible to enlarge the sample volume in 2–3 times. But if we could take a sample not from the ice-core but from walls of the borehole, the volume of sample can be increased 100 times and more, practically without limit.

From 1987, St. Petersburg Mining Institute developed the PSM borehole microbiological sample unit. The experiments show that the diameter of the melting cavity in ice may be 4 m and more.

In creating this unit, it has been taken into account that the temperature regime in the

Table 1. Quantitative changes of microorganism viability in ice of central Antarctica.

Investigated ice levels			Total quantity of samples, on nutrient media	The quantity of samples with viable microbes	% of samples with viable microbes
Intervals of depths (m)	Length of ice core (cm)	Approximate age (years)			
0 - 105.10	105.10	0 - 3000	144	29	20
105.10 - 206.70	101.60	3000 - 7400	129	18	14
206.70 - 320.80	113.10	7400 - 12500	250	24	10
330.00 - 1500.00	1170.00	12500 - 107000	207	14	6
1500.00 - 2405.00	905.00	107000 - 200000	176	6	3

unit and in the sampling zone must keep microorganisms alive in every steps of sampling, beginning from melting of ice to collecting material on the microbiological filters.

The sample unit includes a heater (3) (Fig. 5) for melting ice of borehole and creating an ice insulator above the sampling spot, a little tank (13) with bactericidal liquid (for example–ethyl alcohol) and compressed air. The tank has an electromagnetic valve and is connected to a heater (17) by a tube.

The pump (14), heater (17) and valve (4) are connected to heater nozzles (2) by tubes.

The unit also includes the container (5) with microbiological filters, electric valves (4) and the pump (15).

After placing the unit in the borehole, the heater (3) melts the borehole wall ice. Then the heater (3) is switched off, and melted water is frozen to insulate the sampling zone from the borehole. In the sampling zone, the bactericidical liquid is brought from the tank (13) where it was under the pressure of compressed air. After it the heater (17) and pump (14) are switched on. Melted water begins to circulate through nozzles (2) and melts a cavern of the required volume. Then the heater (17) and pump (14) are switched off and melted water from the cavern is pumped throught the container with microbiological filters (5) by pump (15). When sampling is over, the heater (3) is switched on once more, and the unit is released from the ice insulator and lifted up from the borehole.

The model PSM-152 borehole sampling unit was tested in 1991 in the model borehole at Vostok Station at air and ice temperature –45°C with the following parameters:

–Speed of introducing the unit into the borehole face, m/h 0.3
–Time of cavern formation, h 0.85
–Ice cavern diameter, mm 400
–Cavern height, mm 90
–Heater power, kW 1

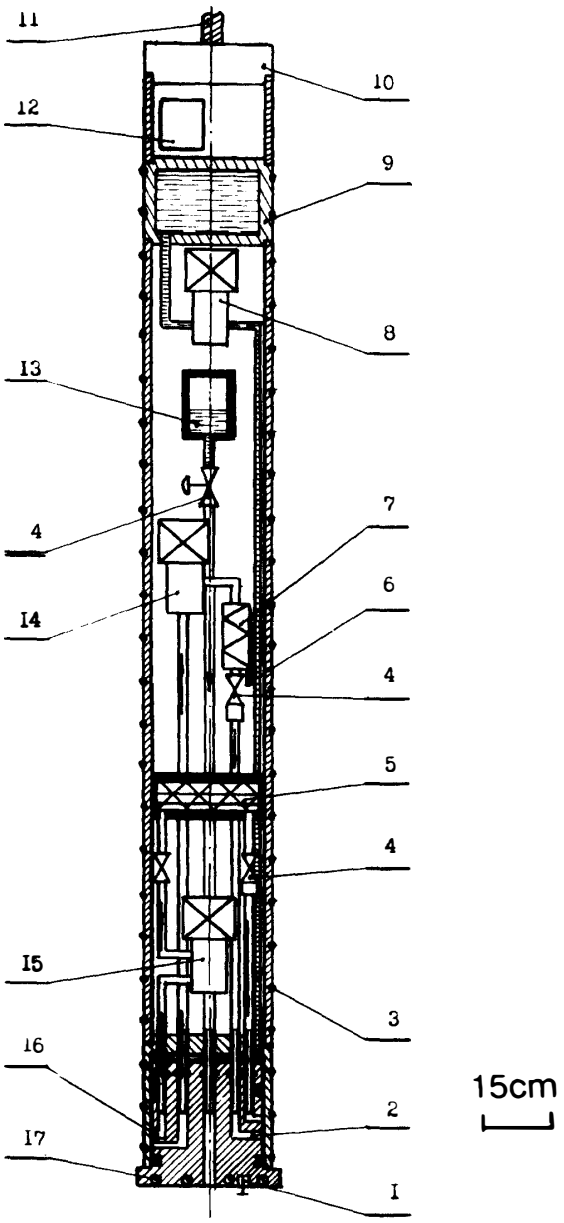

Fig. 5. The scheme of borehole sampling unit PSM-152.
1-Signalizator of borehole face; 2-Nozzles; 3-Heater; 4-Electric valve; 5-Microbiological filter container; 6-Temperature regulator in circulating water; 7-Heater; 8-Pump; 9-Tank; 10-Cable lock; 11-Cable; 12-Control block; 13-Bactericidal liquid; 14-Water pump for making cavern; 15-Water pump for water sampling; 16-Channel for sampling; 17-Heater.

–Ice packer running time, h 0.5

The microbiological research with the PSM-152 borehole unit is planned in the near future.

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(Received April 27, 1993; Revised manuscript received January 31, 1994)