



## **Precautions and controls in studying microbial contents of accretion ice of the subglacial Lake Vostok**

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The recent molecular biological studies of accretion ice of Lake Vostok deeply buried under the East Antarctic ice sheet testify for the very low biomass and ultra low DOC. Therefore, the ice core decontamination and subsequent melt water treatment procedures represent a crucial issue and must be performed according Forensic Biology and Ancient DNA research criteria. Our ice decontamination and melt water treatment protocols are based on stringent physical-chemical procedures followed by ice sample rinsing with 5000 Da filtered low DOC water. Such a treatment reliably removes not only contaminant cells but even extraneous dissolved DNA. However, further studies request to use commercial DNA extraction kits and PCR reagents including disposable plastic articles which are not yet certified bacterial DNA-free. That is why at present the only way to secure results from wrong interpretation is to establish a contemporary contaminant database in order to decipher potential contaminants from the original ice signals. In our work five types of overlapping controls for contaminant cells and foreign dissolved DNA were employed: (i) sham DNA extraction; (ii) negative PCR (with no template DNA added); (iii) ice core wash water; (iv) Vostok kerosene-based drilling fluid; (v) laboratory environment and wares. For all five controls corresponding rDNA libraries were constructed and relevant numbers of clones were sequenced. By this, contaminant database consisting of numerous bacterial phylotypes was established. In addition, any contaminant clones known from other works were included in the list. Bacterial phylotypes recovered from ice samples were always indexed on the basis of six criteria especially applicable for Vostok accretion ice. Since some physical and chemical features of the Lake Vostok environment are known, a degree of confidence was assigned to each criterion allowing us to discard obvious cases with the highest score as contaminants while those with lowest score refer to as

likely contaminants. These criteria (ordered respective their score decrease) include: (i) phylotypes recorded in the contaminant database, (ii) taxa/phylotypes proved to be contaminants in other studies, (iii) bacteria capable to degrade hydrocarbons, (iv) human/animal/plant saprophytes, commensals and pathogens, (v) taxa/phylotypes revealed in e.g. organic reach waste water and (vi) taxa/phylotypes revealed in other (physically separate) glaciers. Application of these contaminant criteria for bacteria recorded in Vostok accretion ice will be exemplified by some findings.