## B83 Products of Microbial DNA Amplification: Risks of False Results During DNA Typing of Decomposed Bodies and Skeletal Remains

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This presentation will discuss the observation of bacterial STR artifacts during DNA testing of skeletal remains and methods to overcome the problems associated with such peaks.

DNA extracted from decomposed human remains frequently contains not only fragmented human DNA but also microbial DNA. Human DNA specific extraction techniques, especially for very low quantities of DNA, are not available and so the presence of microbial DNA in extracts is unavoidable. Some widely used human forensic multiplexes have the ability to amplify various microbial DNAs and thus generate non-specific PCR products. As a result, it is necessary to have a tool for identification of these "microbial peaks" in order not to assign false allele numbers. By testing thousands of bone samples, the ICMP (International Commission on Missing Persons) has become aware of several noticeable patterns that are associated with various bacterial strains. These recognizable patterns are a clear signal of the presence of bacterially induced peaks. In addition, the resulting GeneScan® profiles of suspected bacterial peaks display an absence of artificial repeat slippage, sometimes called n-4 bands, stutter, or shadow bands. Even if there were described repeat sequences in bacterial, yeast and fungi DNAs, the detection of a "microbial peak" with a loss of a repeat unit has not yet been witnessed in the ICMP DNA laboratories.

It is beneficial to clone and sequence "microbial peaks," especially those that display peak patterns that have not been previously observed. Once the bacterial strain has been identified, it can be cultivated and tests performed on that corresponding microorganism to verify the observed patterns. Tests performed with microbial DNAs of a discrete species can enable the creation of a table of the most commonly found "microbial peaks" with highlighted "dangerous sizes" that have the potential of interfering with calling true alleles. Obtained values are continuously added to a special Genotyper® macro that flags possible problem peaks.

Studies of mixed ratios of microbial DNA and human DNA shows that microbial DNA present in a sample of human DNA can influence the characteristics of the resulting electrophoretogram. The level of influence is strongly connected with a primer 's complementarily to the binding site of a particular strain. Some of microbial DNAs generate artificial peaks only in very high quantities. It has been observed that the presence of microbial DNA in a sample of human DNA can lead not only to allelic drop-out (primers used for the amplification of microbial DNA) but also to the appearance of false alleles that are not generated while amplifying only microbial DNA. On the other hand, the presence of microbial DNA in a low quantity sample of human DNA (0.1ng) can help to "visualize" alleles that disappeared due to the stochastic effect.

Changing primer sequence would seem to be a reasonable approach in overcoming the appearance of the observed "microbial peaks" but it is difficult to predict how the changed sequence would affect the robustness of the kit. It would also require considerable testing of degraded and bacterially infested samples to determine whether different bacterial peaks would appear. However, once characterized, the bacteria species and their STR amplification patterns can be documented and steps taken to compensate for the presence of such bacteria.

**STR, Bacterial DNA, ICMP**

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