B15 Potential Contamination When Wearing Sterile Gloves During PCR Preparation: Pass-Through Contamination From Skin

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Contamination during DNA analysis based on PCR is a serious concern that usually happen in genetic labs. Although protocols are developed to avoid this issue, it still happen and most of the cases is not possible to find out the source of contamination. This paper present how, even wearing sterile gloves, contamination from the user can happen. It means that some extra-means should be considered in this sense.

This presentation will impact the forensic community and/or humanity by solving some issues regarding to contamination and crosscontamination, and, at the same time, providing knowledge and focus attention to some circumstances that are not frequent, but when they happen can affect the result of a case

1. Introduction: Mitochondrial DNA (mtDNA) analysis has became a routine procedure in human identification and in anthropological studies. One of the advantages of analyzing mtDNA is the enhanced sensitivity afforded with the technique. But this feature must be considered because contamination can affect the final results of a study. Quality control and quality assurance procedures are enacted to minimize and monitor contamination. However, sometimes it is not easy to identify the source of spurious or inconsistent. One vector for contamination is the gloves worn during experimentation. It is imperative to wear sterile gloves and change the gloves as needed. Clinical and epidemiological studies have demonstrated that bacterial and viral contamination can occur on the surface of sterile gloves after being worn for a length of time. Thus, DNA may get on gloves and be transferred during handling (i.e., cross contamination). It also is possible that if gloves are worn for certain periods of time that DNA may leach from the user's hand through the glove (i.e., pass through contamination). While contamination of this nature is not a routine concern, it may explain rare circumstances of undefined contamination. Therefore, we designed a set of experiments to determine if gloves could be conduits of contamination.

2. Material and Methods: To study pass-through contamination gloves were worn for different time periods compatible with labs tasks. Gloves were worn without touching anything for 5, 10 and 20 minutes by different users. Only intermittent gentle rubbing between the thumb and index finger was carried out to mimic general manipulations.

After each time frame a sample was taken from the areas usually in contact with the tubes using a wet cotton swab and a negative control was taken from a zone where no manipulations occur. After the swabbing the gloves were discarded.

All the swabs were extracted using an organic method (PCIA) and amplified for HVIB and HVIIA according to Wilson et al., we also included some nuclear DNA amplification. Post-amplification of the nuclear and mtDNA product was carried using capillary electrophoresis as previously described.

3. Results and Discussion: The experiments show that the length of time gloves are worn is an interesting factor to be considered. In some samples even after five minutes some DNA leached through the gloves, even in the apparent negative controls. These results are compatible with the clinical studies which have shown that after a time, even with careful washing, bacterial and virus contamination on the surface of the gloves can occur related to time and user.

These findings do not suggest that new practices in contamination control are warranted. They do suggest possible sources for contamination when it occurs. If gloves are not changed between cases, cross contamination may occur and explain why DNA types from unknown sources may be observed. However, cross contamination is not a serious concern under current practices. Pass through contamination may explain the presence of the operators mtDNA in a sample. Sensitivity of mtDNA analysis requires special care during the handling of samples and reagents, and particularly in extreme situations where sample manipulation is for prolonged times. If contamination persists, one may consider changing gloves every five-ten minutes or using double gloves. Also, washing the hands prior to putting on gloves could remove dead cells or their products from skin surface.

DNA, Contamination, Gloves

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