P29: Effects of different storage conditions of sequencing products with formamide in the quality of sequences.

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Introduction: DNA sequencing is widely used in molecular diagnosis and good sequence quality is crucial to a correct interpretation. It has been described that formamide quality decrease and sequencing reactions exposure to light, heat and/or oxygen can cause irregular peaks of cytosine and guanine in electropherograms. In a previous study, we concluded that despite the presence of this artifact when formamide is stored under non-ideal conditions, it does not significantly reduce the quality of the sequences.

Objectives: The aim of this study was to understand the impact of different storage conditions of sequencing mixes (formamide plus sequencing product) in sequence interpretation.

Materials and Methods: Three distinct PCR products were used: a wild-type product smaller than 200 bp; a wild-type product larger than 300bp; and a product with a point mutation. The sequencing mixes were stored under different conditions (exposure to light, air and distinct temperatures) during certain periods of time. The products were sequenced by Sanger method and the electropherograms were scored by evaluation of the background noise degree and presence/absence of irregular cytosine and guanine peaks.

Results and Discussion: When sequencing mixes were exposed to light and air, the sequences obtained at the end of the first day of exposure were illegible. Sequence quality can only be ensured when sequencing mixes are stored at room temperature (if properly protected from light and air) or at a temperature of 4ºC for a maximum period of 3 days. At a temperature of -20ºC, the sequencing mixes may remain stored for a maximum period of 1 week.

Conclusion: The sequencing mixes are affected by non-ideal storage conditions, being particularly affected by exposure to light and air. Even when properly protected from light and air, the stability of sequencing mixes can be affected by the temperature at which they are stored.

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