B107 Evaluating Casework Profiles When Traces of DNA Are Detected In the Reagent Blank

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After attending this presentation, attendees will learn quality based guidelines for evaluating the impact, on casework DNA profiles, when low levels of DNA are detected in the associated reagent blank. The presence of trace DNA in a reagent blank does not necessarily invalidate the associated case sample results.

This presentation will impact the forensic community and/or humanity by demonstrating a quality approach for assessing the overall impact on forensic casework samples of low level DNA contamination in reagent blanks.

In forensic DNA analysis, a reagent blank is processed within a batch of case samples as a negative control, to expose the possible presence of contaminating DNA. A reagent blank consists of all the reagent components of the extraction process, without the added DNA. Possible sources of contamination include the reagents themselves, the staff handling the samples, the equipment, or the consumables used within a laboratory. While a quality assurance program ensures that procedures are designed to minimize the risk of sample contamination, no system is effective in completely eliminating this risk. Forensic DNA analysis has evolved into a very robust, sensitive DNA detection system; therefore it should not be unexpected to occasionally find traces of DNA in negative controls. The key is to develop a mechanism to evaluate the impact of a contaminated reagent blank on casework samples.

At the Centre of Forensic Sciences (CFS), a reagent blank for a particular extraction batch is treated exactly the same as the sample within the batch that has the lowest amount of amplifiable DNA. For instance, if the sample with the lowest amount of amplifiable DNA in an extract volume of 15 µL must be concentrated to 6uL for amplification (in a 15 µL total amplification volume), so too would the reagent blank. On the other hand, if the sample with the lowest amount of amplifiable DNA in an extract volume of 15 µL requires dilution prior to amplification, the reagent blank would be diluted to the same extent. The reagent blank is carried through the entire process, from extraction through to detection.

Occasionally trace amounts of amplified product are detected, most often when a reagent blank is concentrated prior to amplification. The product detected usually ranges from a single peak to two or three peaks. In most of these instances, the corresponding case sample that requires this treatment is one with a minimal amount of DNA, all of which must be committed to the amplification. However, many more samples within the same batch may not require this treatment. Hence, the degree to which the ensuing result in the blank may have an impact on the interpretation and reporting of the case profile is dependent on a number of factors which vary sample by sample throughout the batch.

The CFS has developed guidelines for reporting DNA profiles from batches where a trace amount of DNA has been detected in the reagent blank. The key question is whether a low level of DNA, such as that observed in the reagent blank, would be detectable in the case sample. The following factors must be considered: the total amount of DNA detected within the case sample and hence the manner in which it was treated in preparation for amplification, the amount of DNA actually amplified the appearance of the ensuing profile, and the possible presence of corroborating findings from other samples within the same case. There are two possible outcomes to the evaluation of a reagent blank contaminant in relation to the quality of the casework profiles. Based upon the four factors described above, one can either exclude an impact or not. When one is able to exclude an impact, then the quality of the casework profiles is not at issue and these are reported in the normal fashion, with the rationale documented in the case file. When, on the other hand, one is not able to exclude a possible impact, then additional work is undertaken where possible, including reanalysis or resampling. If ultimately one is still not able to exclude an impact, then this finding is indicated in the report sent to clients. In this presentation actual examples of both outcomes, drawn from casework experience, will be discussed.

The approach that has been developed at the CFS for dealing with low levels of DNA in the reagent blank is scientifically sound and faithful to the principles of an open and effective quality system. In fact, the implementation of these guidelines has improved the CFS quality system by ensuring that evaluations of controls are based on their scientific merit rather than on an arbitrary all-or-nothing basis.

Contamination, DNA Analysis, Reagent Blank

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