Investigating factors affecting stability of synthetic DNA reference material at frozen temperatures and optimizing conditions for improved stability

A. Baoutina, NMI, Australia

Project summary

1. We propose to test several sample processing protocols following samples thawing (heating, mixing and digestion with a suitable restriction enzyme) to identify a procedure that ensures full homogeneity of thawed samples and their efficient amplification in PCR.
2. Once such protocol is found, we will use it to test if the presence of background nucleic acid in the RM formulation improves its stability at -20°C and -80°C.
3. In addition to these two main objectives, we propose to continue testing stability of the RM at two additional time points (fifteen and twenty four months).
4. Finally, as the best storage condition identified in our previous study was 4°C, we would like to test if RM ultrafiltration or addition to it of sodium azide as antibacterial measure for prolonged storage at 4°C affect its analysis by real-time PCR or digital PCR.

Results and Conclusions

This study was a continuation of an earlier WADA-funded project on the development of a synthetic DNA reference material (RM) for use in testing for EPO gene doping. The main aim of the study was to investigate the factors that affect stability of the RM during storage at freezing temperatures. Firstly, we confirmed that the previously observed decrease in the concentration of the RM stored frozen was, indeed, the result of the RM degradation and not due to its inadequate reconstitution upon thawing or compromised amplification in polymerase chain reaction. Secondly, we demonstrated that addition to the RM formulation of background nucleic acid improves its stability at freezing temperatures. In the optimal formulation, the material was stable at 4°C, -20°C and -80°C for one year. Finally, we showed that conventional methods to prevent bacterial growth during prolonged storage of the RM at 4°C do not affect its intended application.

As the outcome, we developed optimal protocols for RM formulation, storage and preparation for use. This outcome is important for generating a fit-for-purpose RM for EPO gene doping detection, but also for future development of RMs for detecting other doping genes. Availability of such RMs will facilitate the development of routine tests for direct gene doping detection that are reliable, robust, and able to withstand legal scrutiny.